Contributions from Healthcare Facilities to the overall Mass Loading of Pharmaceuticals on Wastewater Treatment Plants

by

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

The presence of human pharmaceuticals in the aquatic environment is now becoming a well-established fact. The identified problems associated with their presence include the fact that these compounds are biologically active, some of them are toxic in nature, and a number of compounds have potential to foster and maintain drug resistant microorganisms. They are discharged into the aquatic environment from a variety of sources, but mainly by the excretion of incompletely metabolized pharmaceuticals by individuals into the wastewater. This situation makes finding a source-control strategy difficult. However, healthcare facility (hospitals and long-term-care homes) effluents are suspected to have relatively higher concentrations of these compounds, as such facilities use pharmaceuticals in large amounts for diagnostic, cure and research purposes. It is expected that controlling discharges from these facilities may provide a cost-effective solution to reduce the pharmaceutical loads entering the aquatic environment.

Published literature indicates that very few studies have exclusively investigated the relative contribution of pharmaceutical compounds by hospitals to wastewater treatment plants (WWTPs). No study known to this author explores either discharges from or contributions by long-term-care homes. The current study investigates both types of healthcare facility effluents for occurrence and mass flows of nine therapeutic compounds and the corresponding relative contribution of these compounds to the respective downstream WWTPs.

Results support the idea that healthcare facility effluents may contain elevated concentrations of pharmaceutical compounds. The maximum concentrations of the antibiotic compounds detected in the hospital effluents were Sulfamethoxazole (10.9 μg/L), Trimethoprim (10.3 μg/L), and Ciprofloxacin (1.24 μg/L). The maximum concentrations of these antibiotics in the long term care facility effluent were 2.3 μg/L, 6.5 μg/L and 1.4 μg/L, respectively. The concentration of Acetaminophen was detected in levels of up to 134 μg/L in the hospital and 116 μg/L in the long-term-care home effluents. The contributions of pharmaceutical loads by healthcare facilities to their downstream WWTPs were found to be affected by the size of the facility, its service spectrum, and the size of the community contributing to the loads of these compounds to the same WWTPs. Relatively higher contributions were observed for antibiotic compounds; the maximum contributions of Ciprofloxacin were 26.6% for hospitals and 37% for long-term-care homes.

As hospitals vary considerably in the services they provide and thus the drugs they use, the findings of this study may not be representative for all the hospitals. Long-term-care homes, on the other hand, do tend to provide similar services, a fact supported by statistical findings

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Dedication

I dedicate my thesis to my parents, my father Syed Ghazi and my mother Gulshan Baigum, whose prayers and love paved the way in every field of my life.

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Chapter 1
Introduction

1.1 Background

In recent years, the occurrence of the pharmaceuticals in various environmental compartments has extensively been reported (Heberer, 2002a; Kolpin et al., 2002; Mompelat et al., 2009; Rabiet et al., 2006; Stackelberg et al., 2004). Their presence in the aquatic environment is of great concern mainly because these compounds are designed to be biologically active (Lissemore et al., 2006), and some have the potential to foster drug-resistant bacteria.

For both risk assessment and risk management it is important to identify the major sources of pharmaceuticals emissions. The main pathway whereby human pharmaceuticals enter the aquatic environment is patient excretion of incompletely metabolized pharmaceuticals (Brown et al., 2006), which then enter the sewerage system and subsequently reach water bodies either though direct WWTP effluent discharge or though sludge disposal sites.

Healthcare facilities (i.e., hospitals and long term care facilities) are suspected to be substantial point sources of many pharmaceuticals as a considerable amount of pharmaceuticals are administered in these facilities. The main entrance route of human pharmaceuticals into the water sources is WWTPs (Daughton, 2004). Therefore, determining the relative contribution of healthcare facilities to the total pharmaceutical load of the WWTPs was identified by the US EPA as an important research need (Daughton, 2004).

The emission of pharmaceuticals from healthcare units is still not well investigated. Limited reports characterizing hospital effluents are available but are not consistent in terms of experimental conditions, target compounds and extraction methods to precisely define these specialized streams. In addition, climatic conditions and pharmaceutical use trends that vary from country to country make it difficult to extrapolate results and draw reasonable and general conclusions based on these studies.

Very few studies have exclusively investigated the relative contributions of hospitals to downstream WWTPs, but they only consider discharges from hospitals and assume that households are the only other contributors to the influent load of WWTPs. Thus they largely underestimate the total healthcare facility contribution to the WWTPs, through ignoring long-term-care homes.

This study is the first to date that considers both types of healthcare facilities for the occurrence and mass flows of PhACs in their effluents, and their relative contributions to downstream WWTPs.

1.2 Project Objectives

The goal of this project was to determine the relative contribution of healthcare facilities (hospitals and long-term-care-homes) to the overall mass loading of selected human pharmaceuticals to WWTPs. The specific objectives of the project were to

1. Determine the occurrence of target pharmaceutical compounds in healthcare facility effluents and their downstream WWTP influents.
2. Determine day-to-day variability of pharmaceutical compounds in healthcare facility effluents.
3. Determine the mass flows of pharmaceutical compounds in healthcare facility effluents.
4. Investigate the relative contributions of target pharmaceuticals by healthcare facilities to the overall mass loading of WWTPs.

Chapter 2
Literature Review

2.1 Organization

This literature review consists of three sections. Section-1 covers background information about pharmaceuticals, section-2 examines studies on the occurrence of pharmaceuticals in healthcare wastewaters and section-3 deals with the existing theories used to predict the concentration of pharmaceutical compounds in raw wastewater.

2.2 Pharmaceuticals as Emerging Environmental Contaminants-EECs

The term Emerging Environmental Contaminants (EECs) refers to compounds of domestic, municipal, industrial or agricultural origin which are not currently regulated or monitored but possess eco-toxic potential, and may be future candidates for regulation. (Glassmeyer et al., 2008; Petrovic et al., 2008). Pharmaceutically Active Compounds (PhACs), among all other EECs, have received more attention because of their special physicochemical and biological properties (Glassmeyer et al., 2008; Kümmerer, 2008b).

The presence of pharmaceuticals in the aquatic environment was initially noticed in the 1970s (Kümmerer, 2001a; Santos et al., 2010) but received more attention in the mid 1990s with the advancement of analytical methods to detect chemicals at very low concentration (parts per billion) (Glassmeyer et al., 2008; Mompelat et al., 2009). Since then, the occurrence of pharmaceuticals in various environmental compartments has frequently been reported, i.e., in surface water (Loos et al., 2009; Mompelat et al., 2009; Zhang et al., 2008), ground water (Barnes et al., 2008; Batt et al., 2006; Rabiet et al., 2006), drinking water (Heberer, 2002b; Heberer, 2002a; Putschew et al., 2000; Ternes, 2001; Wasik et al., 2007) and even finished drinking water supplies (Benotti et al., 2009; Reddersen et al., 2002; Stackelberg et al., 2004; Stackelberg et al., 2007) in a ng-μg/L range. The first extensive study of the occurrence of pharmaceuticals, hormones, and other organic compounds in water sources was carried out by the US Geological Survey. This study revealed the presence of 82 out of 95 target compounds in 135 US streams (Kolpin et al., 2002).

The characteristics of PhACs that make them different from the other environmental pollutants include 1) a tendency of the parent neutral compound and its salts to form polymorphic solid states,

2) they mostly enter into the environment after human metabolism, 3) the frequent presence of large, complex molecular structure with multiple ionizable sites spread throughout the molecule, and 4) higher water solubility relative to molecular weight (Cunningham, 2008).

The entrance of PhACs into the aquatic environment is constant and unavoidable (Santos et al., 2010) because these compounds are considered necessary to life as they are used to maintain and restore human health. Thus, unlike other contaminants, they are marketed as an important product for use, and in contrast to conventional contaminants which have well defined point sources, they are discharged from widespread sources, from individual households to communal service facilities (Daughton, 2007).

The identified problems associated with the presence of PhACs in the aquatic environment include the intrinsic toxicity of PhACs like cytostatic agents and antibiotics; the fact that they are biologically active as these compounds are designed to produce biological responses in the receptors (Boillot et al., 2008; Christen et al., 2010), and the possibility that the drug effectiveness could be compromised, especially antibiotics as bacteria can develop and maintain resistance from their constant exposure to low concentrations of these drugs.

In addition there are concerns about the magnitude of the issue and the difficulty in the removal of some of these compounds in wastewater treatment processes. PhACs are produced and used in huge amounts- about 60,000 compounds are in use worldwide (Tropsha, 2000) with wide variations in their physicochemical properties (Kümmerer, 2008b). The difficulty in source identification and control arises because these compounds are discharged from wide spread point and non-point sources; furthermore, most of these compounds are very polar and mobile. Persistency in wastewater treatment processes is usually due to the resistance to biodegradation e.g. antiepileptic Carbamazepine and most of the antibiotics (Alexy et al., 2004; Martins et al., 2008).

2.3 Background Concepts of Pharmaceuticals

The U.S. Food and Drug Administration Center defines a drug as "A substance intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease" (F.D.A, 2004). Pharmaceuticals include a large number of compounds, prescription and non-prescription drugs, and diagnostic aids for both human and veterinary use. They include antibiotics, anti-inflammatories, antiepileptics, beta-blockers, anti-depressants, painkillers, lipid regulators, antineoplastics, antihistamines, tranquilizers diagnostic aids, and cytostatic agents. Approximately 60,000 drug compounds are used worldwide (Tropsha, 2000), and in Canada, 15,331 approved drug compounds are currently in the market (DPD April, 2010). The world wide consumption of antibiotics has been reported to be in the range of 100,000 – 200,000 tons per year, with an average annual per capita consumption of 15 g (Wise, 2002).

Drugs are classified in various ways including by origin, action, therapeutic use, site of action, and, by chemical structure (Nadendla, 2005). In addition, they can be sub-divided based on their chemical molecules. For example β-lactams, fluoroquinolones, sulfonamides, macrolides, etc., are all antibiotics. All β-lactam antibiotics share a common β-lactam ring; the parent compound of fluoroquinolones is nalidixic acid, with a fluorine atom attached to central ring; and sulfonamides contain a sulfonamide functional group in their structure. From an environmental perspective, considering drug sub-groups based on chemical molecules is more helpful, although differing behaviors of drug molecules belonging to the same subgroup of compounds in water treatment systems has been observed for certain compounds (Choi et al., 2008; M. C. Dodd et al., 2005). For analytical purposes PhACs are often grouped based on functional groups such as acidic (compounds containing carboxylic moieties and one or two phenolic hydroxy groups), basic and neutral (containing no acidic functional groups (Ternes, 2001).

The properties of a drug molecule are typically designed to facilitate transport from the site of administration to the site of action. For chemical interaction with the target receptor without binding with other receptors, drug molecules are developed with an appropriate size, shape, electrical charge, and atomic composition (Correia, 2007). The molecular size of the drugs varies widely from less than 10 to about 60,000 (Diaz-Cruz et al., 2007) Dalton; most of the drugs are in the range of 100 to 1000 Dalton (Correia, 2007) . The molecules can exist in anionic, cationic or zwitterionic states under

various environmental conditions. Furthermore they often have acidic or basic functionalities (Kümmerer, 2001b).

2.3.1 Active Pharmaceutical Ingredient-API

It is important to note that from an environmental perspective, the term pharmaceutical generally refers to the active component in a pharmaceutical composition. This component of the drug is measured in different environmental compartments, and prediction models use consumption data on this component (model input data) to estimate the concentration of this portion of the drug (tablets, capsules, etc). Pharmaceuticals are composed of Active Pharmaceutical Ingredients or APIs, and inactive or inert ingredients e.g. excipients, adjuvant, and, in some cases, pigments and dyes. Inactive ingredients are typically considered to be less important to the environment (Kümmerer, 2008b), although in some cases, they can affect the absorption or metabolism of APIs (Daughton, 2007). APIs, are complex molecules having different physicochemical and biological properties. They are used because of their specific biological activity and usually characterized by their ionic nature. This component is of interest to researchers, in the environment (Kümmerer, 2008b).

2.3.2 Metabolism and Transformation Products

Pharmaceuticals are eliminated from the human body by metabolism and subsequent excretion. Drugs are xenobiotics (compounds that do not belong to or are not expected to be in organisms bodies), and metabolism is the process that either breaks them down or transforms these foreign compounds when they come in contact with organisms so that they can be easily removed (King, 2009). Knowledge of drug metabolism can facilitate evaluating the environmental concentrations of pharmaceuticals, risk assessment strategies, and an understanding of consumption and excretion relationships.

Drugs, once administered, are metabolized in the human body. During metabolism the lipophilic compounds are changed into more water- soluble (hydrophilic) compounds, and are more easily excreted (Stephen et al., 2004). Drug metabolism is usually divided into two phases: phase-I functionalization reactions, including oxidation, reduction, hydrolysis, and hydration and phase-II conjugation reactions (Gibson et al., 2001). Phase-I reactions convert the drug into more-polar metabolites by adding or unmasking a functional group (-OH, -NH_2, -SH) (Correia, 2007). The

increased polarity helps the body to readily excrete these compounds. These metabolites are mostly biologically inactive; however, in some cases though activity can be changed or enhanced. For example O-desmethylvenlafaxine is a major active metabolite of the antidepressant venlafaxine (Merck & Co, 2004). Drug metabolites are typically believed to be more persistent in the aquatic environment than the original compounds because of their increased polarity (Petrovic et al., 2008).

If Phase-I reactions do not produce metabolites that are sufficiently polar, to be excreted from the body, then the metabolites often undergo a second reaction that involves; attaching a polar and ionizable endogenous molecule such as glucuronic acid, sulfate, glycine, or glutamine to form highly polar conjugates (Stephen et al., 2004) that can be then excreted. Some compounds which already have a required functional group can directly form conjugates (Correia, 2007). Phase-II reactions are considered to be true detoxification reactions (Gibson et al., 2001) ; however, there are exceptions. Some conjugates have proved to be even more potent than the parent compound, for example, glucuronidation of the analgesic morphine. In fact morphine-6-glucuronide is twice as potent as morphine itself (Smith et al., 2001). Conjugates especially formed with glucuronic acid, have the tendency to be cleaved during sewage transit and during sewage treatment processes to produce their parent compound (Alder et al., 2006; Khan et al., 2004).

Factors that affect drug metabolism include physicochemical properties of the drug compound, route of administration, genetics, sex, age, heath condition, diet, and environmental factors (people exposed to certain environment e.g. industrial workers) (Correia, 2007; Nadendla, 2005). The administered parent compound may be excreted via urine and feces as unchanged, major metabolites, glucuronide or sulfate conjugates and a complex mixture of metabolites (Kümmerer, 2008b).

Significant amounts of administered drugs are excreted though urine (70%) (Alder et al., 2006). Other excretion routes include saliva, sweat, and mother's milk; however these are not typically significant. The metabolism and excretion step may not be present in external (dermal) drug applications. Drugs are metabolized in human bodies to differing extents, and the excretion of unchanged drugs as a percentage of an administered dose varies from less than 5% (acetaminophen, carbamazepine) to more than 90% (contrast agents, e.g., Iohexole) (Sweetman et al., 2007; Glassmeyer et al., 2008).

2.4 Consumption Pattern of PhACs and Occurrence in the Aquatic Environment

There is a direct relationship between the consumption of pharmaceuticals and their occurrence in the aquatic environment; generally, higher concentrations are expected for highly used compounds. The drug consumption varies between countries. Hence, recognizing these differences is important when the findings about the occurrence of pharmaceutical compounds of any study carried out in one country are extended to others without taking into account these differences.

The use pattern of pharmaceuticals varies from region to region and country to country, depending upon existing legislation, treatment guidelines, marketing strategies, regulations, climatic conditions, personal preference and healthcare systems (Alder et al., 2006; Corcoran et al., 2010; Daughton, 2007; Goossens et al., 2005; Kümmerer, 2008b). Therefore, understanding these differences is required as background to any evaluation of concentrations reported in studies carried out in different parts of the world. For example, Vancomycine is a widely used antibiotic in the USA, but its use in Europe is very restricted (Kümmerer, 2001b). In Japan, only 0.4% women of reproductive age take contraceptive pills (ethinyl estroadiol), compared to 16% in North America (Kümmerer, 2008b). Furthermore, colfibric acid, a metabolite of some fibrate lipid regulators and widely detected in Europe, is seldom detected in the USA because these compounds are not often used there (Alder et al., 2006). In addition for the year 2004, the use of 81 antibiotic compounds has been reported in USA compared to 153 of such compounds in Europe (Goossens et al., 2007) Wide differences in the use of antibiotic compounds within European countries have also been reported (Goossens et al., 2005). Such differences have been cited in the use of certain compounds and also access to these compounds. For example, a number of drugs that are only available on prescription in some countries can be purchased over the counter in others (Kümmerer, 2008b), which could affect their environmental concentrations. Again there are variations in the trends of pharmaceutical use; consumption of certain compounds is increasing in some countries while decreasing in others (Alder et al., 2006). All the above differences suggest that regional situations need to be considered by any researcher extending study results carried out in one country to another.

Seasonal variation in consumption patterns is also found to be important (Alder et al., 2006), hence affecting the concentration of compounds entering into the aquatic environment at different times of the year. For example a higher consumption of antibiotic compounds in ten European countries was

observed during the first and last quarter of the year than during the third and fourth (Goossens et al., 2005). Castiglioni (2006) observed higher WWTP influent loads of his target compounds (including Sulfamethoxazole and Ciprofloxacin) in winters than in summer.

2.5 Healthcare Facilities

A variety of sources contribute to the emission of PhACs to the aquatic environment and include pharmaceutical manufacturers, healthcare facilities, long term care homes, individual households, dumps and land-filling of discarded pharmaceuticals, veterinary and agricultural sources, and wastewater treatment plants.

The main pathway of pharmaceuticals to the aquatic environment is excretion of these compounds by patients (Chang et al., 2010). Therefore, healthcare facilities (hospitals and long term care homes) are considered important source of pharmaceuticals as a considerable amount of PhACs are used within these facilities for diagnostic, cure and research purposes (Emmanuel et al., 2005). Higher concentrations of pharmaceuticals, especially antibiotics, cytostatic agents, and iodinated contrast media have been reported in hospital wastewaters (Alder et al., 2006). Patients in long -term care homes also often receive several medications, and their prescriptions are likely to be changed frequently (Daughton, 2007). Furthermore unlike hospitals the patients stay in these facilities for longer periods. This may result in high discharges of certain compounds from these facilities.

2.5.1 Hospitals

This study has been carried out in Ontario, Canada; therefore this section presents information about the hospital sector in Ontario and some key terms used for hospital data that may have a connection to drug discharges.

The Canadian Institute of Health Information (CIHI., 2009) defines a hospital as *"an institution where patients are accommodated on the basis of medical need and are provided with continuing medical care and supporting diagnostic and therapeutic services and which is licensed or approved as a hospital by a provincial government or is operated by the government of Canada".* Ontario has four different types of hospitals: Public, private, federal, and cancer care. According to Public Hospital Act-964, hospitals are classified as general hospitals, convalescent hospitals, hospitals for chronic patients, active treatment teaching psychiatric hospitals, active treatment hospitals for alcoholism and drug addiction and regional rehabilitation hospitals, which are then further graded from "A to V" depending upon the size, care services offered by the facility, and their affiliation.

There are a total of 234 hospitals in Ontario, including seven private hospitals (Ministry of Health and Long-term care, Ontario). Hospitals in Canada operate under the health authorities in some provinces and as separate entities in other provinces (CIHI., 2009).

Some hospitals also have beds allocated for long term care, known as hospital based continuing care. The residents in these facilities are a diverse population with complex health needs, mostly in a clinically unstable condition and dependent on others for daily activities. Data from 2004-2005 shows that 80% of the patients were admitted from the acute care beds in the hospital. Their average length of stay in these care centers is less than three months (CIHI, 2006).

The key indicators in hospital data which will likely have a connection with the drug discharges from these facilities include number of beds staffed (i.e., "beds and cribs available and staffed to provide services to inpatients")(CIHI, 2000) which is also a measure of hospital size, bed density (beds available in hospitals /1000 population); and average length of stay for inpatients (days).

2.5.2 Long term care homes

The Ministry of Health and Long-Term Care defines a long term care (LTC) home as *"a home-like facility that provides care and services for people who no longer are able to live independently or who require onsite nursing care, 24-hour supervision or personal support"*. In Ontario such facilities are owned and operated by various organizations i.e., municipalities, private corporations, and charity organizations. They operate under the regulatory authority of the Ministry of Health and Long-Term Care (http://www.health.gov.on.ca/en/).

Long term care facilities are also known as nursing homes, residential care facilities, and personal care homes. The residents in these facilities are a more homogenous group of people, than in the hospital based continuing care facilities, older in age and stay there for a longer period of time. These residents are clinically stable and moderately dependent in their daily activities. Cognitive impairment and reduced physical function is mostly observed among these residents (CIHI, 2006).

Drug use in long term care homes is relatively high, especially of antibiotics and antidepressants. This situation occurs because older people are highly susceptible to infections (Mody et al., 2007; Monette

et al., 2007; Moro et al., 2007). Over one year in the US, 400,000 deaths in nursing homes were found to be infection related (Crnich et al., 2007). Pneumonia and urinary tract infections (UTIs) are common (Nicolle et al., 2000). The chance of pneumonia in elderly people in a long-term-care home setup is ten times greater than in a normal community setup. In the US the annual Medicare cost for nursing-home-acquired pneumonia is estimated to be over $3 billion (Bonomo et al., 2002).

One important factor that contribute to the high drug use in LTC homes is the physiological responses that sometimes change with age create uncertainties in diagnostics, often resulting in more drugs being prescribed (Nicolle et al., 2000); for example 20-30% of elderly patients may not present fevers even with severe infections (Bonomo et al., 2002).

Antibiotics have been reported to account for 40% of the total prescribed systemic drugs, and 50-70% of residents receive at least one antibacterial drug in any particular year (Nicolle et al., 2000). Some studies indicate that 15% of the population is prescribed antibiotics at any time (Bonomo et al., 2002). (Mylotte, 1996) studied a 150 bed nursing home facility and found that Trimethoprim, sulfamethoxazole, and ciprofloxacin together made up 55% of the all prescribed antibiotics.

Depression develops among nursing home residents because of the various losses in old age, physically illness, and disability (Llewellyn-Jones et al., 2007; Kramer et al., 2009) leading to high use of antidepressants in these homes.

The intensive use of antibiotics has led to LTC homes acting as a reservoir for drug resistant organisms (Bonomo et al., 2002; Nicolle et al., 2000; Fluit et al., 2006). Crnich et al., (2007) have indicated that the number of infections related to antibiotic resistant bacteria in US nursing homes showed an increasing trend from 2000 to 2004. Additionally, an increasing trend in the prevalence of resistance to sulfamethoxazole, trimethoprim and ciprofloxacin in nursing homes in the Netherland was reported by (Vromen et al., 1999).

2.6 Characteristics of Hospital Effluents

Assessing the significance of hospitals as a point source of human pharmaceuticals requires data regarding the occurrence of these compounds in the hospital effluents which has been gained by a review of the related literature and the concentrations that have been reported are summarized in Table 2-2 and Table 2-3.

The discharges of wastewaters from hospitals depend upon several factors, such as the number of beds, medical care services available, and location (Askarian et al., 2004). Hospitals generate large amounts of wastewater (between 400 and 1200 L/bed/day, Emmanuel et al., 2005) that contain elevated concentrations of chemicals, biological liquids, drug residues, heavy metals, and radionuclides (Boillot et al., 2008).

Qualitatively hospital wastewater can be divided into two classes: one that is similar to municipal wastewater and comes from kitchen, laundry, and personal hygiene of patients and staff, and a second that is more specific to hospitals, and contains physical, chemical, and microbiological loadings (Boillot et al., 2008). Chemical characterization of hospital wastewaters has indicated that they can contain a variety of chemical compounds, pharmaceuticals, disinfectants, diagnostic aids, and heavy metals (Kümmerer, 2001b).

The genotoxicity potential of hospital effluent has also been demonstrated (Gautam et al., 2007; Giuliani et al., 1996; A. Hartmann et al., 1999). Eco-toxicological risk assessment studies have confirmed the presence of hazardous materials in hospital wastewater and suggested that they can affect aquatic ecosystems (Emmanuel et al., 2005). Gautam et al. (2007) demonstrated the occurrence of cytostatic agents in hospital effluents and their potential as eco-toxicological hazards and the possible effects on the WWTPs process.

In addition most hospitals have laboratory facilities for diagnostics, recovery monitoring, and research purposes, which generate liquid residues that are heavily contaminated with patient blood

and urine and test reagents, and are generally discharged into sewers (Kümmerer, 2004a). Seven out of nine samples taken from the laboratory effluent of a hospital were found to be mutagenic, indicated by the Ames and hamster cell tests (Gartiser et al., 1996).

Hartmann et al. (1998) found that fluoroquinolone antibiotics are the main source of genotoxicity in umuC tests. The umuC test is a short-term bacterial test, that uses Salmonella typhimurium (TA 1535/pSK 1002) and monitors the induction of a umuC'-'lacZ fusion gene after DNA damage (Giuliani et al., 1996; Oda et al., 1985; Steger-Hartmann et al., 1997).

The characterization of hospital effluent based on conventional wastewater parameters (COD, BOD, TSS) shows that hospital effluent is not much different than municipal wastewater (Kümmerer et al., 1997). The concentrations between 43 and 2464 mg/L for COD, 50 and 530 mg/L for TSS, 16 and 3000 mg/L for TOC, and 15 and 1560 mg/L for BOD_5 have been reported in hospital effluents (Table 2-1).

Chlorine is present in certain disinfectants that are used in hospitals, and when discharged into wastewater, chlorine can react with organic matter producing organic chlorine compounds that can be measured as Adsorbable Organic Halogens (AOX). These compounds are toxic to aquatic organisms, and their properties make them persistent environmental contaminants (Emmanuel et al., 2004). Higher concentrations of these organochlorine compounds are reported and up to 14mg/L concentration are measured in German hospital effluent (Kümmerer et al., 1998). The presence of AOX in hospital effluent can also include iodinated contrast media released by radiology departments (Emmanuel et al., 2004). Oleksy-Frenzel et al. (2000) detected a high concentration (9970 µg/L) of an iodinated fraction i.e., adsorbable organic iodine AOI in hospital effluent, apparently contributed by X-ray contrast agents. The higher concentrations of contrast agents in wastewater occur because of the higher consumption of these compounds in hospitals for diagnostic purposes and because they are metabolically stable in human bodies and therefore excreted mostly unchanged (Hartmann et al., 2002; Perez et al., 2007).

Table 2-1: Physico-chemical and microbiological characteristics of hospital effluent.

Parameter	Unit	concentrations	Cuntry	Reference
TSS	mg/L	484	Brazil	(Vasconcelos et al., 2009)
		531	India	(Gautam et al., 2007)
		155-298	France	(Emmanuel et al., 2005)
		197-446	Mauritius	(Mohee, 2005)
		50-80	France	(Boillot et al., 2008)
		339	Spain	(Suarez et al., 2009)
		225	France	(Emmanuel et al., 2004)
TDS		1540	India	(Gautam et al., 2007)
COD	mg/L	658	Brazil	(Vasconcelos et al., 2009)
		43-270	France	(Boillot et al., 2008)
		1067	India	(Gautam et al., 2007)
		362-2664	France	(Emmanuel et al., 2005)
		479-636	Mauritius	(Mohee, 2005)
		362-1492	France	(Emmanuel et al., 2004)
		2464	Spain	(Suarez et al., 2009)
BOD_5	mg/L	15-120	France	(Boillot et al., 2008)
		149-333	Mauritius	(Mohee, 2005)
		200-1559	France	(Emmanuel et al., 2005)
TOC	mg/L	16-82	France	(Boillot et al., 2008)
		160-3095	France	(Emmanuel et al., 2005)
AOX	mg/L	0.18-0.82	France	(Boillot et al., 2008)
		0.17-1.61	France	(Emmanuel et al., 2005)
		0.41	Germany	(Gartiser et al., 1996)
		0.7	France	(Emmanuel et al., 2004)
		14.2	Germany	(Kümmerer et al., 1998)
Free Chlorine	mg/L	0.09-0.55	France	(Boillot et al., 2008)
Bacterial count	CFU/mL	$2.5X10^7$	India	(Gautam et al., 2007)
Staphylococci	/100mL	608	France	(Boillot et al., 2008)
Fecal bacteria	NPP/100mL	$1x\ 10^6$	France	(Emmanuel et al., 2005)

Hospital effluents contain lower bacterial concentrations (Table 2-1) than are typically present in municipal wastewater (10^8/100 mL) (Metcalf & Eddy, 1991). The reduced microbial concentrations have been attributed to the presence of disinfectants and antibiotics in the hospital effluents (Emmanuel et al., 2005; Gautam et al., 2007). Among the most frequently detected microorganisms are virus and pathogenic bacteria, including those with the resistant characteristics (Emmanuel et al., 2005; Schwartz et al., 2003).

2.6.1 Drug discharges

In this section, antibiotic drugs are discussed separately from the rest of the PhACs because antibiotics are of particular interest to the scientific community based on their potential to develop and maintain antibiotic resistance in addition to their biological activity.

The published data on the likelihood of antibiotics in hospital wastewater, the amount of antibiotics consumed in hospitals and the occurrence of these compounds in hospital effluents have been reviewed. The maximum detected concentrations are presented in a tabulated form (Table 2-2).

2.6.1.1 Antibiotics

The term antibiotic refers to any antimicrobial agent that can come from either natural or synthetic sources which can act against micro-organisms (Diaz-Cruz et al., 2007). Antibiotics are widely used in human and veterinary medicine to treat microbial infections. In the live stock industry, they are also used as growth promoters. Other uses include agricultural and aquaculture, i.e., for fruits (Streptomycin), crops, poultry, beekeeping, and fish farming (Kümmerer, 2008b).

More than ten antibiotic classes (based on chemical structure) are currently used for human therapeutic applications (Huang et al., 2001) and this includes aminoglycosides, ansamycins, carbapenems, carbacephem, cephalosporins, glycopeptides, macrolides, monobactams, pencillins, polypeptides, quinolones, sulfonamides and tetracyclines. Among these classes; aminoglycosides, macrolides, ß-lactams, fluoroquinolones, sulfonamides, and tetracyclines are often detected in hospital wastewater with fluoroquinolones and sulfonamides present in higher concentrations (Table 2-2).

The global use of antibiotics per annum ranges from 100 to $200x10^6$ kg (Wise, 2002). The substantial consumption of antibiotics has led to their presence in various environmental compartments Antibiotic compounds are partially metabolized in the human body and excreted mostly via urine and discharged into hospital wastewater to varying degrees (Kümmerer, 2004b). For example 40 - 50% of a ciprofloxacin oral dose is excreted unchanged in the urine and about 70% of a parenteral dose may be excreted unchanged within 24 hours. About 80-100% of a dose of sulfamethoxazole is

excreted in urine, of which 60% is in the form of acetyl derivatives. For trimethoprim 40 to 60% of the dose is excreted in urine (Sweetman et al., 2007). Many of the antibiotic compounds have been reported to be resistant to biodegradation (Martins et al., 2008; Martins et al., 2008; Alexy et al., 2004), and hence they make their way to the aquatic environment either though WWTP effluents or sludge disposal sites (Kümmerer et al., 2003). The detection of these compounds in wastewater treatment effluent indicates partial removal in wastewater treatment processes hence they enter the water bodies (Miao et al., 2004).

The presence of antibiotics in the aquatic environment is of concern because of their potential to cause genotoxic effects, disturbances of the aquatic ecology, human health risks and formation of antibiotic resistant strains of bacteria (Brown et al., 2006; Lindberg et al., 2004). For instance, the antibiotic ciprofloxacin has been reported to be the main source of genotoxicity in hospital effluent using a umuC test (Hartmann, 1998). Furthermore, their presence in the aquatic environment can challenge water reuse technologies. The continuous exposure to even low level concentrations of antibiotics (ng/L-μg/L) is expected to develop resistance in bacteria (Chang et al., 2010). There is evidence of antibiotic resistant organisms in sewers receiving wastewater from hospitals (Brown et al., 2006). The importance of keeping existing drugs effective is highlighted by the fact that introducing new drugs takes about seven to nine years time on average for approval with an approximate cost of $700 million to $1 billion (Jambhekar et al., 2009).

The excretion of incompletely metabolized antibiotic compounds is the primary source of antibiotics to the aquatic environment (Chang et al., 2010; Alder et al., 2006). Hospitals are believed to be a significant point source of antibiotics as considerable amounts of antibiotics are consumed in hospitals. Data for various countries indicate that hospitals are responsible for 20 to 70% of the total antibiotic consumption of a country (Schuster et al., 2008). In Germany, 1998 pharmaceutical sale figures indicated that the fraction of the total antibiotics sales attributed to hospitals varied from a few percentages to more than 90% depending upon the compound (Alder et al., 2006).

In summary, drugs have been found to making their way into the aquatic environment: The probability of detection of a pharmaceutical in the aquatic environment is a function of its use (initial

concentration), extent of metabolism in the human body, and persistency in the aquatic environment. Highly used and more persistent compounds are often detected in aqueous samples while those that are extensively metabolized have less chances to be detected. For example tetracycline antibiotics are highly metabolized in the human body and are therefore barely discharged into wastewater as in the form of the parent compound (Kümmerer, 2001a).

Sulfonamide, fluoroquinolone, and macrolide antibiotics show highest persistency in the aquatic environment and are frequently detected in wastewater (Brown et al., 2006). The half life of antibiotic compounds in the aquatic environment varies from a few days for some ß-Lactams to several hundred days for Tetracycline or quinolone antibiotics (Kümmerer, 2001a).

The measured concentrations of the sulfonamide Sulfamethoxazole in hospital effluents were 2µg/L in the USA (Brown et al., 2006), 12.8 µg/L in Sweden (Lindberg et al., 2004) and 81 µg/L in India (Diwan et al., 2009; Brown et al., 2006). Up to 15 µg/L of Trimethoprim has been detected in a hospital effluent (Thomas et al., 2007). Higher concentrations of the fluoroquinolone Ciprofloxacin have been reported in many studies, with the measured concentrations in hospital effluents found to be around 87 µg/L in Switzerland, 100 µg/L in Sweden, 124 µg/L in Germany, and 236 µg/L in India. The highest concentrations of the fluoroquinolone ofloxacin and norfloxacin measured in hospital effluent were 35 µg/L and 44 µg/L respectively (Table 2-2). Up to 83 µg/L of the macrolide Erythromycin-H_2O (a degradation product of erythromycin in aqueous solution) was found in the effluent of a German hospital. The concentrations of antibiotic compounds found in hospital effluents in various studies are summarized in Table 2-2

Table 2-2: Maximum antibiotic concentrations measured in hospital effluents

Class, Compound	Conc (ng/L)	Country	Reference
Sulfonamide			
Sulfamethoxazole	4107[a]	Norway	(Thomas et al., 2007)
	2100[b]	USA	(Brown et al., 2006)
	1060[c]	China	(Chang et al., 2010)
	12800[c]	Sweden	(Lindberg et al., 2004)
	81100[c]	India	(Diwan et al., 2009)
	7350[c]	Taiwan	(Lin et al., 2009)
	300[d]	Australia	(Watkinson et al., 2009)
	6000[a]	Germany	(Ohlsen et al., 2003)
Sulfadiazine	253[c]	China	(Chang et al., 2010)
Trimethoprim	14993[a]	Norway	(Thomas et al., 2007)
	5000[b]	USA	(Brown et al., 2006)
	174[c]	China	(Chang et al., 2010)
	7600[c]	Sweden	(Lindberg et al., 2004)
	6000[a]	Germany	(Ohlsen et al., 2003)
	25[e]	Spain	(Gomez et al., 2006)
	300[d]	Australia	(Watkinson et al., 2009)
	40[e]	Spain	(Gomez et al., 2007)
Fluoroquinolone			
Ciprofloxacin	2000[b]	USA	(Brown et al., 2006)
	136[c]	China	(Chang et al., 2010)
	2927[a]	Portugal	(Seifrtova et al., 2008)
	101000[c]	Sweden	(Lindberg et al., 2004)
	124500[e]	Germany	(Hartmann et al., 1999)
	29400[a]	Switzerland	(Alder et al., 2004)
	155000[e]	Brazil	(Martins et al., 2008)
	25800[c]	Vietnam	(Duong et al., 2008)
	236600[c]	India	(Diwan et al.,)
	54049[a]	Norway	(Thomas et al., 2007)
	87000[c]	Switzerland	(Hartmann et al., 1998)
	15000[d]	Australia	(Watkinson et al., 2009)
	5120[e]	Spain	(Gomez et al., 2007)
	51000[a]	Germany	(Ohlsen et al., 2003)

[a] 24-hr composite samples
[b] 4-hr composite samples
[c] Grab samples
[d] 18 hr composite samples with 3h interval
[e] 10 & 14 hr composite samples

Class, Compound	Conc (ng/L)	Country	Reference
Ofloxacin	35500[b]	USA	(Brown et al., 2006)
	4240[c]	China	(Chang et al., 2010)
	9451.9[b]	Portugal	(Seifrtova et al., 2008)
	7600[a]	Sweden	(Lindberg et al., 2004)
	31000[a]	Germany	(Ohlsen et al., 2003)
Norfloxacin	1620[a]	China	(Chang et al., 2010)
	7900[b]	Switzerland	(Alder et al., 2004)
	334[a]	Portugal	(Seifrtova et al., 2008)
	15200[c]	Vietnam	(Duong et al., 2008)
	100[d]	Australia	(Watkinson et al., 2009)
	44000[a]	Germany	(Ohlsen et al., 2003)
Lomefloxacin	1162[c]	China	(Chang et al., 2010)
Enrofloxacin	100[d]	Australia	(Watkinson et al., 2009)
Tetracycline			
Tetracycline	4178[b]	Norway	(Thomas et al., 2007)
Tetracycline	40[e]	Australia	(Watkinson et al., 2009)
Tetracycline	455[a]	Taiwan	(Lin et al., 2009)
Oxytetracyline	3743[b]	Norway	(Thomas et al., 2007)
Democlocycline	52[b]	Norway	(Thomas,et al. 2007)
Chloroetracycline	69[b]	Norway	
Iso-Chlorotetracycline	20[a]	China	(Chang et al., 2010)
Doxycycline	403[b]	Norway	(Thomas et al., 2007)
Doxycycline	6700[a]	Sweden	(Lindberg et al., 2004)
Doxycycline	200[e]	Australia	(Watkinson et al., 2009)
Meclocycline	<7[b]	Norway	(Thomas et al., 2007)
Sulfadiazine	253[c]	China	(Chang et al., 2010)
Cefuroxime	<125[b]	Norway	(Thomas et al., 2007)
Lincosamides			
Lincomycin	2000[b]	USA	(Brown et al., 2006)
Lincomycin	1700[e]	Australia	(Watkinson et al., 2009)
Clindamycin	90[e]	Australia	(Watkinson et al., 2009)
ß-lactams			
Penicillin G	5200[b]	USA	(Brown et al., 2006)
Penicillin V	10[e]	Australia	(Watkinson et al., 2009)
Amoxycillin	900[e]	Australia	(Watkinson et al., 2009)
Cephalexin	10000[e]	Australia	(Watkinson et al., 2009)
Macrolides			
Erythromycin	261[a]	China	(Chang et al., 2010)
Erythromycin	27000[e]	Germany	(Ohlsen et al., 2003)
Erythromycin	150[e]	Spain	(Gomez et al., 2007)
Erythromycin-H_2O	827[c]	China	(Chang et al., 2010)
Erythromycin-H_2O	6110[c]	Taiwan	(Lin et al., 2009)

Class, Compound	Conc (ng/L)	Country	Reference
Erythromycin-H_2O	83000[e]	Germany	(Ohlsen et al., 2003)
Roxithromycin	2189[c]	China	(Chang et al., 2010)
Roxithromycin	400[e]	Australia	(Watkinson et al., 2009)
Oleandomycin	40[e]	Australia	(Watkinson et al., 2009)
Aminoglycoside			
Gentamicin	7600[f]	Germany	(Loffler et al., 2003)
Gentamicin	5000[e]	Germany	(Ohlsen et al., 2003)
Metronidazole	90200[c]	Sweden	(Lindberg et al., 2004)
	3800[c]	China	(Chang et al., 2010)
	5900[e]	Spain	(Gomez et al., 2006)
	3760[e]	Spain	(Gomez et al., 2007)

Both antibiotic use and concentrations that have been reported in previous studies suggest that hospitals can be considered to be major point sources of antibiotic compounds. The material from this review was used to determine target compounds in the current study.

2.6.1.2 Pharmaceutical Compounds

Compounds other than antibiotics, that are used in hospitals and which have received special attention include cytostatic agents, anesthetics, antiepileptics, non-steroidal anti-inflammatory drugs (NSAIDs), and diagnostic aids, especially x-ray contrast agents (Kümmerer, 2001a).

Cytostatic agents are mostly used in hospitals (Kümmerer, 2001a) for cancer treatment and their carcinogenicity, and mutagenicity have been reported by several researchers (Hessel et al., 2001; Skov et al., 1990). Anesthetics can have ozone depletion and global warming potential (Kümmerer, 2001a). Propofol is an important anesthetic compound which is excreted about 90% as an unchanged compound (Kümmerer, 2004a). The antiepileptic drug carbamazepine is frequently detected in the aquatic environment (Zhang et al., 2008). X-Ray contrast media is one of the widely used pharmaceutical for imaging purposes during diagnostic tests. They are mostly derivatives of 2,4,6 tri-iodobenzoic acid with carboxyl and hydroxyl moieties in their chains (Perez et al., 2007). A single dose of an X-ray contrast agent may contain 60-120 g of drug substance (Christiansen, 2005). The excretion half life of contrast agents is 2 hours and they are mostly excreted as the unchanged

[f] Composite sample (10 minutes interval), length is not clear

compound (Kümmerer, 2004a). It has also been suggested that facilities with specialized radiology could contribute more than 50 % of the load to the municipal WWTPs (Kümmerer, 2004a).

The NSAIDs were found in higher concentrations in hospital effluents; with highest detected concentration of acetaminophen 329 μg/L, diclofenac 70 μg/L, ibuprofen 74 μg/L, naproxen 18 μg/L, and metamizole 77 μg/L. The Beta-blocker metoprolol was measured in relatively higher concentrations (5.8 μg/L). Up to 4 μg/L concentration of the anticancer agent cyclophosphamide was detected in a German hospital effluent. Carbamazepine which is mostly excreted as its metabolites was measured up to 700 ng/L. The concentrations of these compounds that have been detected in hospital effluents in different countries are summarized in Table 2-3

Table 2-3 Concentrations (μg/L) of pharmaceuticals reported in literature

Compound Name	Concentration	Country	Reference
Antineoplastic (anticancer)			
Cyclophosphamide	4.48	Germany	(Hartmann et al., 1997)
Ifosfamide	0.056	Norway	(Thomas et al., 2007)
	1914	Germany	(Kümmerer et al., 1997)
Anthracyclines (anticancer)			
Epirubicin	0.1-1.4	Austria	(Mahnik et al., 2006)
Doxorubicin	0.1-0.5	Austria	(Mahnik et al., 2006)
NSAIDs			
Acetaminophen	186.500	Taiwan	(Lin et al., 2009)
	16.02	Spain	(Gomez et al., 2006)
	329.85	Norway	(Thomas et al., 2007)
	3.13	Spain	(Gomez et al., 2007)
	11.27	Spain	(Gomez et al., 2007)
Diclofenac	70	Taiwan	(Lin et al., 2009)
	1.4	Spain	(Gomez et al., 2006)
	2.73	Norway	(Thomas et al., 2007)
	0.51	Spain	(Gomez et al., 2007)
Ibuprofen	0.3	Taiwan	(Lin et al., 2009)
	19.77	Spain	(Gomez et al., 2006)
	2.44	Norway	(Thomas et al., 2007)
	74.7	Spain	(Suarez et al., 2009)
	4.57	Spain	(Gomez et al., 2007)
Naproxen	1.01	Taiwan	(Lin et al., 2009)
	18.1	Spain	(Suarez et al., 2009)
Metamizole	77.4	Germany	(F.D.A, 2004)
ß-blocker			
Propranolol	0.225	Taiwan	(Lin et al., 2009)
	1.35	Spain	(Gomez et al., 2006)
Atenolol	3.4	Spain	(Gomez et al., 2006)
	2.4	Spain	(Gomez et al., 2007)
Metoprolol	5.8	Norway	(Thomas et al., 2007)
Anti-tumour			
Ifosfamide	1.914	Germany	(Kümmerer et al., 1997)
Lipid Regulator			
Gemfibrozil	1.1	Taiwan	(Lin et al., 2009)
Antiepileptic			
Carbamazepine	0.04	Spain	(Gomez et al., 2006)
	0.07	Spain	(Gomez et al., 2007)
Antidepressant			
Fluoxetine	0.06	Spain	(Gomez et al., 2007)
Contrast agents			
Iopromide	1400	Spain	(Suarez et al., 2009)

2.7 Approaches to Predict Concentration of Pharmaceuticals in Wastewater

The use of models to predict the concentrations of pharmaceutical compounds in the aquatic environment is receiving increasing interest. This is because; about 60,000 pharmaceutical compounds are used worldwide (Tropsha, 2000). The biotransformation products of these compounds (metabolites and conjugates) are also of interest because, certain metabolites may possess reduced or similar biological activity to that of the parent compound, and the conjugates have the tendency to cleave back into the original compound during sewer transit and WWTP processes. Therefore, it is almost impossible to test every single compound of interest.

The physico-chemical properties (molecular structure, molecular weight, and functional groups) of PhACs vary widely (Kümmerer, 2001b); moreover, different behavior of the PhACs belonging to the same class during water treatment processes has been reported (Choi et al., 2008; Dodd et al., 2005). This finding makes it difficult to define indicator compounds.

In addition, the lack of analytical methods for many of the PhACs and the unavailability of deuterated standards for PhACs restricts the analysis to certain compounds. Furthermore, the resources, time and cost required to detect low concentrations (µg-ng/L) in different environmental matrices is a constraint in carrying out such studies on a large scale or with untargeted detection.

Despite the above challenges, there is also an opportunity, as most of the drugs used today are pure materials; therefore, it is expected that pharmacokinetic information about these pharmaceuticals can be achieved quite accurately using pharmacokinetic principles (Rowland et al., 1995).Thus, although prediction models cannot replace experimental studies they may be an attractive option for initial screening studies, to identify important PhACs to be monitored (Carballa et al., 2008).

There is a relationship between the consumption of PhACs and their discharge into wastewater (Ternes et al., 2006). Therefore, the consumption data along with some pharmacokinetic characteristics of the PhACs and daily wastewater flows are required to estimate their concentrations in wastewater. Pharmaceutical compounds are xenobiotics that are not normally found in human bodies. Therefore, after administration, PhACs pass though different phases such as absorption,

distribution, and metabolism and are then eventually eliminated from the body through excretion. These processes are generally referred as ADME (Jambhekar et al., 2009). The excreted compounds may be present in an unchanged form, or as metabolites or in a conjugated form.

One important consideration in PhAC modeling is the route of administration, which affects the extent of PhACs metabolism. The sites of administration of PhACs are classified into two categories: 1) Intravascular, which refers to introducing the PhACs directly into the blood and 2) extravascular, which includes oral, intramuscular, subcutaneous, sublingual, and rectal administration; transdermal application; and inhalation. The main difference between the two types of administration from a pharmacokinetic point of view is that the absorption step is not present in the case of intravascularly administered doses. Therefore, the excretion of the unchanged PhAC in urine, as a percentage of the administered dose, is affected by the route of administration. Higher excretion rates for the intravenously administered dose than oral application has been reported. For example the antibiotic ciprofloxacin is excreted 40-50% as unchanged compound in the case of oral doses and up to 70% in the case of parenteral (administration other than through the digestive tract) doses. Figure 2-1 show a schematic view of the pharmacokinetic processes for both intravascularly and extravascularly administered doses.

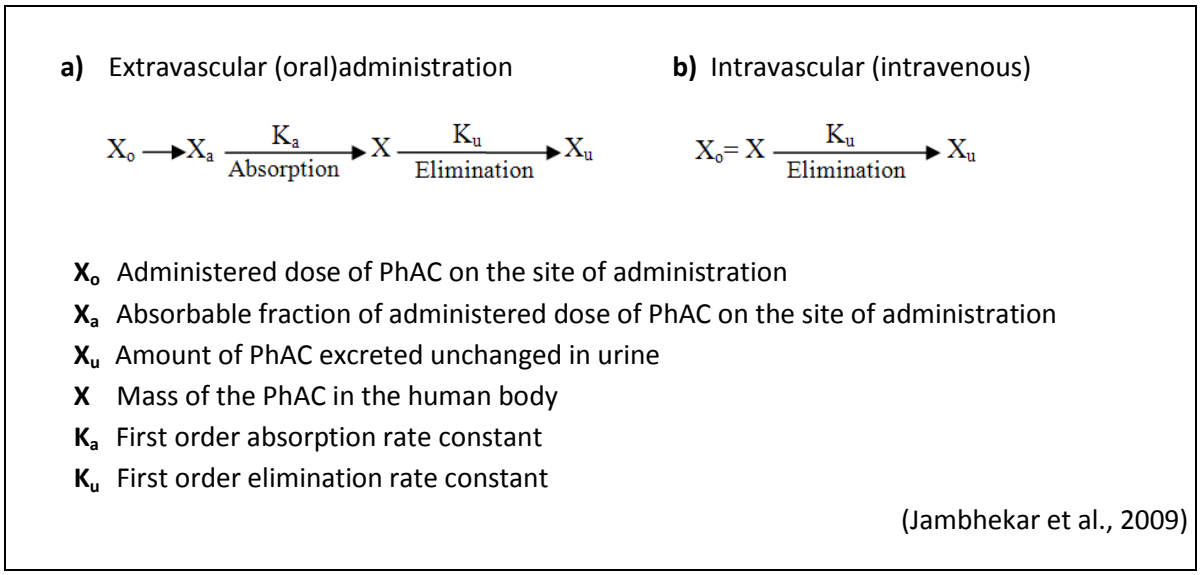


Figure 2-1 Elimination of the PhACs via urinary excretion in different routes of administration

2.7.1 Existing Models

Various approaches to predicting pharmaceutical concentrations in wastewater have been published. Data on consumption of PhACs for a region or a country is typically not readily available therefore different methods have been employed to utilize available data to estimate the consumption of PhACs. Data on annual sales and prescription rates have generally been used to calculate PhACs consumption. The fractions of the unchanged PhACs that are excreted into wastewaters after administration of PhACs along with the typical wastewater volume per capita are used in order to convert consumed masses into concentrations in the wastewater. Some authors have used mass balances and fugacity calculations to predict the concentrations and behavior in the aquatic environment.

A simple prediction model based on annual consumption of target compounds and excretion data was presented by Alder et al. (2006). The annual consumption was estimated using annual sales data. The model assumes that the pharmaceutical consumption is uniformly distributed over the year, and throughout the geographical area. In addition, it is assumed that the compounds are not biodegraded in the sewer system. The concentrations of the target compounds in the environment are predicted using the equation 2.1.

$$PEC_{STPin} = \frac{F_{API} \times 10^{12} \times E}{365 \times P_{op} \times AWW} \quad (2.1)$$

Where;

PEC_{STPin}	Predicted concentration in raw sewage (ng/L)
F_{API}	Amount of active pharmaceutical ingredient consumed in the area per year (Kg/year)
E	Fraction excreted unchanged in urine and faeces.
P_{op}	Population of the target area (persons)
AWW	Wastewater flow per capita per day (200-400 L/person/day)

The uncertainties associated with estimating pharmaceutical consumption by this include;

1. Purchased PhACs may not be used during the same year that they were purchased.
2. Seasonal variations along with regional differences in use of PhACs may result in wide variations in actual consumption.

Furthermore

3. Degradation or hydrolysis processes during sewer transit are not taken into account.
4. Substances that are excreted in the conjugated form that have the tendency to cleaved back to the parent compound in sewers and wastewater treatment are not considered.

(Carballa et al., 2008) estimated the environmental concentrations of the pharmaceuticals in raw sewage using the following equation (2.2).

$$PEC = \frac{I \times P \times f}{Q \times 365} \quad (2.2)$$

Where

PEC Predicted environmental concentration(μg/L)

I Consumption of the target pharmaceutical compound (mg/capita/year)

P Population served by wastewater treatment plant (Persons)

f Total fraction of unchanged PhACs that arrives at WWTPs

Q Average wastewater flow (m^3/day)

In the study of (Carballa et al., 2008) the annual consumption I (equation 2.2) of the PhACs was calculated using annual prescriptions. The author obtained the prescription data through personal communication. The mass of target PhACs consumed per year (M) was calculated using equation 2.3, and then using M, per-capita/year mass consumption (I) was determined.

M = (Number of prescriptions/year) x (number of doses per prescription) x (active compound per dose) 2.3

The sources of variability in this model include;

1.) Calculation of PhAC consumption using prescription data excludes purchases without prescriptions. For example, over-the -counter and internet purchases.
2.) All the prescribed doses are not always administered.
3.) The routes of administration of PhACs are not considered separately which may affect the value of "f" in the equation (2.2).

4.) Degradation processes in the sewer systems are not considered.
5.) While comparing the predicted and measured concentrations, it is important to consider the sorption behavior of the PhACs especially when only aqueous samples are measured.

Carballa et al. (2008)attributed the differences between predicted and measured concentrations to;

1.) Variation in the consumption estimates.
2.) Incomplete release of PhACs to the sewer systems
3.) Elimination of compounds by various elimination processes (degradation, dilution, sedimentation) between the consumption point and sampling point.

Some authors (Golet et al., 2002; Jones et al., 2002; Kümmerer et al., 1997; Kümmerer et al., 2003; Lauridsen et al., 2000) have estimated wastewater treatment plant influent concentrations of PhACs in raw sewage using equation (2.4) that was originally presented in the draft guidelines for risk assessment of the new pharmaceuticals in the European Union (EU 1994,1995) to predict environmental concentrations in surface water.

$$PEC = \frac{A \; x \; (100 - R)}{365 x \; P \; x \; V \; x D \; x 100} \qquad (2.4)$$

Where

PEC Predicted environmental concentration (g/Liter)

A Amount of the compound consumed per year (kg.year $^{-1}$)

R Removal efficiency (%). The removal considers both human metabolism and the loss by other processes, i.e. adsorption, volatilization, hydrolysis and biodegradations

P Population of the area considered (persons) and (beds) in case of hospitals

D Dilution factor (Hospital effluent to communal sewage for estimating concentrations in hospitals effluent or wastewater flow to surface water when predicting concentrations in surface water and was set to zero while estimating concentrations in raw wastewater)

V Wastewater flow (m^3/person/day) & (m^3/bed/day) for hospitals

The equation (2.4) is a general equation to predict concentrations in wastewater and surface water. The strength of the prediction depends on the accuracy of the individual parameters that can be obtained. For example the amounts of PhACs consumed, as discussed in the previous models. Furthermore this equation does not consider the portion of the biotranformation products which have

the tendency to cleaved back to original compound. Additionally different routes of administration which affect the extent of metabolism are not accounted for.

Johnson et al., (2004) presented a model to predict concentrations of steroid estrogens, which could be extended to pharmaceuticals with slight modification i.e., by excluding the Ss term in equation (2.5) which represents the transformation of one estrogen to other during sewer transit and considering only glucuronide conjugates in the total mass. The model addresses excretion of estrogens in feces in the deconjugated form, and additionally assumes that all the excreted glucuronide conjugates via urine are deconjugated in sewer transit to WWTPs.

$$S_T = (1 - K_T)(U_T + F_T) + S_S \tag{2.5}$$

- S_T Total estrogens arriving in WWTP in all forms
- K_T Fraction that is lost during the transit
- U_T Total amount of steroid estrogen in urine arrived at the WWTP
- F_T Amount of estrogen excreted in feces,
- S_S Internal generation of estrogens from other estrogens.

Johnson et al., (2004) used excretion data of estrogens for 5 different population groups (pregnant, menstrual, and menopausal females, females taking hormonal replacement therapy and males) in estimating total excreted mass. In equation 2.5 U_T and F_T were calculated as shown in equations (2.6) and (2.7).

$$U_T = \sum_{i=1}^{n} f_i (U_i' + U_i^g + U_i^s) \tag{2.6}$$

- f_i Fraction of the group i (population)
- U'_i Amount of estrogen excreted by ith fraction of population in particular form(µg/day)
- U^g_i Amount in free glucuronide form of estrogen
- U^s_i Amount in sulfate form of estrogen

$$F_T = \sum_{i=1}^{n} f_i F_i \tag{2.7}$$

F_i Amount of estrogen excreted by i^{th} fraction of the population (µg/day)

Substituting values of U_T & F_T from equations (2.6) and (2.7) in equation (2.5) yields

$$S_T = (1 - K_T) \sum_{i=1}^{n} f_i (U_i' + U_i^g + U_i^s + F_i) + S_s \tag{2.8}$$

The equation (2.7) was then further simplified by (Johnson et al., 2004) for their three target estrogens (EE2, E2, and E1) by substituting their respective values for K_T and S_S.

Johnson et al., (2004) assumed that the consumption of estrogens in the UK would be similar to the USA. This assumption may not hold true based on the fact that significant differences exist in the pharmaceutical consumptions between different countries (As discussed earlier in this report in section 2.4). To extend this model to compounds other than steroidal estrogens including excretion rates of different age groups and routes of administrations would be helpful.

Khan et al., (2004) presented a model to predict pharmaceutical concentrations in raw sewage using 1) data describing pharmaceutical consumption by population, 2) human metabolism and excretion of pharmaceutical residues. They used the Australian Health insurance commission reimbursement data to find the total prescriptions in the selected region and calculated the total number of quantities dispensed (T) as

$$T = N \times Q \tag{2.9}$$

Where N is the number of prescriptions dispensed per year and Q is the average dispensed quantity per prescription. The active mass of the dispensed amount was calculated by multiplying total quantities dispensed (T) with the active mass strength per dose (S)

$$M = T \times S \tag{2.10}$$

Since prescriptions may have different active mass, the total active mass was obtained by addition of the different active mass strength per dose.

$$M_{TOTAL}=M_1+M_2+M_3+\ldots\ldots\ldots\ldots..$$

The WWTP influent concentrations (C) were calculated using equation (2.11)

$$C\ (gm^{-3}) = \frac{M_{exc}\ x\ P_{STP}\ x\ 10^3}{R\ x\ 24\ x\ T\ x\ P_{surey}} \qquad (2.11)$$

Where

M_{exc} Mass excreted during study period (Kg)

P_{STP} Population served by WWTP (Persons)

R Average wastewater inflow (m^3/h)

T Study period (days)

P_{survey} Population contributed to the prescription data(Persons),*(contributed to mass M_{exc})*

The consumption of the target PhACs was calculated using prescriptions submitted to claim subsidies for the purchased pharmaceuticals. Consumption data for the compounds which were not eligible for the subsidy was collected from the pharmacy sources. Using this data the per-capita excretion was estimated. That was then used along with the population upstream to the WWTP to calculate the inflow concentrations.

Khan et al., (2004) found that these calculations highly underestimated the consumption of compounds like paracetamol (Acetaminophen) that are often purchased from markets. In addition, the estimated annual per-capita consumption was used to determine the consumption of target compounds during the study period. This may lead to wide differences in the actual consumed amounts, because seasonal and regional differences in pharmaceutical consumption, as discussed in section 2.4. Furthermore only oral route of administration is assumed and median values for excretion rates were used which may underestimate the actual excreted amounts.

Heberer et al., (2005) presented an equation (2.10) to calculate the weekly load of PhACs from hospitals and households to the municipal wastewater. The equation (2.12) considers consumption data, route of administration, and human metabolism to predict the concentration in wastewater. The weekly load of carbamazepine and Diclofenac by a hospital to WWTP was predicted. Actual consumption data was collected from the hospital wards during the study period.

$$M_{totweek} = \sum_{i=1}^{n} \underbrace{a_i \, x \, b_i \, x \, m_i \, x \, s_i}_{\text{Amount administered}} ((1 - R_p) + R_p (x_p + x_c))_i \, x 10^{-6} \qquad (2.12)$$

Where

- a_i Number of administered packages per week for brand i
- b_i Number of units per package of brand i
- m_i Content of active compound per unit (mg)
- S_i Release rate of pharmaceuticals compound in brand i
- R_p Absorption rate (%)
- X_p Portion of pharmaceuticals excreted unchanged after absorption (%)
- X_c Percentage excreted in conjugated form (%)

Since the data regarding the absorption (Rp) and exertion rates (Xp & Xc) for pharmaceutical compounds are normally available in range therefore it was suggested that maximum and minimum concentrations should be calculated for each compound. Equation (2.11) contains the required parameters to predict concentrations in wastewater.

The concentrations of PhACs in the aquatic environment are a function of the initial concentration (consumption), the extent of metabolism, and their persistency in the aquatic environment. For accurate prediction of these concentrations all three components need to be considered. Typically it is difficult to obtain data regarding the consumption of pharmaceuticals so relatively simple models have been reported in the literature, consequently their use has relied upon a number of assumptions in order to convert consumption data into environmental concentrations. Therefore many uncertainties are associated with these calculations. For example consumption estimated using country level annual sales data doesn't consider unused medicines, which could either directly reach the aquatic environment in the case of improper disposal (flushing into toilets, or draining in the sink) or leaving the consumption data as over estimated. Estimated consumption using prescription data, excludes the over the counter, and internet purchases. Seasonal and regional differences in use of PhACs made it difficult to use country level annual consumption data to target regions. Furthermore many formulations are possible for each prescription of drug, so the mass of the PhAC may be different for each dose.

The extent of metabolism is affected mainly by the sites of administration of the PhACs. For example the antibiotic ciprofloxacin is excreted 40-50% as unchanged compound in case of oral dose and up to 70% is excreted unchanged in case of parenteral (administration other than through the digestive tract) dose in 24 hours ((Sweetman et al., 2007). In addition external applications of PhACs are expected to reach wastewater by washing and bathing without going through a metabolism step. It is important to note that, for the compounds which are extensively metabolized in the human body the contribution of the improper disposal of these compounds to the wastewater is expected to be significant. The data about the excretion rates of PhACs is usually available in a range because the extent of metabolism varies among individual patients, depending upon age, sex, and health condition. Therefore the selection of the right values needs personal information of the patient (sex, age, health condition) in addition to administered dose.

The administered dose is excreted either as the unchanged compound, a major metabolite, or in a conjugated form (mostly as glucuronide and sulfate conjugates) through urine and feces. Amounts excreted in feces are usually considered as unchanged compounds because of the ability of the intestinal bacterial flora to de-conjugate the conjugates through bacterial hydrolysis. The conjugates excreted via urine, especially glucuronide conjugates are suggested to include along with the

unchanged portion to calculate the total load of the compound, because of the tendency of these transformation products to cleave back to the original compound in sewer systems.

After excretion to the sewer, depending upon the physico-chemical properties of the compound itself and the sewer conditions (residence time, aerobic or anaerobic conditions, sewer biofilms) a compound may either biodegrade, be hydrolyzed, adsorb to solids, etc. For instance, Sulfonamide, fluoroquinolone, and macrolide antibiotics show highest persistency in the aquatic environment and are frequently detected in wastewater (Brown et al., 2006).The half life of antibiotic compounds in the aquatic environment varies from a few days for some ß-Lactams to several hundred days for Tetracycline or quinolone antibiotics (Kümmerer, 2001b). ß-Lactams are rapidly hydrolyzed after excretion into wastewater (Kümmerer, 2008a). Fluoroquinolone antibiotics posses strong sorption tendency to solids (Golet et al., 2002). All these processes need to be accounted for to predict concentrations of these compounds.

For potential point sources of pharmaceuticals like hospitals and nursing homes where self-medication is not allowed quite accurate consumption data should be available. Furthermore the information about the route of administration, patient age, sex, health condition and the length of the patient stays is usually maintained in hospitals. This suggests that the emissions of PhACs from these healthcare facilities (hospitals & long term care homes) could be reasonably predicted using prediction models.

The eco-toxicity of the hospital effluent is well documented and some researchers have attributed its genotoxicity to the presence of antibiotic compounds (Giuliani et al., 1996; A. Hartmann et al., 1998) The relative contribution from hospitals to WWTPs was identified as an urgent research need (Daughton, 2004) but still very little data is available. No data is available for long term care homes which employ a considerable amount of PhACs on a regular basis. In addition pharmaceutical use varies from country to county making it difficult to draw any conclusions based on these studies.

For both risk assessment and risk management it is important to identify the contributions from potential sources of emissions of pharmaceuticals to the aquatic environment. Control of sources like health care facilities would be expected to reduce the emission of PhACs into the aquatic environment by a considerable amount, which will reduce the unanticipated risks associated with these to the environment, aquatic and human health.

Chapter 3
Experimental Design

3.1 Selection of Target Compounds

Since 15,331 approved pharmaceutical compounds are currently used in Canada (Health Canada, 2010), the first task in this study was to prepare a priority list of compounds of interest. The focus was on the occurrence of PhACs in healthcare facility effluents (hospitals and long term care homes). A direct relationship exists between the consumption of PhACs and their discharge into wastewater (Ternes et al., 2006) therefore, the PhACs consumption data for healthcare facilities was preferred, but was not readily available. Instead the drug purchase data for Ontario hospitals in "extended units" (number of tablets, capsules, mg etc) for the year 2008 was obtained from the International Medical Statistics (IMS) Canada database through Health Canada. The collected data was then subdivided on a monthly basis, and it was assumed that the purchase of new PhACs would be indicative of the current consumption of PhACs. In addition, through the literature review, fourteen therapeutic classes of PhACs were chosen based on their occurrence in and potential risks to the aquatic environment, and on their high excretion rates as the parent compound. Then 46 PhACs, that were most purchased by Ontario hospitals in 2008, according to IMS database, and belonging to the 14 pre-defined therapeutic classes were included in a preliminary list. A short list was then established using the following criteria:

1. Specific mode of action, i.e., possible health risks (Ternes 2004).
2. Persistence in aqueous solution (Bendz et al., 2005).
3. Representation of different therapeutic groups.
4. Presence in the Canadian environment as indicated by previous studies (Metcalfe et al. 2004; Lissemore et al., 2006).
5. Analytical capabilities of the laboratory at Trent University.
6. Availability of deuterated standards.

Eventually seven compounds representing five different therapeutic classes and two metabolites of venlafaxine were selected for this study (Table3-1). Antibiotics were deemed to be the most important class of PhACs because of their potential to foster antibiotic-resistant bacteria in addition to undesirable biological activity. The antibiotic ciprofloxacin has been reported to be a major source of genotoxicity in hospital wastewater (Hartmann et al., 1998). The beta-lactum antibiotics were not

included because they are rapidly hydrolyzed after excretion (Gobel et al., 2005). The anesthetic propofol (Kümmerer, 2004a) was excluded from the final list because an analytical method was not readily available for this compound and the development and validation process was expected to take longer than the project duration. X-ray contrast agents were also excluded because of the unavailability of deuterated standards for these compounds in Canada at the time of compound selection.

Table 3-1 Pharmaceutical compounds selected for the study, their class, CAS registry numbers and therapeutic use

Class/Compound	CAS #	Internal Standards	Therapeutic use[g]
Fluoroquinolones			
Ciprofloxacin	85721-33-1	Ciprofloxacin-^{13}C	Antibacterial
Sulfonamides			
Sulfamethoxazole	723-46-6	Sulfmathoxazloe-$^{13}C_6$	Antibacterial; antipneumocystic
Neutral drugs			
Trimethoprim	738-70-5	Trimethoprim-$^{13}C_3$	Antibacterial.
Carbamazepine	298-46-4	Carbamazepine-d_{10}	Antiepileptic, Anticonvulsant
Acidic drug			
Acetaminophen	103-90-2	Acetaminophen-d_3	Analgesic; antipyretic
Beta-blocker			
Metoprolol	37350-58-6	Propranolol-d_7	Antihypertensive; antianginal; antiarrhythmic (class II).
Anti-depressant			
Venlafaxine	93413-69-5	Venlafaxine-d_{10}	Antidepressant; anxiolytic
Metabolite			
O-des-Venlafaxine	93413-62-8	Venlafaxine-d_{10}	Antidepressant (active metabolite)
N-des-Venlafaxine		Venlafaxine-d_{10}	Metabolite antidepressant

3.2 Identification and Selection of Sampling Sites

A list of existing health care facilities (hospitals and long term care homes) in the target area was obtained though the Internet. The information about care services that these facilities are currently offering, was collected from the official website of each facility. Discharges from hospitals depend upon various factors, including size of the facility, location, and types of the services available (Askararian 2004). Therefore, two hospitals of different size and service spectrum, located in different areas, were selected in consultation with the regional municipality of the study area. The

[g] Martindale & Merck Index

long-term care facilities provided similar services, so their selection was based on size and location. The two largest facilities (by number of beds) in the project area were selected for sampling. Additional factors considered during site selection were availability of access to sampling points, available space to install sampling machines, and the possibility of keeping this area reserved for a week-long sampling.

To estimate the mass contribution of target PhACs by the selected healthcare facilities, the downstream wastewater treatment plants (that receive discharges from the selected facilities) were identified for same day sampling. The identified facilities, i.e., hospitals, long term healthcare facilities and their corresponding WWTPs are referred to in this report as HS_1, HS_2, LTC_1, LTC_2, WWTP-HS_1, WWTP-HS_2, and WWTP-LTC_1, WWTP-LTC_2 respectively. Table 3-2 defines the terms used in the report and provides information describing the size of the selected facilitates.

Table 3-2 Description of selected healthcare facilities[h]

Facility description	Facility label	Facility size (No of beds / population served)
Hospital -1	HS_1	365
Hospital -2	HS_2	263
Wastewater treatment plant downstream hospital -1	WWTP-HS_1	51,218
Wastewater treatment plant downstream hospital -2	WWTP-HS_2	171,000
Long term care home-1	LTC_1	228
Long term care home-2	LTC_2	200
Wastewater treatment plant downstream long term care home-1	WWTP-LTC_1	80,000
Wastewater treatment plant downstream long term care home-2	WWTP-LTC_2	33,000

[h] This information was collected either from the official website of the facilities or provided by the regional municipality.

Manholes located on the property line of each facility were selected as sampling points to cover all discharges from the facility and ensure representative samples. Regional staff helped to identify sampling points. At the second facility, effluent was discharged at two locations to the municipal sewer lines so both streams were sampled and blended for extraction.

3.3 Sampling Protocols

Twenty-four hour time proportionate composite samples were collected over five consecutive week days from each sampling point (Monday to Friday). A total of nine sampling points were selected at the eight facilities. Table 3-3 indicates the sampling point details and dates.

Table 3-3 Sampling point description and dates.

S.No	Sampling site	Sampling point	Sampling dates
1	HS_1	HS_1 effluent	July 22 to 27, 2009
2	WWTP-HS_1	WWTP-HS_1 influent	July 22 to 27, 2009
3	HS_2	HS_2 effluent (manhole-1) *Main facility effluent*	November 3 to 7, 2009
4	HS_2	HS_2 effluent (manhole-2) *Cancer clinic effluent*	November 3 to 7, 2009
5	WWTP-HS_2	WWTP-HS_2 influent	November 3 to 7, 2009
6	LTC_1	LTC_1 effluent	February 23 to 27, 2010
7	WWTP-LTC_1	WWTP-LTC_1 influent	February 23 to 27, 2010
8	LTC_2	LTC_2 effluent	March 9 to 13, 2010
9	WWTP-LTC_2	WWTP-LTC_2 influent	March 9 to 13, 2010

3.3.1 Sample Collection

Samples were collected by regional municipality personnel with training for this type of activity. All the selected WWTPs were pre-equipped with refrigerated sampling machines (Auto sampler Sigma SD900) at the inflow points.

The sampling frequency was selected based on the literature review, as variability in the concentrations over the weekdays was expected in both types of healthcare facilities. Furthermore some of the higher compound concentrations were reported based on grab samples or very short sampling events, indicating the possibility of those concentrations being a single even or batch discharge, therefore in this study the samples were collected over five consecutive days. The week long sampling also helped to identify individual peaks, day-to-day variability and trends in target compounds concentrations over the weekdays.

Portable auto samplers (Sigma 900) were used to collect samples from all the healthcare facility effluents. The auto sampler directly collected wastewater from sewer lines, and then stored it either in one large container or 24 bottles in a tray. Ice pads (U-Tek refrigerant pak; Fisher Scientific) were used to maintain the collected sample reasonably cool. The ice packs were placed in the middle hollow portion of the auto-sampler tray in case of 24 bottles, and around the central container where one large container was used. Twenty-four hour composite samples were collected from each sampling point at a rate of two samples per hour, (a total of 48 sub-samples, 125 mL each, was collected over 24 hours). Two 24-hour composite samples, one from the healthcare facility effluent and the second from the WWTP influent point that received its discharge, were collected on each day.

The auto sampler was installed on Monday morning at 9:00 am, and samples were collected, starting on Tuesday and continuing till Saturday for each facility. These samples were then transferred to 1L new wide-mouth pre-labeled amber glass bottles. Sampling information was recorded on the site, and chain of custody forms were maintained (Appendix A). A total of 2-3 liters of sample volume were collected from each sampling point. All the collected samples were stored on ice and transported to the Waterloo Aquatic Toxicology and Ecosystem Remediation Laboratory-(WATER Lab) at University of Waterloo on the same day.

The auto samplers were flushed with DI water each day after transfer of the collected sample to the amber glass bottles. New wide-mouth amber glass bottles were used for sample collection to avoid

any possible sample carryover or photo-degradation of the analytes of interest during storage and transportation. All the samples were immediately transported in coolers to the laboratory within a maximum of three hours after the time of collection.

3.4 Analytical Methods

All the collected samples were prepared and extracted using solid phase extraction (SPE) as described by Miao,(2004) and Hongxia (2010) at the Water Aquatic Toxicology and Ecosystem Remediation laboratory at the University of Waterloo. The analysis of the extracts was done by Trent University in Peterborough, Ontario. Hospital samples were frozen after arrival at the laboratory, and extracted within a maximum of 10 days from sample collection while samples from the long term care facilities were processed (extracted) on the same day of collection and immediately transported to Trent University for analysis.

3.4.1 Sample preparation

The collected samples were vacuum filtered through 1.5 μm glass fiber filters to remove suspended solids. The glass fiber filters were prewashed in a Soxhlet apparatus with hexane and dichloromethane (1:1) for two hours. Three different methods (Table 3-4) were used to extract nine compounds and each method was conducted in triplicate. Therefore each filtered sample was distributed into nine 125 mL wide mouth amber glass bottles, each containing 100 mL of sample. One blank sample containing 100 mL of Milli-Q water was processed with each method. The 125 mL bottles were organized by SPE method in three different trays each containing seven 125 mL bottles (thee replicates of each sample and a method blank). Solutions containing Surrogate standards (200 μL for ciprofloxacin and 100 μL for all other target analytes), each with a concentration of 250 ng/mL were added to each bottle.

3.4.2 Solid Phase Extraction-SPE

Three different extraction methods were used to extract the nine target compounds. Three replicates were processed for each sample, and the order of extractions was fully randomized. Each extraction also included three lab blanks, with 1 blank/method. Surrogate standards were spiked into each sample prior to extraction. Table 3-4 indicates the target compounds and extraction method details.

Table 3-4 Target compounds and extraction method details

Extraction Method	Target compounds
Method-1	Ciprofloxacin
Method-2	Metoprolol, Trimethoprim, Venlafaxine, N-des-Venlafaxine, O-des-Venlafaxine
Method-3	Sulfamethoxazole, acetaminophen, Carbamazepine

3.4.2.1 Method-1

The samples were acidified to a pH of 3 by adding 1.75 M H_2SO_4. In addition, 50 mg of Na_2EDTA was added to each bottle containing 100 mL of the sample, as a chelating agent. Oasis HLB (Hydrophilic-Lipophilic Balanced reversed phase) cartridges were preconditioned sequentially with 6 mL of acetone, 6 mL of methanol and 6 mL of 50 mM Na_2EDTA at pH 4 and left for one hour. The sample was then passed through the cartridges via teflon tubing at a flow rate of approximately 1 mL/min. After passing of the entire sample, the bottles were rinsed with 10 mL of high performance liquid chromatography (HPLC) grade water at pH 3 that was then passed through the cartridges. Once the entire sample passed through the cartridges the teflon tubing was immediately removed and the cartridges were rinsed with 2 mL of HPLC grade water at pH 3. The cartridges were then vacuum dried for 30 minutes. Elution was done using 2 x 3 mL of 2 % ammonium hydroxide in methanol, and then with 3 mL of methanol. The eluant (9 mL) was collected in 15 mL glass tubes and then

subsequently evaporated under a gentle nitrogen stream to almost dryness, and finally reconstituted with 1mL of 40 % aqueous methanol.

3.4.2.2 Method – 2

The pH of the samples was adjusted in the range of 2.5-3 using 1.75M H_2SO_4. Oasis MCX (Mixed mode Cation-Exchange) cartridges were preconditioned sequentially with 6 mL of acetone, 6 mL of methanol and 6 mL of HPLC grade water adjusted to pH 2.5-3. The sample was passed though the cartridge under vacuum via teflon tubing. After passing the entire sample, the bottles were rinsed with 10 mL of HPLC grade water at pH 2.5- 3 and then passed through the cartridges. The Teflon tubing was then removed and cartridges were rinsed with 2 mL of HPLC grade water at pH 2.5-3. The cartridges were then dried under vacuum for 30 minutes. Elution was done three times with 3 mL of 5 % ammonium hydroxide in methanol. The eluents were collected in 15 mL glass tubes which were then evaporated to dryness under gentle nitrogen stream. The reconstitution was done using 400 μL of methanol.

3.4.2.3 Method- 3

The sample pH was adjusted to 8 using 1% NH_4OH. Oasis MAX (Mixed mode anionic–exchanged reversed phase) cartridges were preconditioned using 6 mL of methanol, 6 mL of 0.1 M NaOH and 6 mL of HPLC grade water. The sample was then passed though the cartridges via teflon tubing under vacuum. After complete transfer of the sample from the bottle, the bottles were rinsed with 10 mL of HPLC grade water at pH 8. Once the entire volume had passed through the cartridges, the teflon tubing was removed and the cartridges were rinsed with 2 mL of HPLC grade water at pH 8. The cartridges were aspirated to dryness for 20 minutes under vacuum. The sample was eluted sequentially using 2 mL of methanol and three times with 3 mL of 2% formic acid in methanol. Eluants (11 mL) were collected in 15mL glass tubes and under a gentle nitrogen stream, were evaporated to almost dryness. Finally the extracts were reconstituted using 400 μL of methanol.

3.4.3 Instrumental Analysis

The instrumental analysis was conducted at Trent University in Peterborough, as described by Li et al., (2010). Briefly, Sulfamethoxazole, neutral pharmaceuticals and antidepressants were analyzed by liquid chromatography with atmospheric pressure chemical ionization and tandem mass spectrometry (LC-APCI-MS/MS). The rest of the PhACs were analyzed by Liquid chromatography with electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) using a Micromass Quattro LC triple quadrupole mass spectrometer fitted with a Z electospray interface. The LC-MS/MS instruments were operated in positive or negative mode though multiple reaction monitoring for the transition ions.

3.5 Nomenclature and Pharmacokinetics of Target Compounds

The following section provides detailed descriptions of the properties of the target compounds. Compound nomenclature and pharmacokinetic information were taken from Martindale (Sweetman et al., 2007) and the Merck manual (Merck & Co, 2004), while the chemical structure and dose forms were taken from the Drug Bank (Wishart et al., 2010), a database maintained by the Department of Computing Science and Biological Sciences, University of Alberta Canada.

3.5.1 Sulfamethoxazole

Chemical Name: N1-(5-Methylisoxazol-3-yl) sulphanilamide

Molecular Formula: $C_{10}H_{11}N_3O_3S$

Molecular weight: 253.3

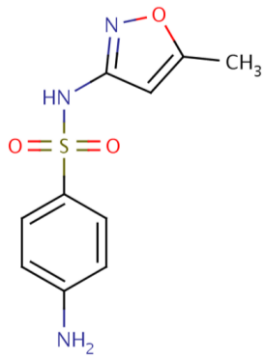


Figure 3-1 Chemical structure of Sulfamethoxazole

Sulfamethoxazole is an important synthetic antibacterial agent, used for both human and animal application; it is also used as growth promoter in animal applications. Its administration routes include oral, intravenous and ophthalmic. The usual adult dose is 2 g initially and then 1g twice daily. About 80 to 100% of the administered dose is excreted in Urine, in which 60% is in acetyle derivative (N^4-acetylsulfamethoxazole). N^4-acetylsulfamethoxazole has been found to be transformed back to the parent compound during wastewater treatment processes (Gobel et al., 2005).

3.5.2 Trimethoprim

Chemical Name: 5-(3,4,5-Trimethoxybenzyl)pyrimidine-2,4-diamine

Molecular Formula: $C_{14}H_{18}N_4O_3$

Molecular weight: 290.3

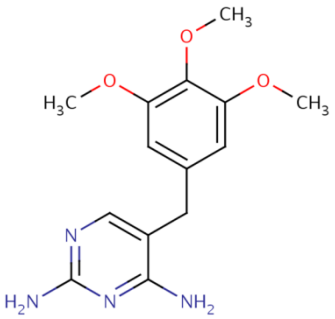


Figure 3-2 Chemical Structure of Trimethoprim

Trimethoprim is broad spectrum synthetic antibacterial agent prescribed as separate therapeutic compounds and with sulafamethoxazole at a ratio of 1:20 . It is orally administered. The usual oral adult dose is 100 or 200 mg twice a day. About 40 to 60% of the administered dose is excreted in urine mostly as the parent compound (Sweetman et al., 2007).

3.5.3 Ciprofloxacin

Chemical Name:

1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid

Molecular formula: $C_{17}H_{18}FN_3O_3$

Molecular Weight: 331.3

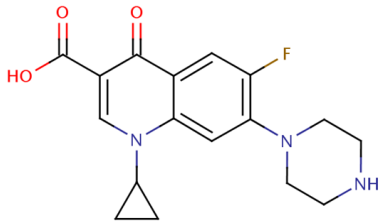


Figure 3-3 Chemical Structure of Ciprofloxacin

Ciprofloxacin is a broad spectrum synthetic antibacterial agent, active against both gram negative and gram positive bacteria. Dose forms include intravenous, oral and ophthalmic. The usual oral adult dose of ciprofloxacin is 250-75mg, twice a day, and the intravenous adult dose is 100-400 mg twice a day.

About 40-50% is excreted as the parent compound and 15% is excreted as the metabolites when oral dose is administered; while up to 70% is excreted as parent compound, and 10% is excreted as metabolites when administered as a parenteral dose in 24 hours. Four active metabolites of ciprofloxacin have been identified; the major urinary and fecal metabolites are Oxociprofloxacin and Sulfociprofloxacin respectively. About 20-30% of oral and 15% of intravenous doses are excreted with feces over 5 days

3.5.4 Acetaminophen

Chemical Name: 4´-Hydroxyacetanilide; N-(4-Hydroxyphenyl)acetamide

Molecular formula: $C_8H_9NO_2$

Molecular Weight: 151.2

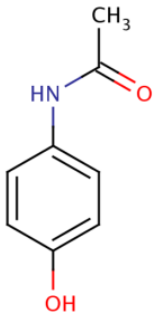


Table 3-5 Chemical Structure of Acetaminophen

Acetaminophen is a para-aminophenol derivative, and has analgesic and antipyretic properties. The routes of administration include oral and rectal. Its usual dose is 0.5 to 1 g every 4 to 6 hours up to a maximum of 4 g daily. It is excreted in the urine mainly as the glucuronide and sulfate conjugates. Less than 5% is excreted as parent compound. Previous studies have revealed the re-transformation of all the conjugated form to the parent compound during batch scale sewage studies (Khan et al., 2004).

3.5.5 Carbamazepine

Chemical Name: 5H-Dibenz[b,f]azepine-5-carboxamide

Molecular formula: $C_{15}H_{12}N_2O$

Molecular Weight: 236.3

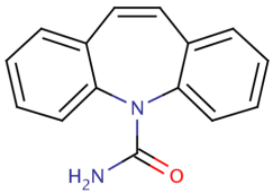


Table 3-6 Chemical structure of Carbamazepine

Carbamazepine is a dibenzazepine derivative having antiepileptic and psychotropic properties that is administered orally. Its initial dose is 100 to 200 mg once or twice daily with a maintenance dose of 800 to 1200 mg daily. Carbamazepine is excreted in the urine almost entirely in the form of its metabolites; (1-2 %) is excreted as parent compound (Alder et al., 2006).

3.5.6 Metoprolol

Chemical Name: -1-Isopropylamino-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol

Molecular formula: $C_{15}H_{25}NO_3$

Molecular Weight: 267.4

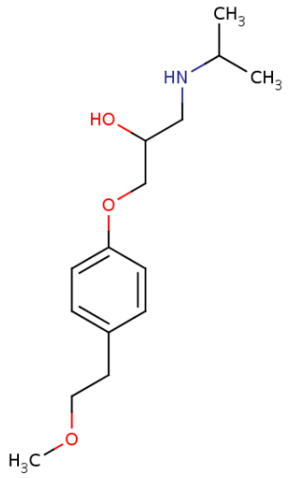


Figure 3-4 Chemical structure of Metoprolol

Metoprolol is a cardio-selective beta blocker; it is administered orally and intravenously. A usual dose is 100 to 200mg daily. It is extensively metabolized in the liver. Metabolites are excreted in the urine together with only small amounts of unchanged metoprolol

3.5.7 Venlafaxine

Chemical Name: -(2-Dimethylamino-1-p-methoxyphenylethyl)cyclohexanol hydrochloride

Molecular formula: $C_{17}H_{27}NO_2$

Molecular Weight: 277.40

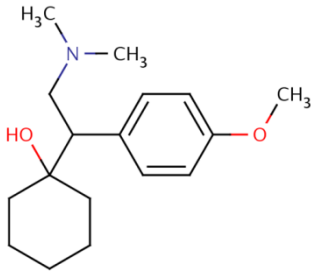


Figure 3-5 Chemical structure of Venlafaxine

Venlafaxine is used to treat depression, and is administered orally with an initial dose of 75mg daily. Venlafaxine is excreted mainly in the urine, mainly in the form of its metabolites, either free or in conjugated forms. The major active metabolite is o-desmethylvenlafaxine and other metabolites include N-desmethyvenlafaxine, and N,O-didesmethylvenlafaxine with about 2% excreted in the faeces.

3.5.8 O-desmethylvenlafaxine

Chemical Name: 1-[2-(dimethylamino)-1-(4-hydroxyphenyl)ethyl]cyclohexanol

Molecular Formula: $C_{16}H_{25}NO_2$

Molecular Weight: 263.38

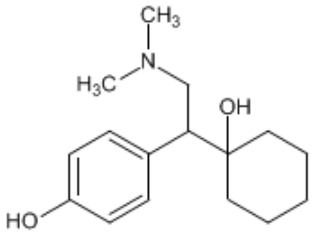


Figure 3-6 Chemical structure of O-desmethylvenlafaxine

O-desmethylvenlafaxine is an active metabolite of Venlafaxine, and possesses antidepressant properties.

The pK_a value of sulfamethoxazole indicates that it is found in anionic form in hospital wastewater where the pH is typically between 7 and 8. Ciprofloxacin, at pH 7.04 (the isoelectic point of Ciprofloxacin) contains both positive and negative charges, although the compound itself is neutral. The $logK_{ow}$ of Ciprofloxacin at pH 7.04 suggests high hydrophilicity but in contrast to this, it is highly sorbed to sludges and sediments. This is presumably due to it planer structure which helps to intercalate into the layers of clay minerals (Kummerer, 2008). Acetaminophen had the highest water solubility among the target compounds (11 g/L). Table 3-5 provides the physico-chemical properties of target PhACs.

Table 3-7 : Physico-Chemical properties of target compounds.

Target Compound	pK_a	Log K_{ow}	Log D *at pH 7*	Melting Point	Water Solubility (g/L)
Sulfamethoxazole	pK_{a1}=1.7 pK_{a2}= 5.6	0.89	-0.43	167^{o}	0.37
Trimethoprim	pK_a= 7.2,6.6	0.91	0.49	199-203^{o}	0.5
Ciprofloxacin	pK_{a1}= 6.2 pK_{a2}= 8.8	-1.74, -0.28 *(at pH 7.04)*	-0.73	318-320^{o}	
Acetaminophen	pK_a = 9.5	0.27-0.5	0.34	169-170.5^{o}	11
Carbamazepine	Neutral	2.45	2.67	190-193^{o}	0.945
Metoprolol	pK_a = 9.7	1.9			
Venlafaxine				215-217^{o}	

(Dodd et al., 2004; Jjemba, 2008; Kümmerer, 2008b; Merck & Co, 2004; Yalkowsky, 2003; Zhang et al., 2008; Feitosa-Felizzola, 2009; Yamamoto et al. 2009)

Chapter 4
Occurrence of PhACs in Healthcare Facility Effluents and WWTP Influents

This chapter presents the measured concentrations of the nine target PhACs in the investigated healthcare facility effluents and in the downstream WWTP influents. It discusses the day-to-day variability in concentration of target compounds in these streams. Further the investigated healthcare facility effluent concentrations are compared. The wastewater treatment influents are also compared for the occurrence of the target PhACs.

To assess the discharges of the nine target PhACs by the healthcare facilities, the effluents of the selected hospitals and long-term-care homes (two of each type) were sampled over five week days. The downstream WWTPs that received discharges from these institutions were also sampled during the same days for mass balance calculations. Twenty-four hour composite samples were collected from each site, extracted using SPE and then analyzed with LC-MS/MS.

The following sampling issues arose during the study:

1. At the HS_1 facility twenty-one sub-samples out of 48 that were intended to be composited were missed on the second day of sampling.
2. At the HS_2 facility two auto samplers were installed because the discharges from the cancer clinic had a second sewer line that was independently connected to the municipal sewer. The Tuesday sample from this facility contained only wastewater discharged from the cancer clinic because the auto sampler on the main facility was positioned high above the wastewater stream and the sampler was unable to draw the effluent.
3. The volume of the Friday sample collected from the main facility (HS_2) manhole was less (about 3 L) than expected (5-6 L). It was not clear whether it was due to low flows condition or the some strainer openings being closed due to suspended matter.
4. Some portion of Monday sample from LTC_2 facility effluent was lost due to leakage in sampler container, therefore only one replicate was processed for that day sample.

4.1 Occurrence of Target PhACs in the Healthcare Facility Effluents

In this study the samples collected on each day were analyzed in triplicate. In the plots that follow, the bars present the average of the triplicates, while the error bars represent the range of the triplicates. In the text, the average value of the triplicate analyses is discussed as this represents the best estimate of the actual values. For example, Table 4-1, shows the calculations for the second day sample (the antibiotic Sulfamethoxazole was not tested for on the first day) from the first hospital facility HS_1 effluent. This sample was analyzed in triplicate as mentioned above and the values recorded are referred to as R_1, R_2 and R_3 in Table 4-1.

Table 4-1 Analytical results for the HS_1 effluent day-2 sample

Compound	HS_1 sample (ng/L)			Mean	Range
	R_1	R_2	R_3		
Sulfamethoxazole	3772	3392	3664	3609	3392- 3772
Trimethoprim	540	544	568	551	540 - 568
Ciprofloxacin	382	408	575	455	382 - 575
Acetaminophen	115200	116400	111600	114400	111600 - 115200
Carbamazepine	586	550	602	579	550 - 602
Metoprolol	41	48	42	44	41 - 48
Venlafaxine	344	321	378	348	321 - 378
N-desmethylvenlafaxine	177	232	149	186	149 – 232
O-desmethylvenlafaxine	2252	3172	1456	2293	1456 - 3172

The day-to-day variability observed in the concentration of target PhACs in the investigated healthcare facility effluents presumably occurs due to variation in the consumption of PhACs and water usage; higher flow reduces concentrations and vice versa provided that compound consumption remains the same. The coefficient of variation (CV = standard deviation/Mean) was used as an indicator of the day-to-day variability. The CV normalizes the standard deviation values and allows comparison between the variability estimates of the target compounds, regardless of their concentration values (Reed et al., 2002). In this study, compounds with higher CV values had relatively higher variability than others; further it was assumed that compounds with CV<10% had the least or no variability.

As mentioned, the Tuesday sample from HS_1 contained only 27 sub-samples out of the 48 to be composited. Similarly, at HS_2 the Tuesday sample contained the discharge from the Cancer clinic only, and the Friday sample had less volume than expected; therefore, the results of these samples are plotted separately.

4.1.1 Sulfamethoxazole

Figure 4-1 shows Sulfamethoxazole concentrations measured in the investigated hospital and long-term-care home effluents (HS_1, HS_2, LTC_1, and LTC_2) in ng/L. Sulfamethoxazole was not tested for in HS_1's Monday sample; therefore, no results are available for this day.

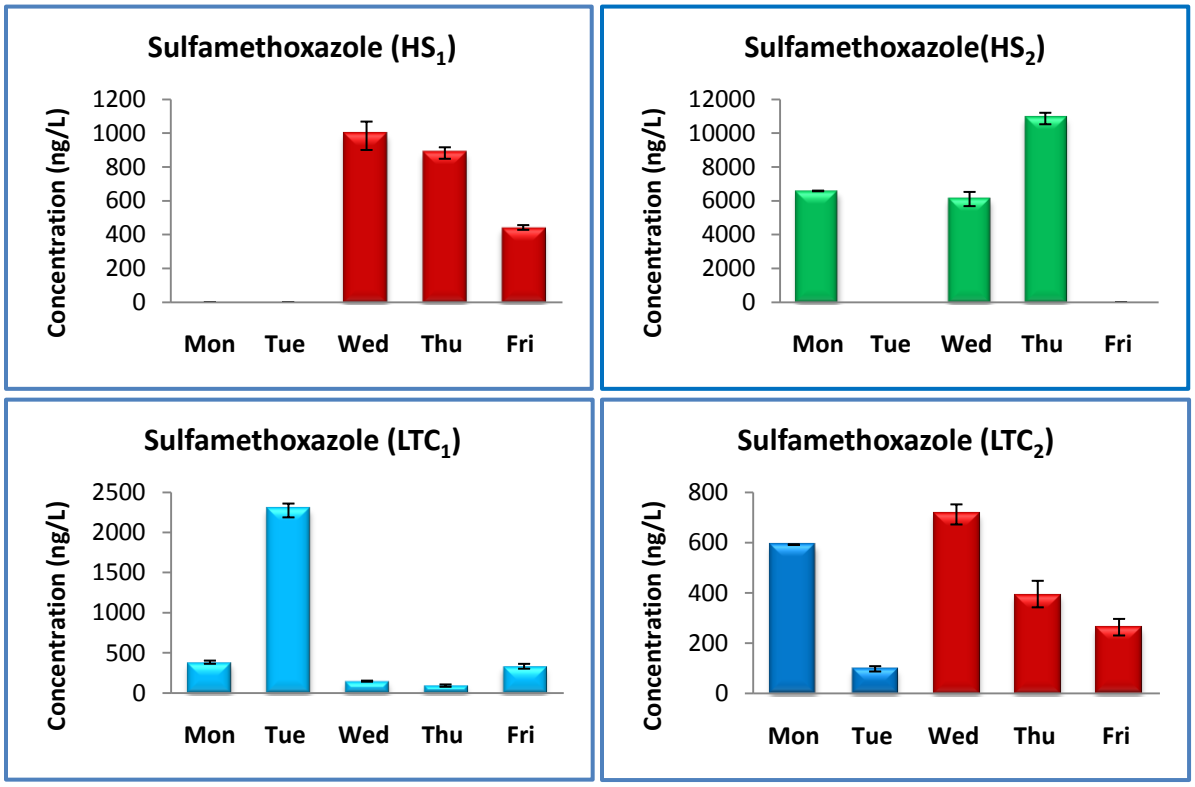


Figure 4-1 Sulfamethoxazole concentrations in healthcare facility effluents

Relatively higher concentrations were observed in HS_2's effluent than in the rest of the facilities. The detected concentration was greater than 6 μg/L in all samples. The highest concentration (11 μg/L) was measured on Thursday (Figure 4-1). The weekly maximum concentrations of Sulfamethoxazole in the other investigated healthcare facility effluents were HS_1 (~ 1 μg/L), LTC_1 (2.3 μg/L) and LTC_2 (0.7 μg/L).

The Sulfamethoxazole concentrations followed similar trends in the HS_1 and LTC_2 effluents from Wednesday to Friday (bars shown in red in HS_1 and LTC_2), with a maximum concentration on

Wednesday and then a decrease over the next the two days (Thursday and Friday). No patterns in concentration were found either in the HS_2 or LTC_1 effluents over the week days.

The day-to-day variability in concentration allows the identification of extreme individual events during week days. Table 4-3 presents the variability in concentrations about the mean values of each facility's effluent.

Table 4-2 Variability in concentrations of Sulfamethoxazole about the mean in the investigated healthcare facility effluents

Facility	Concentrations (ng/L)					Mean	Sd	CV
	Monday	Tuesday	Wednesday	Thursday	Friday			
HS_1			**996**	888	440	775	294.82	0.38
HS_2	6573		6160	**10933**		7889	2645	0.34
LTC_1	378	**2292**	147	95	330	648	926	1.43
LTC_2	592	100	**716**	391	260	412	248	0.60

Sd = Standard deviation
Bold values indicate the maximum measured concentrations

The highest variability in the Sulfamethoxazole concentration over the week days was observed in LTC_1. It occurred due to the individual peak concentration value on Tuesday (Table 4-2), which may have resulted from the disposal of unwanted or expired compounds. Further research is needed to clearly identify the sources of an individual spike. The higher day-to-day variability in Sulfamethoxazole concentrations in HS_2 (263 beds) than in HS_1 (365 beds) may have occurred because smaller facilities are more affected by individual events than larger facilities.

4.1.2 Trimethoprim

The measured Trimethoprim concentrations in HS_1, HS_2, LTC_1, and LTC_2 effluents are presented in Figure 4-2. Higher concentrations were observed in the HS_2 effluent than in others. Up to 10.3 μg/L of Trimethoprim was detected in HS_2 effluent. The weekly maximum concentrations were HS_1 (0.5 μg/L), LTC_1 (2.3 μg/L), and LTC_2 (1.3 μg/L).

No consistent concentration patterns over the week days were observed in the facility effluents (Figure 4-2). In the HS_1 effluent the Trimethoprim concentration was at its maximum (512 ng/L) on

Monday then remained relatively stable between 314 - 336 ng/L from Wednesday to Friday. In the HS_2 effluent, the concentration was at a minimum on Monday, and then increased over the following week days. The maximum concentration was detected on Thursday (10.3 µg/L). In the long-term-care homes the maximum concentrations occurred either on Thursday (LTC_1) or on Wednesday (LTC_2).

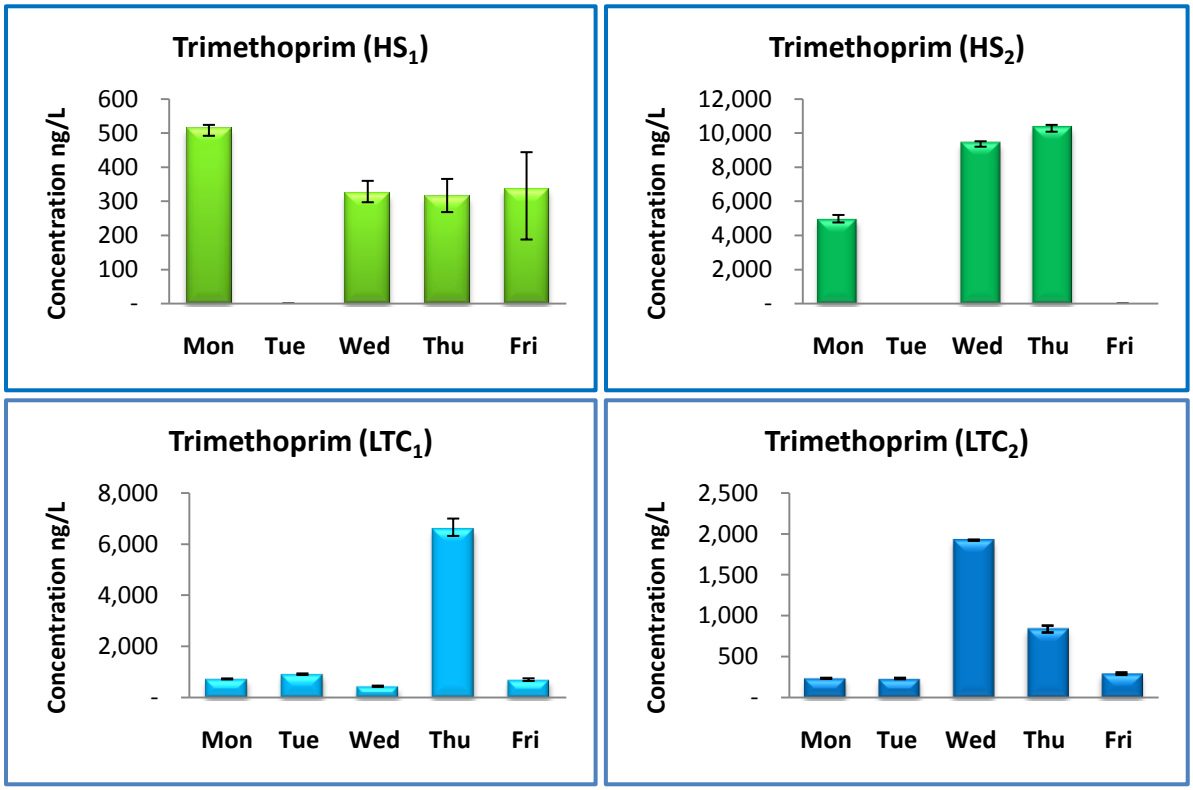


Figure 4-2 Trimethoprim concentrations in the healthcare facility effluents

Substantial day-to-day variability of Trimethoprim concentrations was observed in all the facility effluents (CV >10%) with the maximum variability observed in the long-term-care home effluents (Table 4-3). The higher CV values in LTC_1 and LTC_2 effluents were due to the individual peak concentrations that occurred on a particular day

Table 4-3 Variability in concentrations of Trimethoprim about the mean in the investigated healthcare facility effluents

Facility	Concentrations (ng/L)					Mean	Sd	CV
	Monday	Tuesday	Wednesday	Thursday	Friday			
HS_1	**512**		321	314	336	371	95	0.26
HS_2	4947		9413	**10320**		8227	2877	0.73
LTC_1	736	924	451	**6573**	701	1877	2631	1.40
LTC_2	238	234	**1924**	847	298	708	727	1.03

Sd = Standard deviation
Bold numbers show the maximum concentrations measured

The individual peak concentrations in LTC_1 and LTC_2 (Table 4-3) may have resulted from either the administration of a single therapeutic dose, or disposals of un-needed and expired compounds. Trimethoprim is sometimes used as a single dose (75-450 mg) therapy for urinary tract infections (Sweetman et al., 2007). As mentioned earlier, such infections are among the most common diseases in long-term-care home setups. About 40 to 60% of the administered dose has been reported to be excreted unchanged within 24 hours (Sweetman et al., 2007) ; therefore, the peak concentration value in LTC_2's Wednesday sample (1924 ng/L) may have been the results of a single dose therapy, as about half of this amount showed up on the next day (Table 4-3). The peak concentration in LTC_1 (6573 ng/L) on Thursday was less likely to be a single therapeutic dose, as the concentration on the following day was nine times less than this value. Thus this value was assumed to occur due to the disposal of unneeded or expired compounds.

4.1.3 Ciprofloxacin

The weekly maximum Ciprofloxacin concentrations in the HS_1, HS_2, LTC_1, and LTC_2 effluents were 1.2, 0.16, 0.6, and 1.4 μg/L respectively (Figure 4-3). Relatively higher concentrations over the week days were observed in HS_1 effluent, with the mean concentration of 0.79 μg/L compared to 0.15, 0.31, and 0.32 μg/L in HS_2, LTC_1, and LTC_2 effluents respectively (Table 4-4).

The Ciprofloxacin concentrations showed similar patterns over the week days in HS_1, HS_2 and LTC_1 effluents (bars filled red), with lower concentrations on the first and last day of the week

(Monday and Friday), and maximum concentrations observed on Wednesdays. In LTC_2, the daily concentrations decreased from Monday to Wednesday, with the minimum concentration (31 ng/L) on Wednesday and a spike of 1470 ng/L on Thursday (Figure 4-3).

The findings indicate that the maximum Ciprofloxacin concentrations occurred on Wednesday especially in hospital effluents, regardless of the sampling season; because the investigated hospitals HS_1 and HS_2 were sampled in different seasons (summer and winter respectively) and had their maximum concentrations on Wednesday (Table 4-4).

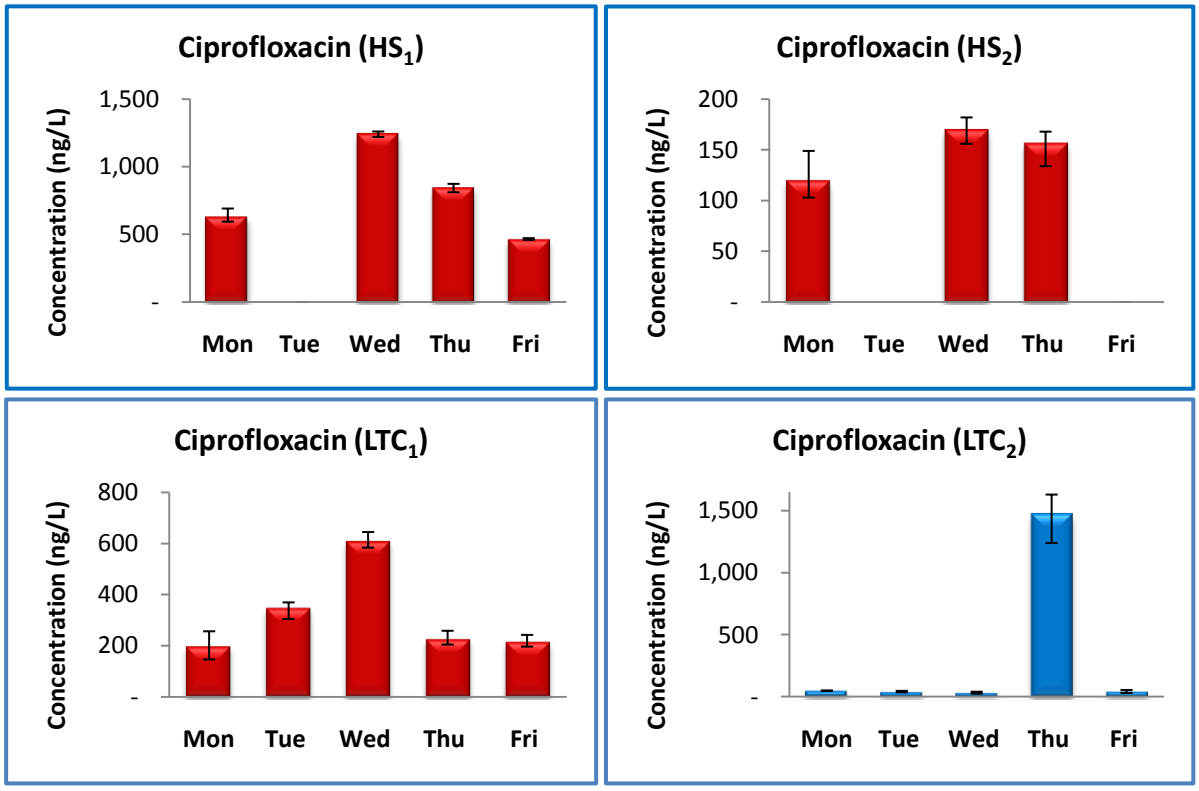


Figure 4-3 Ciprofloxacin concentrations in healthcare facility effluents

The highest day-to-day variability in Ciprofloxacin concentration was observed in LTC_2 and the lowest in HS_2 (Table 4-4). The higher variability in the LTC_2 effluent was due to the individual spike in concentration on Thursday (1.4 µg/L), which presumably happened due to disposal of unneeded or expired Ciprofloxacin compound ; as the concentrations in other weekdays was always less than 50 ng/L.

Table 4-4 Variability in concentrations of Ciprofloxacin about the mean in the investigated healthcare facility effluents

Facility	Concentrations (ng/L)					Mean	Sd	CV
	Monday	Tuesday	Wednesday	Thursday	Friday			
HS_1	632		**1240**	843	465	795	335	0.42
HS_2	119		**169**	155		148	26	0.17
LTC_1	197	345	**604**	224	214	317	171	0.54
LTC_2	49	41	31	**1470**	42	327	639	1.96

Sd = Standard deviation
Bold numbers show the maximum concentrations measured

In LTC_2 (Table 4-4) the individual peak value of Ciprofloxacin (1470 ng/L) on Thursday shows a possible batch discharge, because, this compound is normally administered more than once; therefore, a similar concentration range is expected to show up on the following day sample (Friday). In addition, the pharmacokinetic information suggests that 40-50% of the oral dose is expected to be excreted unchanged in 24 hours (Sweetman et al., 2007). This too supports the idea that a portion of the administered compound will be excreted the next day.

In some health conditions Ciprofloxacin is used as a single oral dose. Up to 8 hours of Ciprofloxacin elimination half life has been reported for the elderly (Sweetman et al., 2007), and 5 half-lives are normally required to eliminate the drug up to 97% (Rowland et al., 1995). This information suggests that some portion of the administered dose may be excreted during the next day and should show up in that day's sample if a single oral dose was used. Therefore it is reasonable to consider the observed peak concentration as being a batch discharge.

4.1.4 Acetaminophen

The measured concentrations of Acetaminophen in the investigated hospital and long-term-care home effluents are shown in Figure 4-8. Relatively higher concentrations were observed in HS_1 (the biggest facility investigated) with a mean concentration of 99.5 μg/L compared to 13.6 μg/L in HS_2 and 82 to 88 μg/L in long-term-care homes. The weekly maximum concentrations were HS_1 (134 μg/L), HS_2 (16 μg/L), LTC_1 (116 μg/L), and LTC_2 (88 μg/L).

No common patterns were observed among the facilities. The Acetaminophen concentration was relatively stable in long term care homes. In LTC_2, the concentration varied between 72.8 to 88.8 µg/L over the week days. While in LTC_1, it varied between 77.7 and 85µg/L with an individual spike on Thursday (116 µg/L).

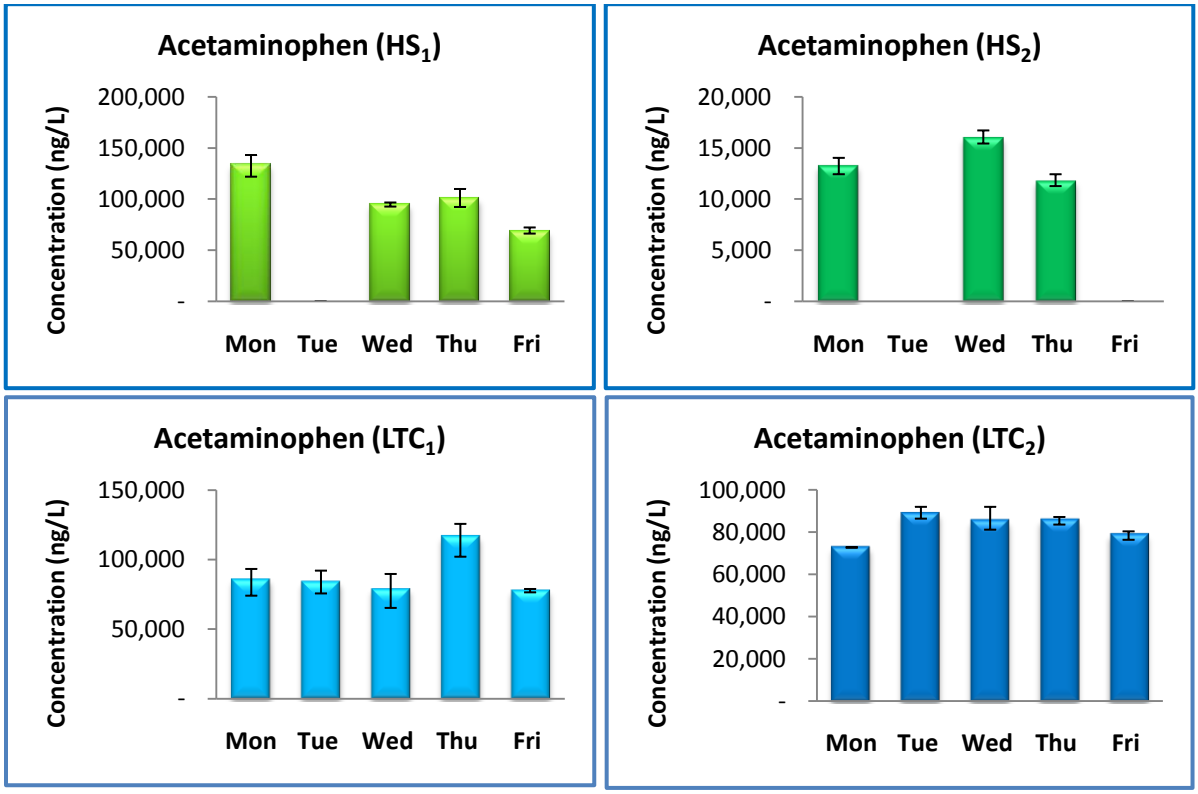


Figure 4-4 Acetaminophen concentrations in healthcare facility effluents

The day-to-day variability in Acetaminophen concentrations (Table 4-5) was lower in long-term-care homes than in hospital effluents, with the least variability in LTC_2 (CV< 10%). Again, there was one peak concentration in LTC_1 on Thursday (bold value); otherwise, there was minimal variability between rest of the week days (CV = 0.05) in this facility. Relatively higher variability was observed in HS_1 (a bigger facility). The lower variability in Acetaminophen concentration in long-term-care homes suggests that this compound may be used more frequently in these facilities.

Table 4-5 Variability in concentrations of Acetaminophen about the mean in the investigated healthcare facility effluents

Facility	Concentrations (ng/L)					Mean	Sd	CV
	Monday	Tuesday	Wednesday	Thursday	Friday			
HS_1	**134133**		95067	100533	68533	99567	26951	0.27
HS_2	13253		**15973**	11773		13667	2130	0.16
LTC_1	85600	83867	78667	**116267**	77733	88427	15917	0.18
LTC_2	72800	**88800**	85333	85733	78933	82320	6419	0.08

Sd = Standard deviation
Bold numbers show the maximum concentrations measured

4.1.5 Carbamazepine

Higher Carbamazepine concentrations were observed in the HS_2 effluent than in the rest of the facilities, with a maximum detected concentration of 676 ng/L. The weekly maximum concentrations in HS_1, LTC_1, and LTC_2 were 144, 527, and 77 ng/L, respectively (Figure 4-5).

Carbamazepine had similar concentration patterns during the weekdays in the HS_2 and LTC_2 effluents (bars filled red), with higher concentrations on Mondays, minimum concentrations on Wednesdays and then increases in the next two days (Thursday and Friday). In the LTC_1 effluent the concentration showed a rising trend from Monday to Thursday with a peak on Thursday and a dip on Friday. The maximum concentration was observed on Monday in both the hospitals (HS_1 and HS_2). In long-term-care homes the maximum concentration occurred on either Thursday or on Friday (Figure 4-5).

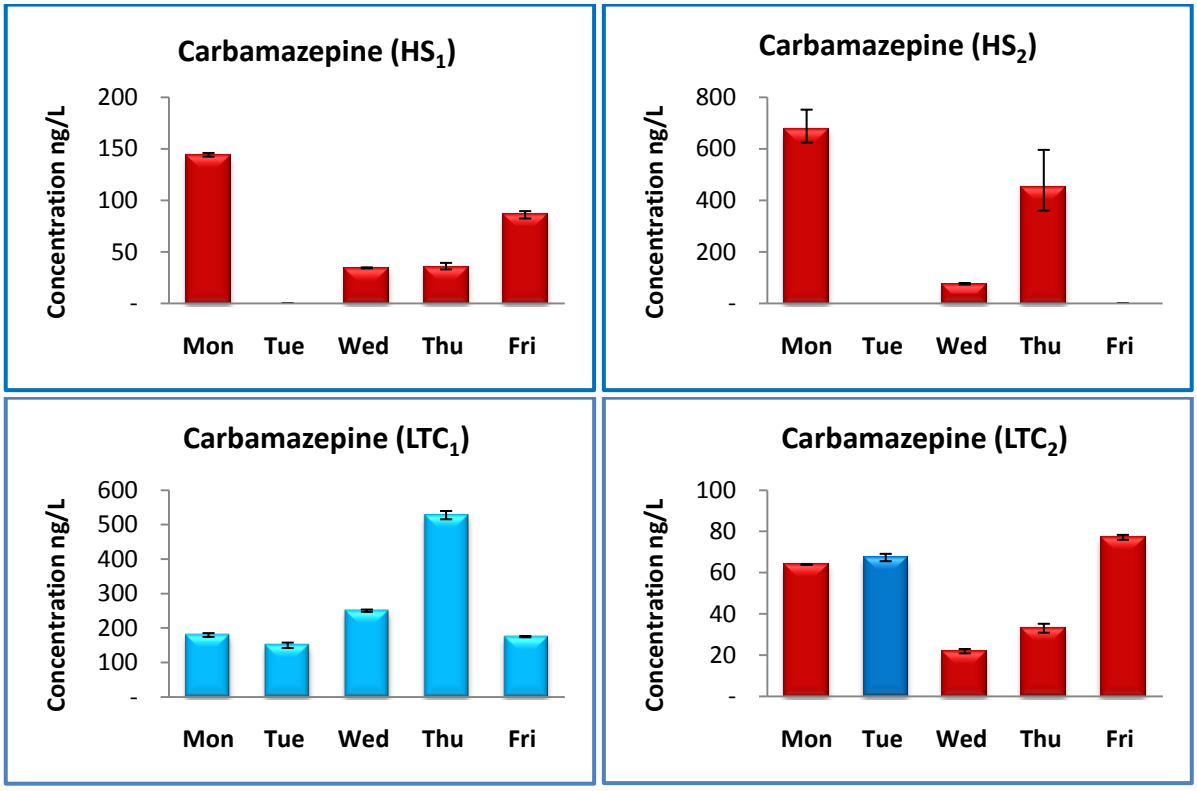


Figure 4-5 Carbamazepine concentrations in healthcare facility effluents

Day-to-day variability (Table 4-6) in concentration was observed in all the facility effluents (CV > 10%). Relatively higher variability (CV = 0.69 and 0.76) was observed in the hospital effluents than in the long-term-care homes (CV = 0.6 and 0.4).

Table 4-6 Variability in concentrations of Carbamazepine about the mean in the investigated healthcare facility effluents

Facility	Concentrations (ng/L)					Mean	Sd	CV
	Monday	Tuesday	Wednesday	Thursday	Friday			
HS_1	**144**		34	36	86	75	52	0.69
HS_2	**676**		76	452		401	303	0.76
LTC_1	182	153	252	**527**	176	258	155	0.60
LTC_2	64	67	22	33	**77**	53	24	0.45

Sd = Standard deviation
Bold numbers show the maximum concentrations measured

4.1.6 Metoprolol

Higher Metoprolol concentrations were detected in the LTC_1 effluent than in all the other investigated healthcare facility effluents (Figure 4-6) with up to 5 µg/L detected in its effluent. The weekly maximum concentrations in the other effluents were HS_1 (493 ng/L), HS_2 (676 ng/L), and LTC_2 (321 ng/L).

The Metoprolol concentrations varied randomly in all the facility effluents. Common patterns were observed between hospital effluents HS_1 and HS_2, with minimum concentrations on the first week day (Monday) and maximum values on Wednesday. The long-term-care home effluents had similar patterns.

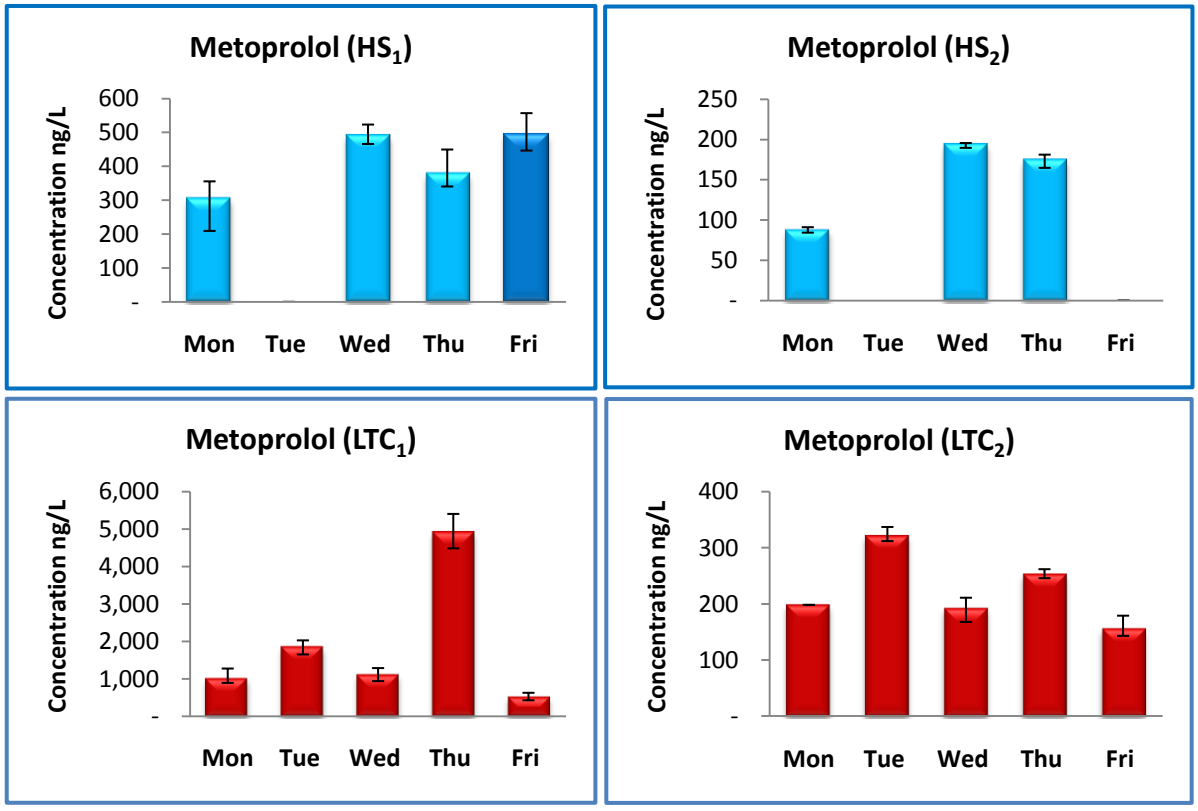


Figure 4-6 Metoprolol concentrations in healthcare facility effluents

The day-to-day variability in concentration of Metoprolol was greatest in LTC_1 (CV = 0.92), because of the individual peak concentration on Thursday ~ 5 μg/L (Table 4-7). The individual peak concentration may have been due to the disposal of expired or unwanted compounds down drains. The higher variability in HS_2 than in HS_1 may be because smaller facilities are more affected by individual events.

Table 4-7 Variability in concentrations of Metoprolol about the mean in the investigated healthcare facility effluents

Facility	Concentrations (ng/L)					Mean	Sd	CV
	Monday	Tuesday	Wednesday	Thursday	Friday			
HS_1	306		490	378	**493**	417	91.17	0.22
HS_2	88		**193**	175		152	56	0.37
LTC_1	1033	1875	1131	**4920**	541	1900	1754	0.92
LTC_2	198	**321**	192	253	157	224	64	0.29

Sd = Standard deviation
Bold numbers show the maximum concentrations measured

4.1.7 Venlafaxine

Venlafaxine was detected in much higher concentrations in HS_2 than in the other healthcare facilities, with daily measured concentrations greater than 4 μg/L except on Monday. Up to 9 μg/L (Wednesday) was measured in the HS_2 effluent (Figure 4-7). The higher concentrations were presumably due to the presence of the cancer clinic in HS_2. A Venlafaxine concentration of 35.4 μg/L was measured in the cancer clinic effluent and this supports this assumption (Figure 4-12). The weekly maximum concentrations measured in HS_1, LTC_1, and LTC_2 were 744, 2275, and 716 ng/L respectively.

No common concentration patterns were observed between the facility effluents (Figure 4-7). The maximum concentrations in hospital effluents were detected either on Thursday or Wednesday (HS_1 and HS_2 respectively). Long-term-care homes had individual peaks on Wednesday in LTC_1 and on Friday in LTC_2, otherwise they followed a similar trend.

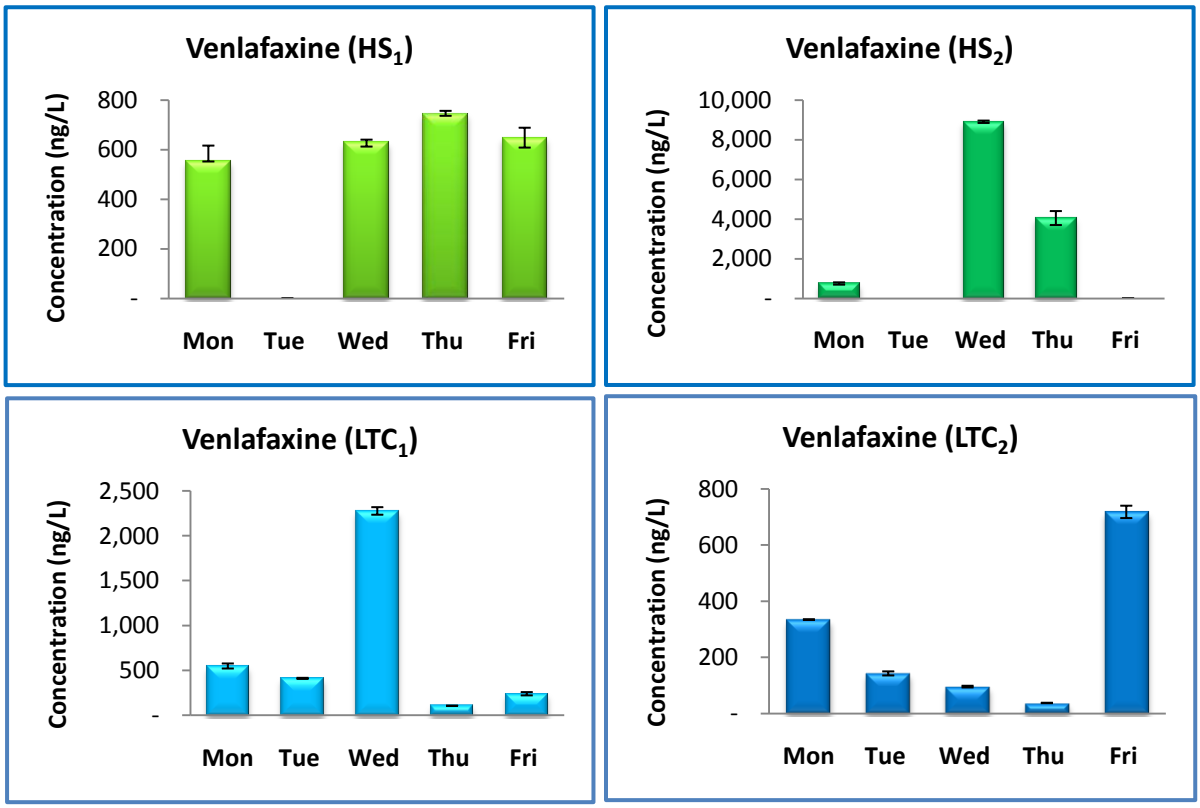


Figure 4-7 Venlafaxine concentrations in healthcare facility effluents

Venlafaxine concentrations showed higher variability in long-term-care homes than in hospitals, due to an individual spike or dip in concentration during week days. For instance, in LTC_1 2275 ng/L was detected on Wednesday; for rest of the weekdays Venlafaxine concentration varied between 108 and 547 ng/L. A similar spike was observed in LTC_2 on Friday (716 ng/L) and for other week days the concentration varied between 38 and 334 ng/L (Table 4-8). The least variability was found in HS_1 effluent (CV = 0.12). The lowest variability in HS1 was perhaps due to the fact that bigger facilities are less affected by individual events.

Table 4-8 Variability in concentrations of Venlafaxine about the mean in the investigated healthcare facility effluents

Facility	Concentrations (ng/L)					Mean	Sd	CV
	Monday	Tuesday	Wednesday	Thursday	Friday			
HS_1	552		629	**744**	647	643	79	0.12
HS_2	761		**8893**	4059		4571	4090	0.89
LTC_1	547	412	**2275**	108	243	717	886	1.24
LTC_2	334	144	97	38	**716**	266	275	1.04

Sd = Standard deviation
Bold numbers show the maximum concentrations measured

4.1.8 N-desmethylvenlafaxine

Relatively higher N-desmethylvenlafaxine concentrations were observed in HS_1 effluents than in the other facilities. The measured concentrations were greater than 200 ng/L in all samples, with a maximum concentration of 416 ng/L (Figure 4-8). The weekly maximum concentrations of N-desmethylvenlafaxine in other investigated facility effluents were HS_1 (457 ng/L), LTC_1 (266 ng/L) and LTC_2 (119 ng/L).

The N-desmethylvenlafaxine concentrations showed similar patterns from Wednesday to Friday in the hospital effluents, with an increasing trend over these days (bars filled red). No trends were found in the long-term-care home effluents.

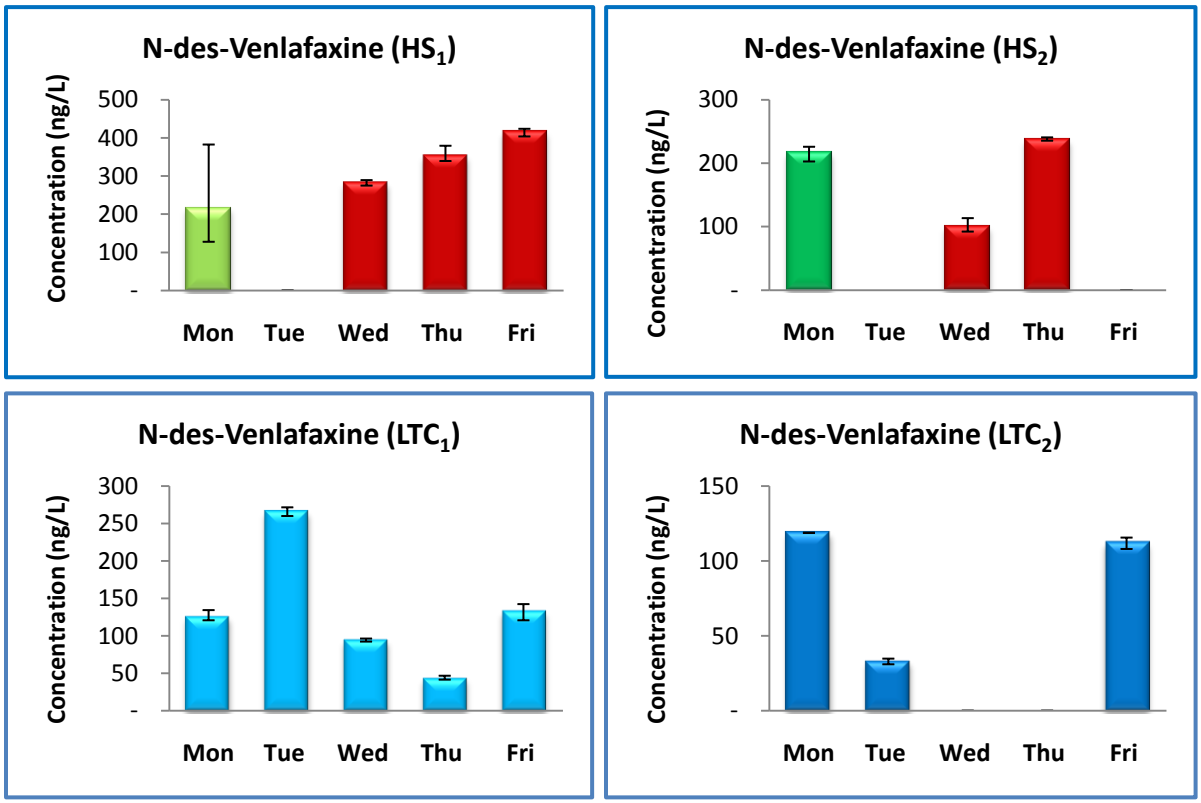


Figure 4-8 N-desmethylvenlafaxine concentrations in healthcare facility effluents

The day-to-day variability in concentration was lower in the hospital effluents than in the long-term-care-home's (Table 4-9). HS_1 had the least variability (CV = 0.27) and the highest variability was observed in LTC_2 (CV = 1.11). The higher variability in LTC_2 may be because this compound was not detected in two samples from its effluent (Wednesday and Thursday).

N-desmethylvenlafaxine is excreted in lesser amounts compared to the parent compound, with 1% of administered dose reported to be excreted as N-desmethylvenlafaxine as compared to 1-10% for the parent compound (Klamerus et al., 1992). The concentration of Venlafaxine on Wednesday and Thursday was 97 and 38 ng/L respectively, so the concentration of N-desmethylvenlafaxine was expected to be close to the instrument detection limit (<10 ng/L).

Table 4-9 Variability in concentrations of N-desmethylvenlafaxine about the mean in the investigated healthcare facility effluents

Facility	Concentrations (ng/L)					Mean	Sd	CV
	Monday	Tuesday	Wednesday	Thursday	Friday			
HS_1	217		283	353	416	317	86.31	0.27
HS_2	217		100	238		185	74	0.40
LTC_1	126	266	95	44	133	133	82	0.62
LTC_2	119	33	0	0	112	53	59	1.11

Sd = Standard deviation
Bold numbers show the maximum concentrations measured

4.1.9 O-desmethylvenlafaxine

The weekly maximum concentrations of O-desmethylvenlafaxine in the investigated healthcare facility effluents were HS_1 (2880 ng/L), HS_2 (2535 ng/L), LTC_1 (6987 ng/L) and LTC_2 (2124 ng/L).

Similar patterns of O-desmethylvenlafaxine concentration were observed in the hospital effluents from Wednesday to Friday (bars filled red). The highest concentrations of O-desmethylvenlafaxine in the long-term-care homes were observed on Fridays (Figure 4-9).

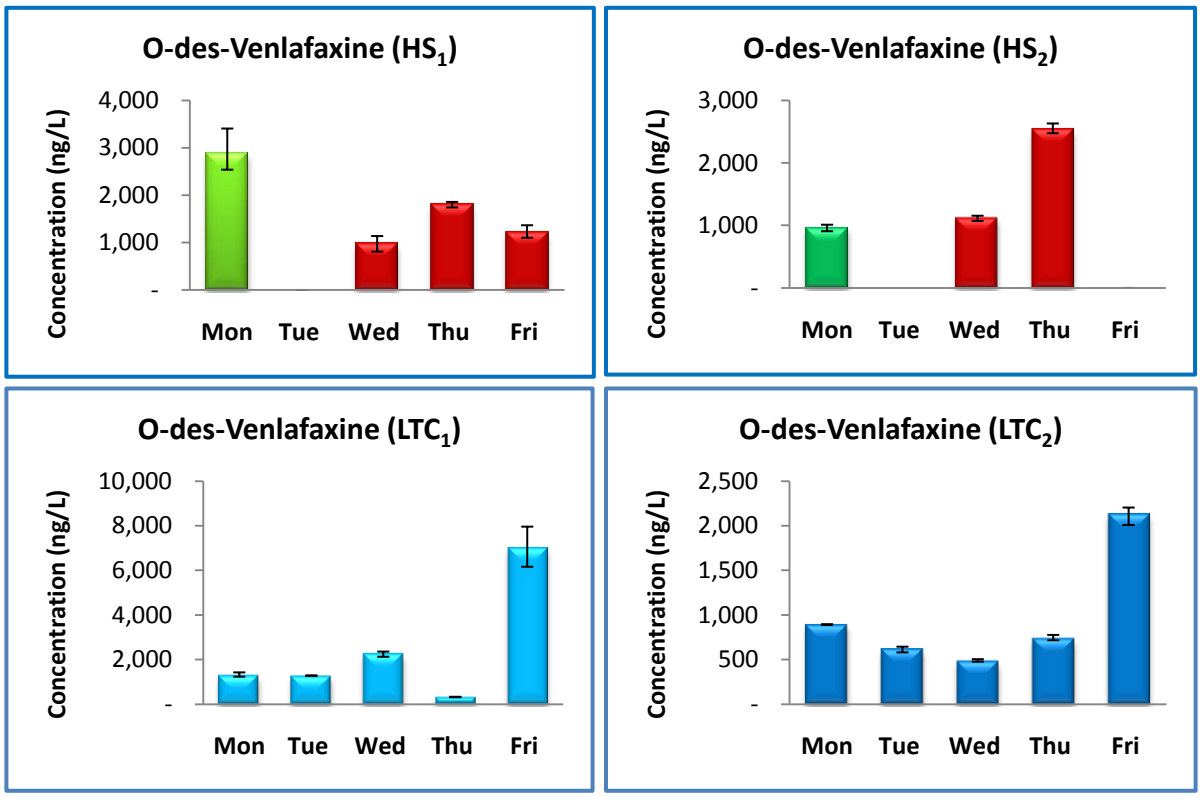


Figure 4-9 O-desmethylvenlafaxine concentrations in healthcare facility effluents

The highest day-to-day variability in the concentration of O-desmethylvenlafaxine was observed in LTC_1 effluent (Table 4-10), due to the individual peak concentration on Friday (6987 ng/L).

Table 4-10 Variability in concentrations of O-desmethylvenlafaxine about the mean in the healthcare facility effluents

Facility	Concentrations (ng/L)					Mean	Sd	CV
	Monday	Tuesday	Wednesday	Thursday	Friday			
HS_1	**2880**		968	1797	1216	1715	851	0.50
HS_2	956		1127	**2535**		1539	866	0.56
LTC_1	1301	1276	2272	323	**6987**	2432	2638	1.08
LTC_2	892	621	485	736	**2124**	972	661	0.68

Sd = Standard deviation
Bold numbers show the maximum concentrations measured

4.1.10 Relationship between Venlafaxine and its Metabolites

Venlafaxine and its metabolites have a pharmacokinetic relation that may vary between individuals, depending upon, their age, sex and health conditions and route of administration in addition to other factors (Correia, 2007). Klamerus et al., (1992) found that the urinary excretion for an oral dose of Venlafaxine in healthy adults was 1-10% in the parent form, up to 30% of the active metabolite O-desmethylvenlafaxine, and about 1% N-desmethylvenlafaxine.

To study whether the concentration of Venlafaxine and its metabolites (N-desmethylvenlafaxine and O-desmethylvenlafaxine) in each sample followed this pattern, the measured concentrations of these compounds in each facility were plotted together (Figure 4-10). This figure shows that the measured concentrations in HS_1, LTC_1 and LTC_2 followed the same order from lowest to highest, as N-desmethylvenlafaxine, Venlafaxine and O-desmethylvenlafaxine, as proposed by Klamerus et al. (1992). In the HS_2 effluent a similar trend was observed only for the Monday sample, while in all other samples, the concentration of Venlafaxine was greater than O-desmethylvenlafaxine's.

To investigate further the relationship between the measured concentration of Venlafaxine and its metabolites, the measured concentrations of these compounds for each day were normalized with the concentration of N-desmethylvenlafaxine for that day, i.e., concentrations were divided by the N-desmethylvenlafaxine concentration for that day. The results are presented in Table 4-11.

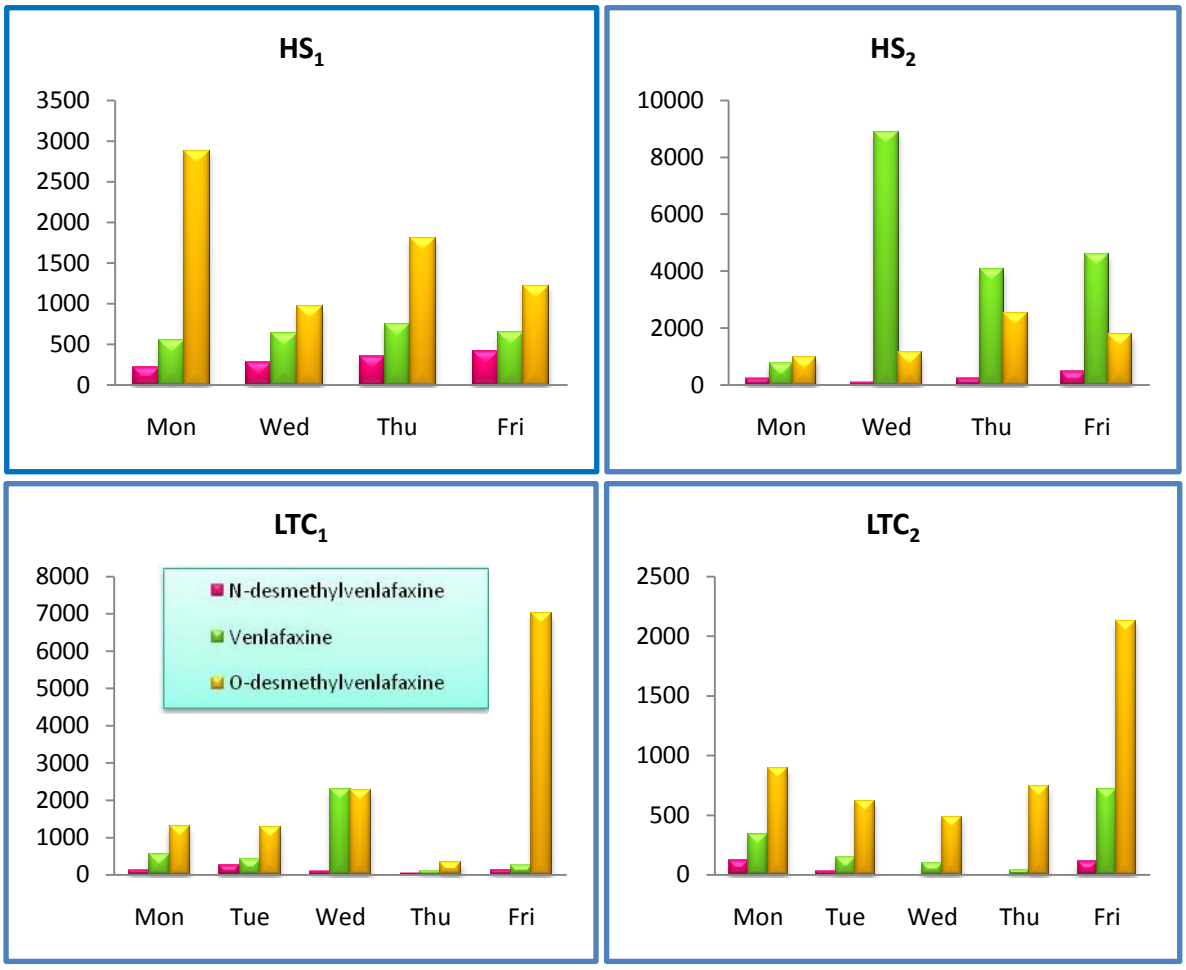


Figure 4-10 Concentration of Venlafaxine and its metabolites in healthcare facility effluents

Table 4-11 Measured Concentration ratios between Venlafaxine and its metabolites in the healthcare facility effluent.

Facility	N-desmethylvenlafaxine : Venlafaxine : O-desmethylvenlafaxine														
	Monday			Tuesday			Wednesday			Thursday			Friday		
LTC_1	1	4	10	1	2	5	1	24	24	1	2	7	1	2	53
LTC_2	1	3	7	1	4	19							1	6	19
HS_1	1	3	13				1	2	3	1	2	5	1	2	3
HS_2	1	4	4				1	89	11	1	17	11	1	10	4

N-desmethylvenlafaxine was not detected in two samples of LTC_2 (Wednesday & Thursday)
Tuesday's samples from HS_1 and HS_2 were not full effluent samples

The differences in the relationship between measured concentrations of Venlafaxine and its metabolites (Table 4-11) may occur due to the difference in the elimination half life of Venlafaxine (4 hours) and its active metabolite O-desmethylvenlafaxine (10 hours). Therefore, a consistent relationship cannot be expected in the healthcare facility effluents, especially in hospitals, where patients are frequently admitted and discharged. The excretion of these compounds depends on the time patients spent in the hospital; for example if Venlafaxine is administered to patients who spend less than 10 hours in the hospital Venlafaxine excretion is expected but not O-desmethylvenlafaxine. Patients, who take Venlafaxine at home and visit the hospital after 5 to 6 hours, will excrete only O-desmethylvenlafaxine in hospital, not Venlafaxine, and vice versa.

The HS_2 effluent's higher concentration of Venlafaxine than O-desmethylvenlafaxine might be explained by the disposal of unwanted compounds down drains. These compounds will not pass thought the human metabolism, resulting in higher concentrations of Venlafaxine. In addition, patients who visit hospitals for less than 10 hours are expected to excrete Venlafaxine only but not O-desmethylvenlafaxine; this may lead to higher concentrations of Venlafaxine than O-desmethylvenlafaxine.

In the long-term-care homes, where patients do not frequently change the relation of venlafaxine and it metabolites was 1% N-desmethylvenlafaxine 2 to 6% of Venlafaxine and 3 to 19% of O-desmethylvenlafaxine except for the Wednesday and Friday samples in the LTC_1 facility. The relatively higher concentrations of Venlafaxine (similar to O-desmethylvenlafaxine concentration) on Wednesday in the LTC_1 may have been due to the disposal of un-wanted compound leading to a higher concentration of the parent compound only.

4.1.11 Concentration of Target PhACs in Day-2 Sample from HS_1

As indicated earlier, on the second day of sampling at the HS_1 facility the auto-sampler missed 21 sub-samples out of 48 that were to be composited. Therefore, the Tuesday results are plotted separately from those of the other days of the week in Figure 4-11.

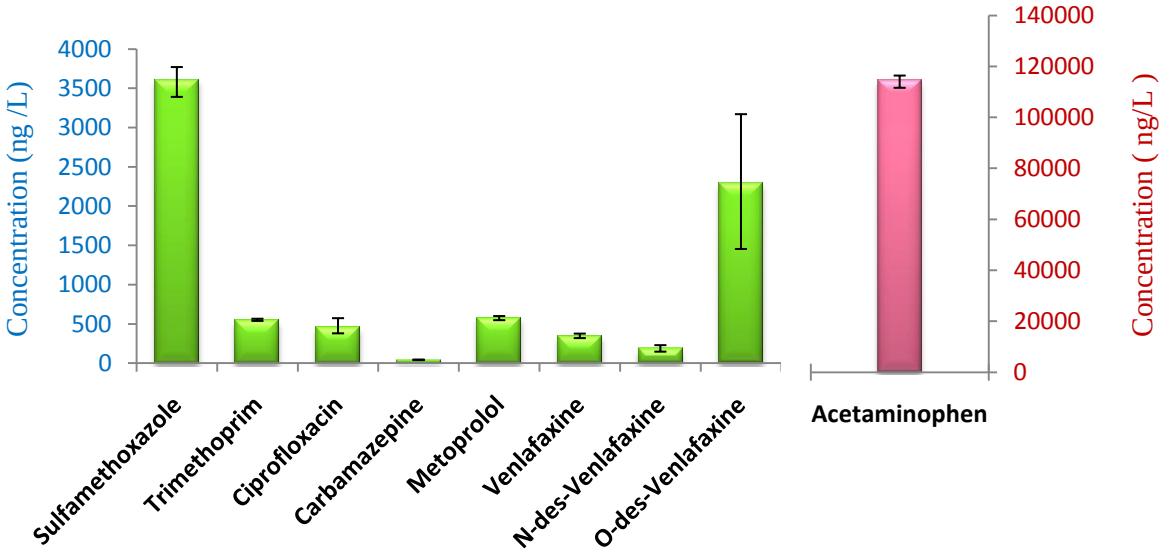


Figure 4-11 Concentrations of target compounds in day-2 sample of HS_1 effluent

A comparison of the results of this sample (HS_1 day-2) with those of the rest of the week (Figure 4-1 to 4-9) shows that the Sulfamethoxazole concentration (3609 ng/L) was higher in this sample than in the other weekdays (varied between 456 and 900 ng/L). In contrast Venlafaxine had lower concentraion (348 ng/L) compared to the other weekdays (552 to 744 ng/L). For all other target compounds, the concentrations were in the same range as found on other weekdays (Table 4-12). This may have been due to differences in the dosing patterns of the differing groups of compounds.

Table 4-12 Comparison between Tuesday sample and other weekday samples

Target Compound	**Concentrations (ng/L)**	
	Tuesday	**Monday to Friday (**Range**)**
Trimethoprim	550	456 - 900
Ciprofloxacin	455	465 - 1240
Acetaminophen	114400	68533 - 134133
Carbamazepine	44	34.5 - 144
Metoprolol	579	305 - 490
N-desmethylvenlafaxine	186	216 - 416
O-desmethylvenlafaxine	2293	968 - 2880

4.1.12 Concentrations of Target PhACs in the Cancer Clinic Effluent and Friday Sample from HS_2

As mentioned earlier, the Tuesday sample from the HS_2 facility effluent contained only discharges from the cancer clinic and the Friday sample had a lesser volume than expected; therefore, these values were plotted separately from the other days in Figure 4-12. In the cancer clinic effluent, the antidepressant Venlafaxine had the highest concentrations; up to 36 μg/L of Venlafaxine and 6.4 μg/L of its metabolite O-desmethylvenlafaxine were measured. Acetaminophen and Carbamazepine concentrations were 13.3 μg/L and 0.6 μg/L, respectively while the concentrations of the antibiotics sulfamethoxazole, trimethoprim, metoprolol and n-desmethylvenlafaxine were in the range of 0.3 to 0.4 μg/L. Carbamazepine levels may be high (628 ng/L) because it also possesses psychotropic properties and can thus be used for neuralgia and other severe pain syndromes connected with neurological disorders (Sweetman et al., 2007). The Carbamazepine concentrations on the other weekdays (full hospital effluent samples) varied between 76 and 452 ng/L except on Monday (676 ng/L). The use of Venlafaxine, Carbamazepine and Acetaminophen in certain cancer treatments has also been reported (Hardy et al., 2005; Lersch et al., 2002; Tasmuth et al., 2002).

The Friday sample contained a full sample from the cancer clinic, but a reduced volume from the discharge point of the main facility; therefore the concentrations of some compounds in this sample were in the same range to those of the cancer clinic (Ciprofloxacin, Sulfamethoxazole and Trimethoprim in Figure 4-12). Comparing the concentrations of the target PhACs in this sample to those of the rest of the week days shows that on Friday the Sulfamethoxazole and Trimethoprim concentrations were much lower (327 and 425 ng/L respectively) than those for other week days (6.5 to 11 μg/L for Sulfamethoxazole and 5 to 10 μg/L of Trimethoprim). All other compound concentrations were in the same range as for other week days.

Day-to-day variability in compound discharge is unavoidable, especially in hospitals, where patients are frequently discharged and admitted and such variability is often expected. In long-term-care homes, the higher variability was due to individual concentration peaks that presumably resulted from the disposal of unwanted compounds.

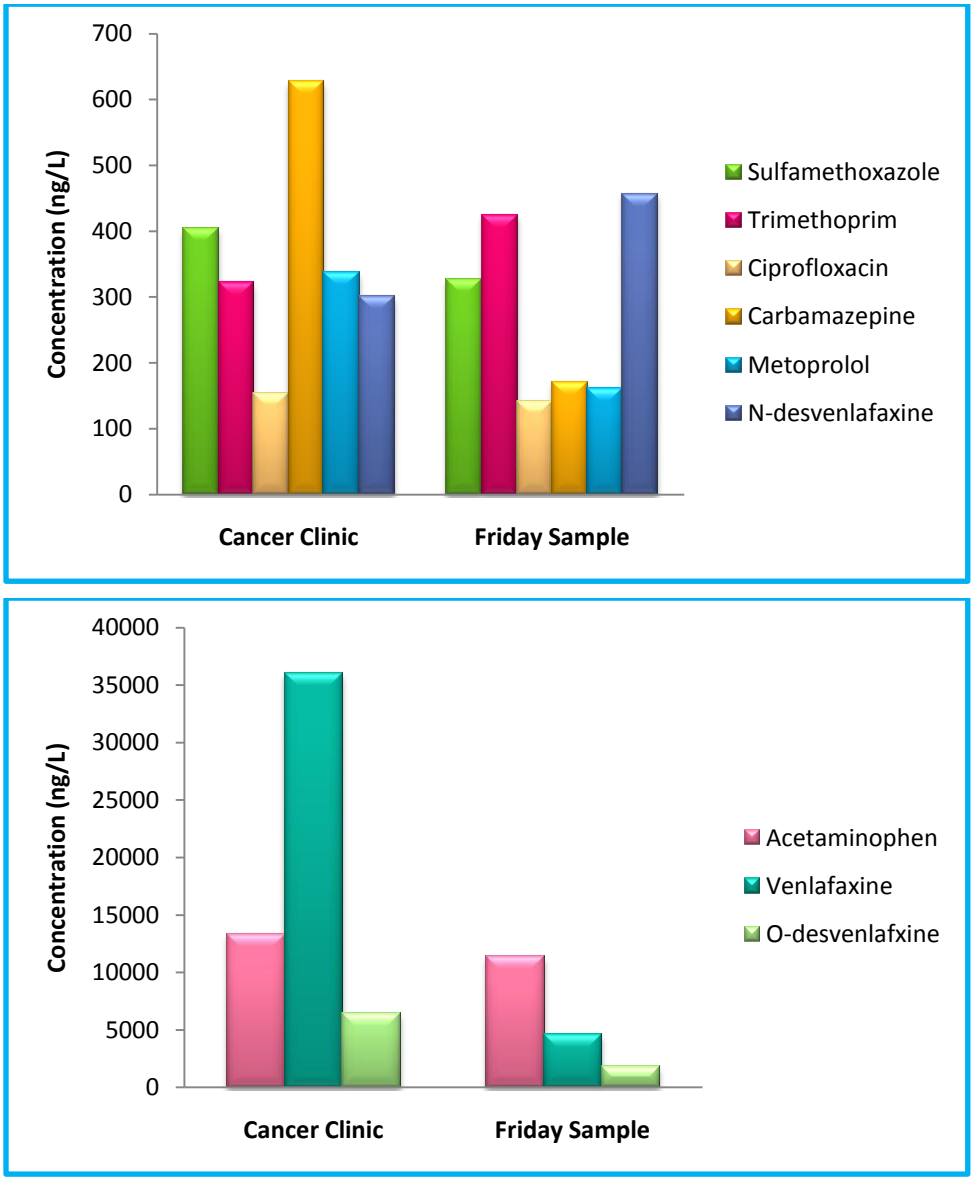


Figure 4-12 Concentration of target PhACs in the cancer clinic effluent and Friday sample from HS_2 effluent.

The measured concentrations of Sulfamethoxazole and Trimethoprim were within the range reported in previous hospital wastewater studies (Table 2-2). Ciprofloxacin was measured in lower concentrations than in hospital effluents studied in Europe; for instance, up to 124 μg/L was reported in the effluent of a German hospital (Hartmann et al., 1999).

The concentrations of Sulfamethoxazole, Trimethoprim, Carbamazepine and Venlafaxine were higher in the HS_2 effluent, while Metoprolol, Acetaminophen, and Ciprofloxacin concentrations were found to be higher in HS_1 effluent. The higher concentration of Sulfamethoxazole (~11 μg/L) and Trimethoprim (10 μg/L) in the relatively smaller facility's effluent (HS_2) than in HS_1's (1 μg/L and 0.5 μg/L respectively) may be explained by seasonal variations in the consumption of these compounds. The HS_1 effluent was sampled in mid-July, while HS_2 was sampled in the first week of November. Therefore, use of the July and October purchases can be assumed. Figure 4-9 shows Ontario hospital purchases for the year 2008 (IMS database), and indicates more Sulfamethoxazole and Trimethoprim was bought in October than in June and July.

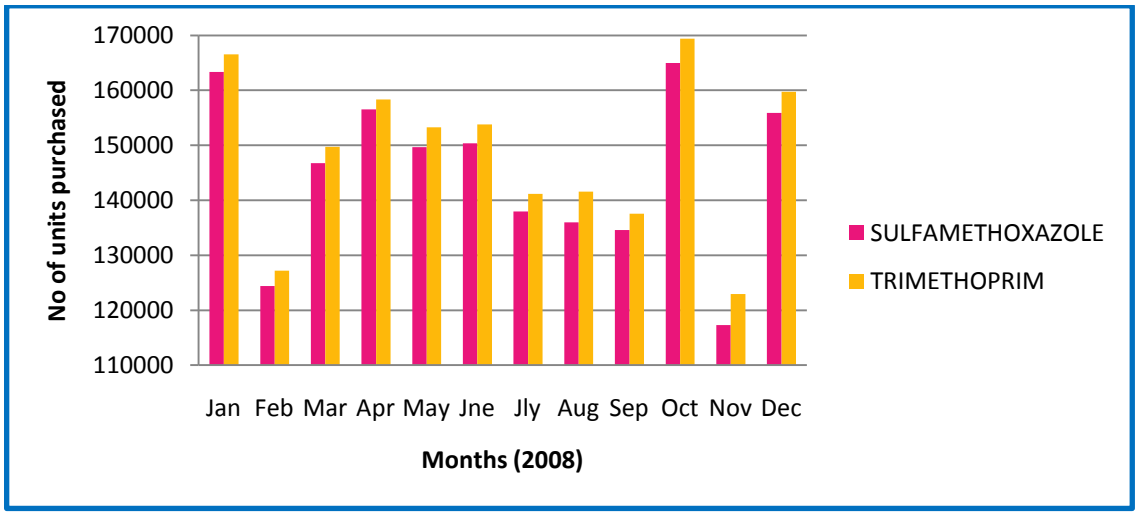


Figure 4-13 Number of individual units (tablets, capsules etc.) of Sulfamethoxazole and Trimethoprim purchased by Ontario hospitals in 2008 (IMS database)

An additional source of variation may be the differences in the service spectrum of the investigated hospitals. For instance, higher concentrations of Venlafaxine and Carbamazepine in HS_2 effluent than in HS_1 were presumably due to the presence of a cancer clinic, where such drugs may be prescribed to patients. The higher concentrations of Venlafaxine (~36μg/L) and Carbamazepine (0.6μg/L) in the cancer clinic effluent supports this hypothesis (Figure 4-12).

The occurrence of target PhACs in the long-term-care home effluents suggests that these streams can also contain elevated concentrations of antibiotics and other compounds. Acetaminophen concentrations were detected up-to 116 μg/L and the maximum concentration of Metoprolol was greater (~5μg/L) than that found in hospital effluents (0.57μg/L). Individual day concentration spikes were observed in the long-term-care home effluents especially for antibiotic compounds; and were attributed to the disposal of un-needed or expired compounds. The increased amounts of un-wanted pharmaceutical compounds in these facilities may occur because the prescriptions for the elderly often change due to the uncertainties associated in diagnostics with age.

All the target PhACs were detected in all samples except for the metabolite of Venlafaxine, N-desmethylvenlafaxine, which was not detected in the Wednesday and Thursday samples from the $LTC_{2.}$ Therefore, the frequency of the target compounds detection in the investigated healthcare facility effluents was almost 100%.

The maximum detected concentrations of the target PhACs are presented in Table 4-13. The maximum concentrations for all the target PhACs in the healthcare facility effluents exceeded 1200 ng/L, with the exception of Carbamazepine and the metabolites of Venlafaxine. Up to 36 μg/L of Venlafaxine was detected in the cancer clinic effluent and 134μg/L of Acetaminophen was measured. The concentrations of the antibiotic compounds Sulfamethoxazole, Trimethoprim and Ciprofloxacin were 10.93 μg/L, 10.32 μg/L and 1.24 μg/L respectively. The results (Table 4-13) support the hypothesis that hospital wastewaters contain elevated concentrations of pharmaceutical compounds.

Table 4-13: Maximum detected concentrations of the target PhACs

Target Compounds	Hospitals (ng/L)		Long-term-Care homes (ng/L)		Maximum Concentrations (μg/L)	
	HS_1	HS_2	LTC_1	LTC_2	Hospitals	Long term care homes
Sulfamethoxazole	3609	10933	2292	716	10.9	2.29
Trimethoprim	550	10320	6573	1924	10.3	6.57
Ciprofloxacin	1240	168	604	1470	1.24	1.47
Acetaminophen	134133	15973	116266	88800	134	116
Carbamazepine	143	676	526	76	0.67	0.52
Metoprolol	579.2	337	4920	320	0.57	4.92
Venlafaxine	744	8893	2275	892	8.89	2.27
N-desmethylvenlafaxine	416	238	265	118	0.45	0.26
O-desmethylvenlafaxine	2880	2535	6986	2124	6.44	6.98

4.2 Comparison of Day- to-day Variability in Concentrations in Healthcare Facility Effluents

Considerable day-to-day variability was observed for all compounds in the investigated facility effluents, and may have been due to either variations in the wastewater flow or differences in the consumption of PhACs during each day. Higher flows would reduce concentrations and vice versa, provided that compound consumption remains the same. If the variability was only from the fluctuations in a facility's wastewater flows, then it should affect all target PhACs similarly (similar CV values). This was not the case; the different CV values (Table 4-13) for each compound suggest that the observed variability was due to variations in both consumption and wastewater flows.

It is important to note that the consumption of the pharmaceutical compounds within the healthcare facility is not directly related to the concentration of these compounds in its effluent; it is strictly the excretion of these compounds within the facility after administration. Therefore an important consideration in understanding the effects of consumption on the compound's variability in the facility effluent is the consumption and excretion relationship of pharmaceutical compounds. The excretion pattern of these compounds depends on various factors. First, it varies between individual patients depending upon age, sex health condition etc, suggesting the possibility of detecting different concentrations over time even when the amounts consumed remain the same. Second, the administered compounds need a certain time for excretion (elimination half-life) (Jambhekar et al., 2009); therefore, concentrations will be affected by the length of patient stays. Third, the excretion rates depend on the route of administration (Khan et al., 2004; Sweetman et al., 2007); similar amounts administered through different routes of administration lead to different excretion patterns. Fourth, excretion patterns also depend on the therapeutic dose. For single dose therapy, half of the administered dose is expected to be excreted during one half-life period, and then a quarter during the next half-life; thus discharge of a compound varies over time. Only in continuous therapeutic regimens will a steady state be attained, where consumption and excretion may be directly related in attempts to maintain a certain amount of compound in the human body (Rowland et al., 1995).

As previously mentioned, the coefficient of variation was used as an indicator of day-to-day variability of the target compounds in the investigated facility effluents. Statistically, the coefficient of variation of two samples is considered significantly different if the absolute difference between them is more than the critical value at the considered significance level multiplied by the standard error of the coefficient of variations (Pal, 1998; Thomas et al., 2007). For example, if v_1 and v_2 are the coefficients of variation of two samples with sample size n_1 and n_2, respectively, their standard error will be calculated using equation 4.1.

$$\sigma(v_1 - v_2) = \sqrt{\frac{v_1^2}{2n_1} + \frac{v_2^2}{2n_2}} \qquad ------(4.1)$$

If the significance level is α, then the CV of the two samples will be significantly different if

$$\text{Absolute difference in CVs} > Z_\alpha * \sqrt{\frac{v_1^2}{2n_1} + \frac{v_2^2}{2n_2}} \qquad ------(4.2)$$

Figure 4-14 shows the CV values for the target compound concentrations over the week days in the investigated healthcare facility effluents. The CV values are taken from Tables 4-3 to 4-11. For the purpose of this report, the day-to-day variability of target compound concentrations were assumed to be significantly different only if

$$\text{Absolute difference in CVs} > t_{0.05, n_1+n_2-2} * \sqrt{\frac{v_1^2}{2n_1} + \frac{v_2^2}{2n_2}} \qquad ------(4.3)$$

A comparison between the investigated hospitals (HS_1 and HS_2), and between the two long-term-care homes for the CV values for all compounds, showed no significant differences in the CV for all compounds either between the hospital effluents, or between long-term-care homes (details are attached in Appendix C).

Table 4-14 Coefficients of Variation of target PhACs in the investigated healthcare facility effluents.

Target Compounds	CV			
	HS_1	HS_2	LTC_1	LTC_2
Sulfamethoxazole	0.38	0.34	**1.43**	0.60
Trimethoprim	0.26	0.35	1.40	1.03
Ciprofloxacin	0.42	**0.17**	0.54	**1.96**
Acetaminophen	0.27	0.16	**0.18**	**0.08**
Carbamazepine	**0.69**	**0.76**	0.60	0.45
Metoprolol	0.22	0.37	0.92	0.29
Venlafaxine	**0.12**	0.89	1.24	1.04
N-desmethylvenlafaxine	0.27	0.40	0.62	1.11
O-desmethylvenlafaxine	0.50	0.56	1.08	0.68

Considerable day-to-day variability in concentration (CV >10%) was observed for all compounds in the hospital effluents. Relatively higher variability in the concentrations of Sulfamethoxazole, Trimethoprim and Venlafaxine of these compounds in HS_1 effluent (CV values) than HS_2 may occur because smaller facilities (HS_2) may be more affected by individual events than larger facilities. In the HS_1 effluent, Venlafaxine had the lowest day-to-day variability; the maximum CV was observed for Carbamazepine concentrations. In the HS_2 effluent, the least variability was observed in Ciprofloxacin concentration, and the highest variability in Carbamazepine concentration.

The least day-to-day variability about the mean in the LTC effluents was observed for Acetaminophen, (CV = 0.08 in LTC_2 and CV = 0.18 in LTC_1), indicating that Acetaminophen is used in long-term-homes more regularly than other compounds. The higher variability in other compounds suggests that their use is less frequent.

To investigate further, the relation between the type of healthcare facility and the compound's variability in its effluent, the maximum and minimum CV values for the target compounds and their corresponding facilities are compared (Table 4-15). This table shows higher day-to-day variability in

Acetaminophen and Carbamazepine concentrations (rows with blue background in Table 4-15) occurred in hospital effluents. For all other target compounds, hospital effluents had lower variability than did long-term-care homes. The minimum variability in Acetaminophen concentration (CV= 0.08) in LTC_2 effluents suggest that this compound is most often used in the long-term-care homes by a certain number of long-term care home residents.

The relatively lower variability for most of the compounds in hospital effluents may be explained by the fact that hospitals have a number of beds designated for each type of treatment, and are continuously filled by a series of new patients; overall drug consumption thus stays the same, as does the discharge of these compounds to the wastewater. This is not the case in long-term-care homes, where very few beds are actually designated for rehabilitation, and most of the beds are occupied by the long-term residents. Further, the higher variability in long-term-care homes was mainly due to the individual peak concentrations of the target compounds in their effluents.

Table 4-15 Maximum and minimum CV values in the investigated healthcare facility effluent

Target Compounds	Maximum		Minimum	
	CV	Facility	CV	Facility
Sulfamethoxazole	1.43	LTC_1	0.34	HS_2
Trimethoprim	1.4	LTC_1	0.26	HS_1
Ciprofloxacin	1.96	LTC_2	0.17	HS_2
Acetaminophen	0.27	HS_1	0.08	LTC_2
Carbamazepine	0.76	HS_2	0.45	LTC_2
Metoprolol	0.92	LTC_1	0.22	HS_1
Venlafaxine	1.24	LTC_1	0.12	HS_1
N-desmethylvenlafaxine	1.11	LTC_2	0.27	HS_1
O-desmethylvenlafaxine	1.08	LTC_1	0.50	HS_1

4.3 Comparison between Healthcare Facility Effluents for the Occurrence of Target PhACs

Differences in the target compound concentrations between the hospital effluents (HS_1 and HS_2) were observed. Similarly, such differences were found between the two long-term-care home effluents. To further investigate whether these differences were statistically significant, ANOVA tests were carried out. The results are summarized as follows.

4.3.1 Comparison between HS_1 and HS_2 Effluents

A comparison of the HS_1 and HS_2 facility effluents showed that significant differences existed between these streams in the concentrations of Sulfamethoxazole, Trimethoprim, Ciprofloxacin, Acetaminophen, and Metoprolol (P-values were 0.01, 0.002, 0.02, 0.003, and 0.007 respectively). No significant differences were found between the two hospital's effluent for the concentrations of Carbamazepine, Venlafaxine and its metabolites, i.e., N-desmethylvenlafaxine and O-desmethylvenlafaxine (P-values were 0.08, 0.1, 0.08, and 0.8 respectively.

These findings suggest that the effluents of different sized hospitals, (number of beds) may contain similar concentrations of certain compounds, and also that the concentration of certain other compounds may differ. Therefore information relating to number of beds only, for a hospital, may not be useful when estimating the concentration of PhACs in effluents.

4.3.2 Comparison between LTC_1 and LTC_2 Effluents

Comparing LTC_1 and LTC_2 facility effluents indicated that these effluents did not significantly differ in terms of target compounds concentrations with the exception of carbamazepine (P-value = 0.02). This finding may be due to the similar services provided by the long-term-care facilities and partly their relatively homogenous population (elderly people) who would be expected to consume similar types of drugs. The differences in Carbamazepine concentrations may occur because this compound is used in specific health conditions.

4.4 Occurrence of Target PhACs in WWTP Influents

The influents of the downstream WWTPs that received discharges from the investigated healthcare facilities (two hospitals and two long-term-care homes) were sampled to facilitate mass balance calculations. Twenty-four hour composite samples were collected at the influents of the four WWTPs during the same week days as that of the respective upstream healthcare facility effluents.

The healthcare facilities were hypothesized to be major contributors of pharmaceutical compounds to the WWTPs. To investigate this hypothesis, the WWTPs were identified based on the respective upstream healthcare facilities, WWTP-HS_1, WWTP-HS_2, WWTP-LTC_1 and WWTP-LTC_2. The concentrations in the wastewater treatment facility influents were evaluated considering the contributions of upstream healthcare facilities.

In addition to the type and size of the upstream healthcare facilities, the investigated WWTPs were different in terms of the community size they serve and the sampling time. The WWTP sizes according to the population served from largest to smallest facility, were WWTP-HS_2 (171000), WWTP-LTC_1 (80000), WWTP-HS_1 (51218), and WWTP-LTC_2 (33000). WWTP-HS_1 was sampled in mid July, WWTP-HS_2 in early November, WWTP-LTC_1in mid February, and WWTP-LTC_2 in the first week of March.

The concentrations of each compound measured in the WWTP influents are presented in Figures 4-15 to 4-24. This presentation allowed comparison of the compound concentrations between WWTPs, and identification of any common patterns over the week days. Further, the day-to-day variability in target compound concentrations in the WWTP influents was investigated and individual concentration peaks were identified. The variability about the mean of the target compounds in the influents is presented in Tables 4-15 to 4-23. The tables are organized with the highest to lowest coefficient of variation values ranked from top to bottom.

4.4.1 Sulfamethoxazole

Sulfamethoxazole concentrations detected in the WWTP influents are shown in Figure 4-14. This compound was not tested for the Monday sample from WWTP-HS_1; therefore, no results are available for this day. The weekly maximum concentrations measured in WWTP-HS_1, WWTP-HS_2, WWTP-LTC_1, and WWTP-LTC_2 influents were 605, 548, 461, and 540 ng/L respectively.

No common patterns of Sulfamethoxazole concentration among the WWTPs were observed. WWTP-HS_1 and WWTP-HS_2 both had their maximum concentrations at the beginning of the week, then a decrease over the next days, with a minimum concentration either on Wednesday (WWTP-HS_2) or on Thursday (WWTP-HS_1), followed by an increase. In WWTP-LTC_1 influent, the Sulfamethoxazole concentration was relatively constant between 378 ng/L to 461 ng/L. In WWTP-LTC_2 the maximum concentration was on Tuesday, and then concentrations decreased over the next weekdays, with a minimum concentration on Friday (Figure 4-14).

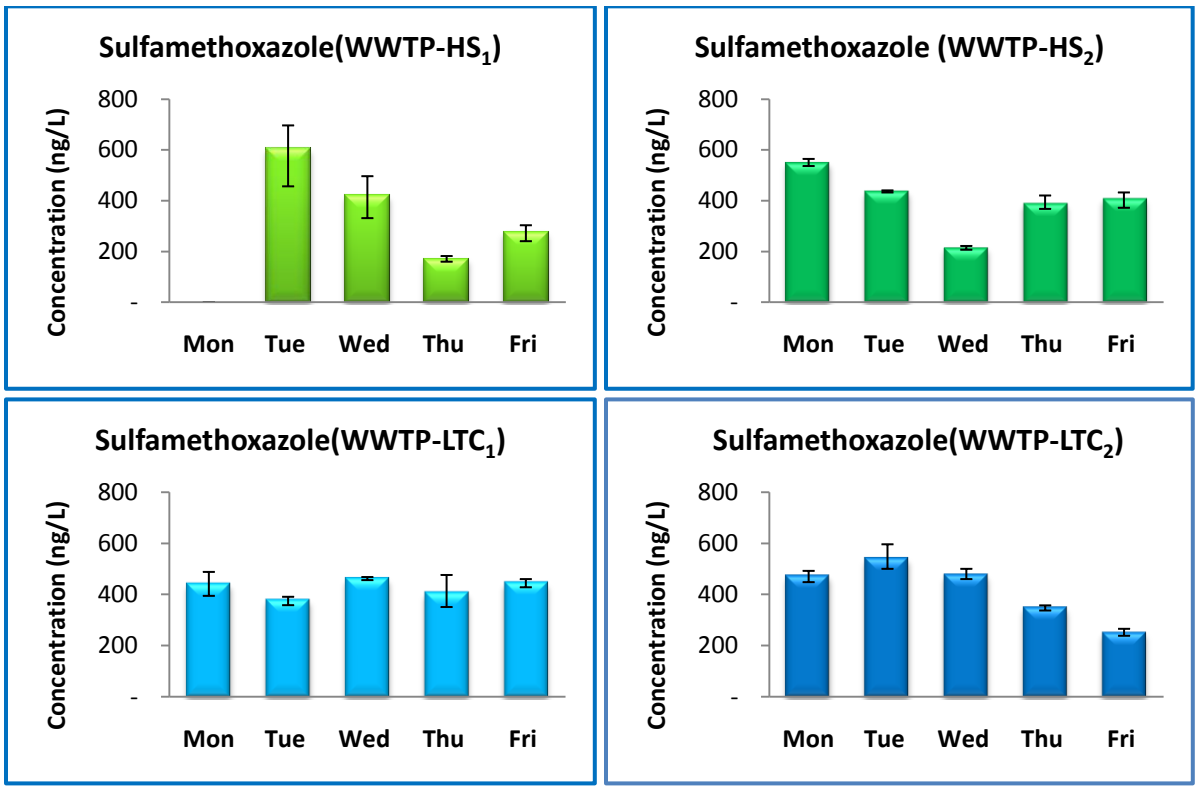


Figure 4-14 Concentration of Sulfamethoxazole in the WWTP influents

Relatively higher variability in Sulfamethoxazole concentration was observed (Table 4-16) in the WWTPs that received hospital discharges WWTP-HS_1 (CV = 0.51) and WWP-HS_2 (CV= 0.33). WWTP-LTC_1 had the least variability (CV<0.1). This wastewater treatment facility also has a hospital upstream, but the size of the hospital was relatively small (68 beds) compared to the community size contributing the PhAC loads to this facility (80000 pop). Therefore no noticeable effect of the hospital was expected. The variability in WWTP-LTC_2 (CV= 0.29) was presumably due to its relatively smaller size (30000 population) as smaller size facilities are more affected by individual events.

Table 4-16 Variability in Sulfamethoxazole concentration about the mean in WWTP influents

Facility	Concentrations (ng/L)					Mean	Sd	CV
	Mon	Tue	Wed	Thu	Fri			
WWTP-HS_1		**605**	420	171	277	368	188.1	0.51
WWTP-HS_2	**548**	436	216	389	408	362	119.6	0.33
WWTP-LTC_2	472	**540**	476	349	252	404	115.4	0.29
WWTP-LTC_1	441	378	**461**	407	445	423	33.56	0.08

Sd = Standard deviation
Bold numbers show the maximum concentrations measured

4.4.2 Trimethoprim

The detected Trimethoprim concentrations in the WWTP influents (Figure 4-15) were in the range of WWTP-HS_1 (153 to 412 ng/L), WWTP-HS_2 (217 to 316 ng/L), WWTP-LTC_1 (226 to 353 ng/L), and WWTP-LTC_2 (100 to 244 ng/L). Relatively lower Trimethoprim concentrations were observed in the WWTP-LTC_2 that served a smaller community (Figure 4-15).

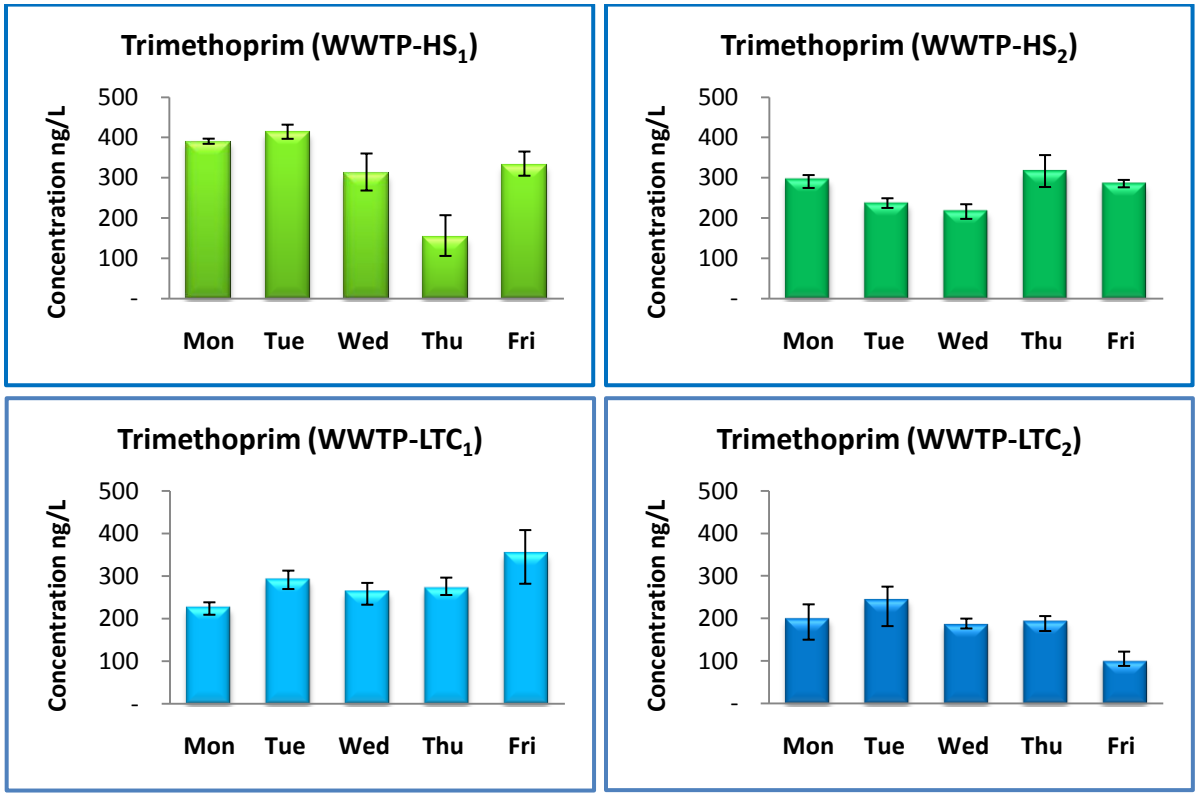


Figure 4-15 Concentration of Trimethoprim in the influents of WWTPs

The highest variability in Trimethoprim concentration about the mean was in WWTP-HS_1 (CV= 0.32), then in WWTP-LTC_2 (CV= 0.28). The higher variability in these facility influents may have been due to the fact that WWTP-HS_1 is relatively smaller in size (51,218 inhabitants) and has a relatively bigger hospital (365 beds) upstream; while WWTP-LTC_2 was the smallest WWTP (30000 inhabitants) investigated and had a long-term-care home (200 beds) upstream. Smaller facilities are usually more affected by individual events. The least variability occurred in WWTP-HS_2, which had a relatively smaller hospital upstream (263 beds) and a large community (171000 inhabitants). Again, the low variability in WWTP-LTC_1influent was perhaps due to its size (80000 inhabitants), as bigger size facilities are expected to be less affected by individual events. These findings suggest that the variability in WWTP influent concentrations is affected by the size of the treatment facility and also the size of any hospitals upstream.

Table 4-17 Variability in Trimethoprim concentration about the mean in WWTP influents

Facility	Concentrations (ng/L)					Mean	Sd	CV
	Mon	Tue	Wed	Thu	Fri			
WWTP-HS$_1$	389	412	309	153	330	319	102	0.32
WWTP-LTC$_2$	200	244	186	194	100	185	52	0.28
WWTP-LTC$_1$	226	292	264	271	353	281	47	0.17
WWTP-HS$_2$	296	237	217	316	285	270	41	0.15

Sd = Standard deviation
Bold numbers show the maximum concentrations measured

Sulfamethoxazole and Trimethoprim are often prescribed together (Sulfamethoxazole: Trimethoprim 5:1), and their elimination half-lives are within the same range (6-12 hrs and 8-10 hrs, respectively)(Sweetman et al., 2007). Therefore, similar trends at the WWTP influent level were expected. Such trends were more obvious in WWTP-HS$_1$, WWTP-HS$_2$ and WWTP-LTC$_2$ (Figures 4-17). In the WWTP-HS$_1$ influent, the concentration of both compounds decreased from Tuesday to Thursday then increased again on Friday. Similarly, in WWTP-HS$_2$ the concentration of both compounds was at a maximum on Monday then decreased over the next two days, and was at a minimum on Wednesday. In WWTP-LTC$_2$ the concentrations of the two compounds slightly increased from Monday to Tuesday then decreased over the next weekdays and reached a minimum concentration on Friday.

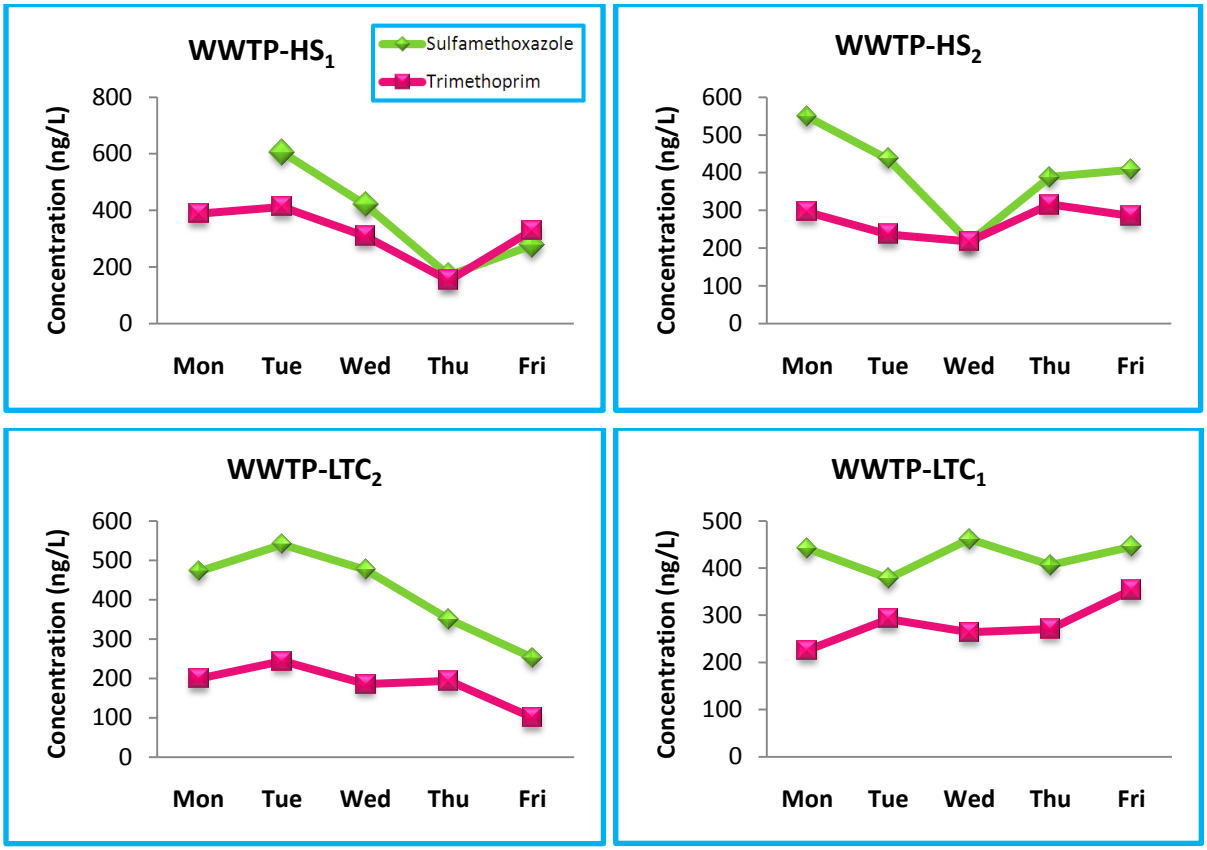


Figure 4-16 Trends of Sulfamethoxazole and Trimethoprim concentrations in WWTP influents

4.4.3 Ciprofloxacin

The weekly maximum Ciprofloxacin concentrations detected in the WWTP influents (Table 4-17) were WWTP-HS_1 (105 ng/L), WWTP-HS_2 (130 ng/L), WWTP-LTC_1 (80 ng/L), and WWTP-LTC_2 (151 ng/L). No common patterns of Ciprofloxacin concentration were observed in the WWTP influents. The maximum Ciprofloxacin concentrations were detected on Thursdays in all the WWTPs except WWTP-LTC_2, where the maximum concentration was found on Tuesday (Figure 4-17). In WWTP-LTC_2, the Ciprofloxacin concentration pattern was similar to those of Sulfamethoxazole and Trimethoprim, with an increase from Monday to Tuesday then a decrease over next the few weekdays. This finding may suggest similar use patterns of these compounds in catchment area of this WWTP-LTC_2.

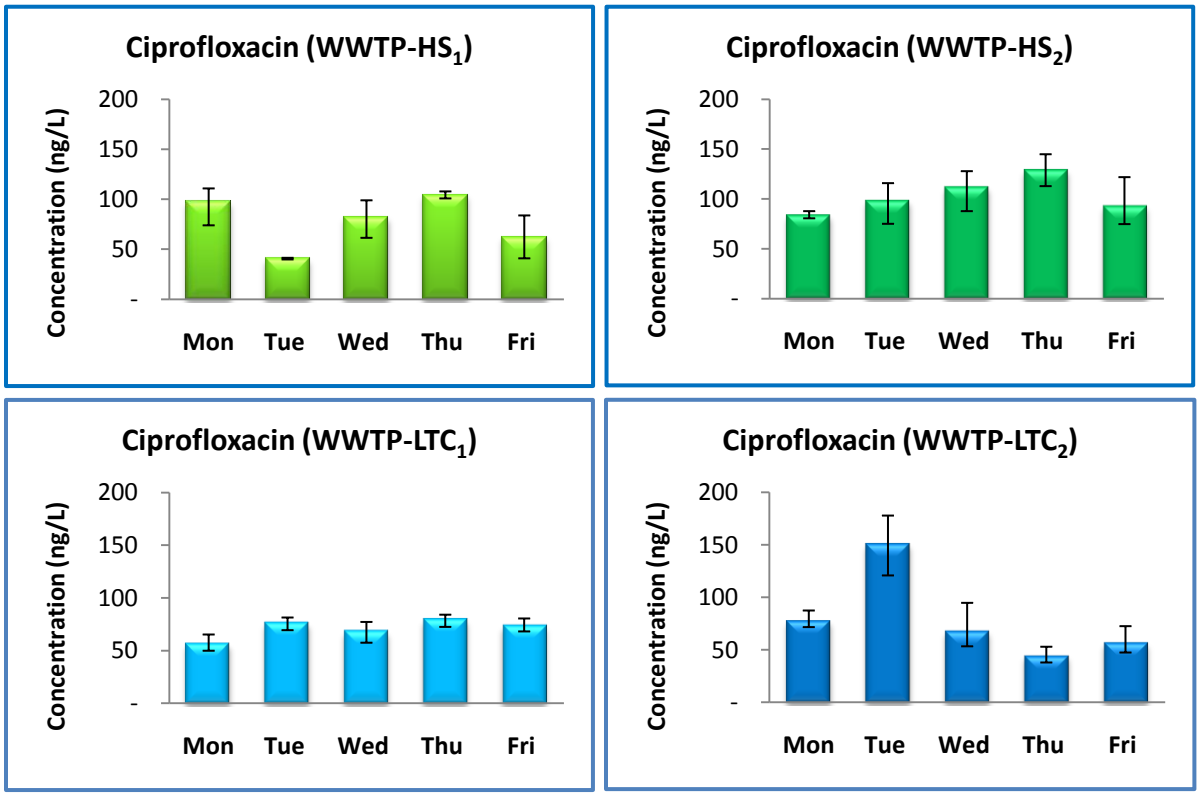


Figure 4-17 Concentrations of Ciprofloxacin in the WWTP influents

The highest variability in the concentration about the mean was observed (Table 4-18) in WWTP-LTC_2 (CV = 0.52), due to the individual peak concentration on Tuesday, then, in WWTP-HS_1, with (CV=0.34), which perhaps occurs because (as mentioned earlier in this section) of the relatively bigger hospital upstream to this WWTP in relation to the community it serves. Lower variability was observed in WWTP-HS_2, which had a relatively smaller hospital upstream and serves a larger community. The least variability was observed in WWTP-LTC_1 which serves 80000 people and receives discharge from a small hospital (68 beds).

Table 4-18 Variability in Ciprofloxacin concentration about the mean in WWTP influents

Facility	Concentrations (ng/L)					Mean	Sd	CV
	Mon	Tue	Wed	Thu	Fri			
WWTP-LTC$_2$	78	151	68	45	57	80	41	0.52
WWTP-HS$_1$	97	41	82	105	63	77	26	0.34
WWTP-HS$_2$	84	91	112	130	103	104	18	0.17
WWTP-LTC$_1$	57	76	69	80	74	71	9	0.13

Sd = Standard deviation
Bold numbers show the maximum concentrations measured

4.4.4 Acetaminophen

Acetaminophen was detected in higher concentrations than all other target compounds in the investigated WWTP influents. The detected influent concentrations varied from 40-83 μg/L for WWTP-HS$_1$, 39.5-47.5 μg/L for WWTP-HS$_2$, 64-70 μg/L for WWTP-LTC$_1$, and 42-68 μg/L for WWTP-LTC$_2$. The average Acetaminophen concentration was lower (43 μg/L) in the biggest WWTP (WWTP-HS$_2$). All other WWTP influent concentrations were in the same range 61 to 67 μg/L (Figure 4-18).

In WWTP-HS$_1$, the Acetaminophen concentration was found to be highest on Monday (83 μg/L) then decreased over the next three days, with a minimum value on Thursday (51 μg/L). The concentrations then started rising again on Friday. In contrast, in WWTP-LTC$_1$ the lowest values occurred on Monday (64 μg/L), and then had an increasing trend over the next week days, with a maximum concentration on Friday (70 μg/L). In WWTP-HS$_2$, the concentration was relatively stable between 39 μg/L and 47 μg/L over the week days. In WWTP-LTC$_2$, the concentrations were stable (between 63 μg/L to 68 μg/L from Monday to Thursday) and then dropped on Friday (42 μg/L). In the WWTP-HS$_1$influent, Sulfamethoxazole and Acetaminophen concentrations had quite similar patterns, which may suggest that the variability of these compounds was contributed by the same sources (Table 4-18).

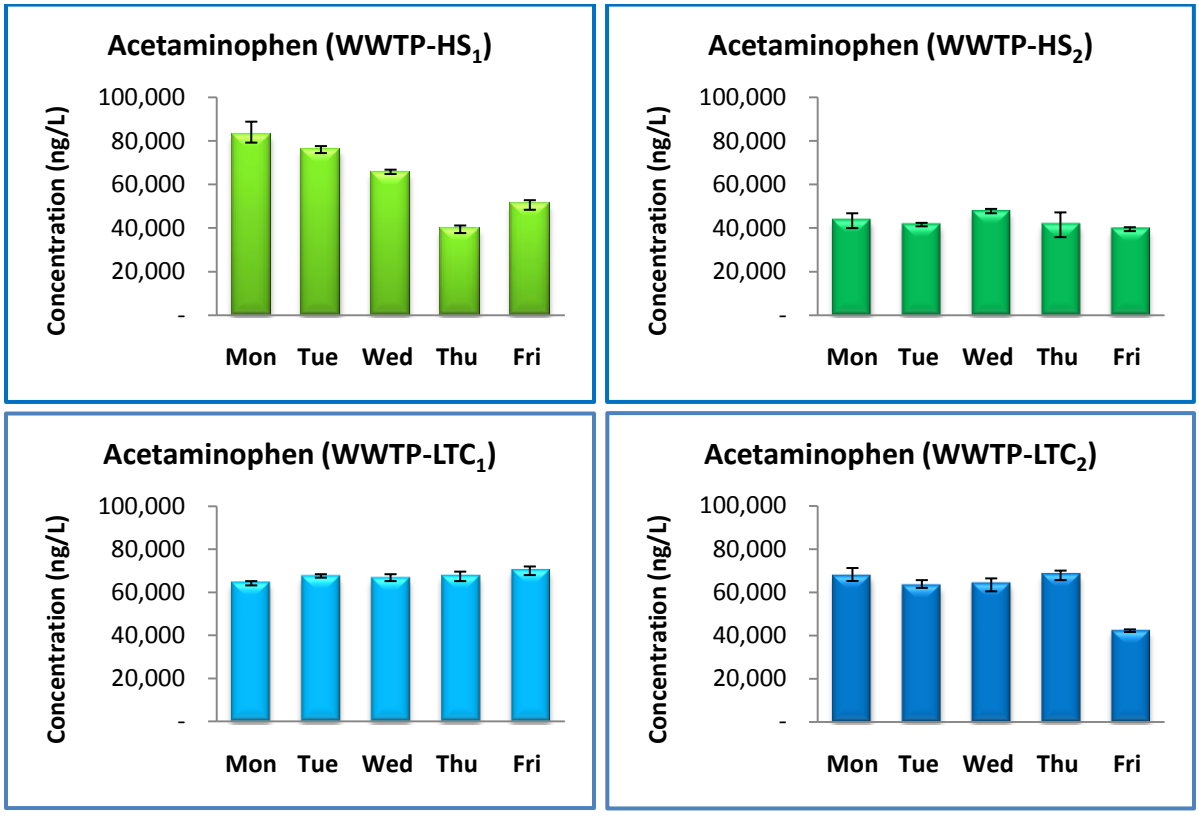


Figure 4-18 Concentrations of Acetaminophen in the WWTP influents

The variability in the Acetaminophen was highest (CV=0.28) in WWTP-HS_1, and was probably caused by the larger hospital upstream (Table 4-19), than in WWTP-LTC_2 (CV=0.18), the smallest treatment facility. WWTP-HS_2 and WWTP-LTC_1 influents had low variability (CV< 0.1%), with the least value for WWTP-LTC_1 (CV = 0.03), perhaps because Acetaminophen is often used in communities, so a consistent discharge of this compound is expected to their treatment plants. WWTP-HS_2 and WWTP-LTC_1 are bigger facilities than the other two, and WWTP-LTC_1 had only a small hospital upstream (68 beds).

Table 4-19 Variability in Acetaminophen concentration about the mean in WWTP influents

Facility	Concentrations (ng/L)					Mean	Sd	CV
	Mon	Tue	Wed	Thu	Fri			
WWTP-HS$_1$	**83067**	75867	65600	39907	51200	63128	17660	0.28
WWTP-LTC$_2$	67600	63200	64133	**68400**	42267	61120	10769	0.18
WWTP-HS$_2$	43600	41600	**47600**	41680	39573	42811	3033	0.07
WWTP-LTC$_1$	64400	67333	66533	67600	**70267**	67227	2113	0.03

Sd = Standard deviation
Bold numbers show the maximum concentrations measured

4.4.5 Carbamazepine

The weekly maximum concentrations in the WWTP influents (Figure 4-19) were WWTP-HS$_1$ (897 ng/L), WWTP-HS$_2$ (719 ng/L), WWTP-LTC$_1$ (184 ng/L), and WWTP-LTC$_2$ (104 ng/L). No common patterns in the Carbamazepine concentrations were observed over the week days. WWTP-HS$_1$ and WWTP-HS$_2$ had individual peak concentrations on Wednesday (897 ng/L) and Thursday (719 ng/L) respectively (Figure 4-19). The concentrations over the other weekdays in these WWTP influents varied between 151-269 ng/L in WWTP-HS$_1$ and 191-255 ng/L in WWTP-HS$_2$. The Carbamazepine concentrations in the WWTP-LTC$_1$ influent were relatively stable over the weekdays, between 158-184 ng/L. WWTP-LTC$_2$ had a higher concentration on Tuesday (104 ng/L), and the concentration during the rest of the week days varied between 69-81 ng/L.

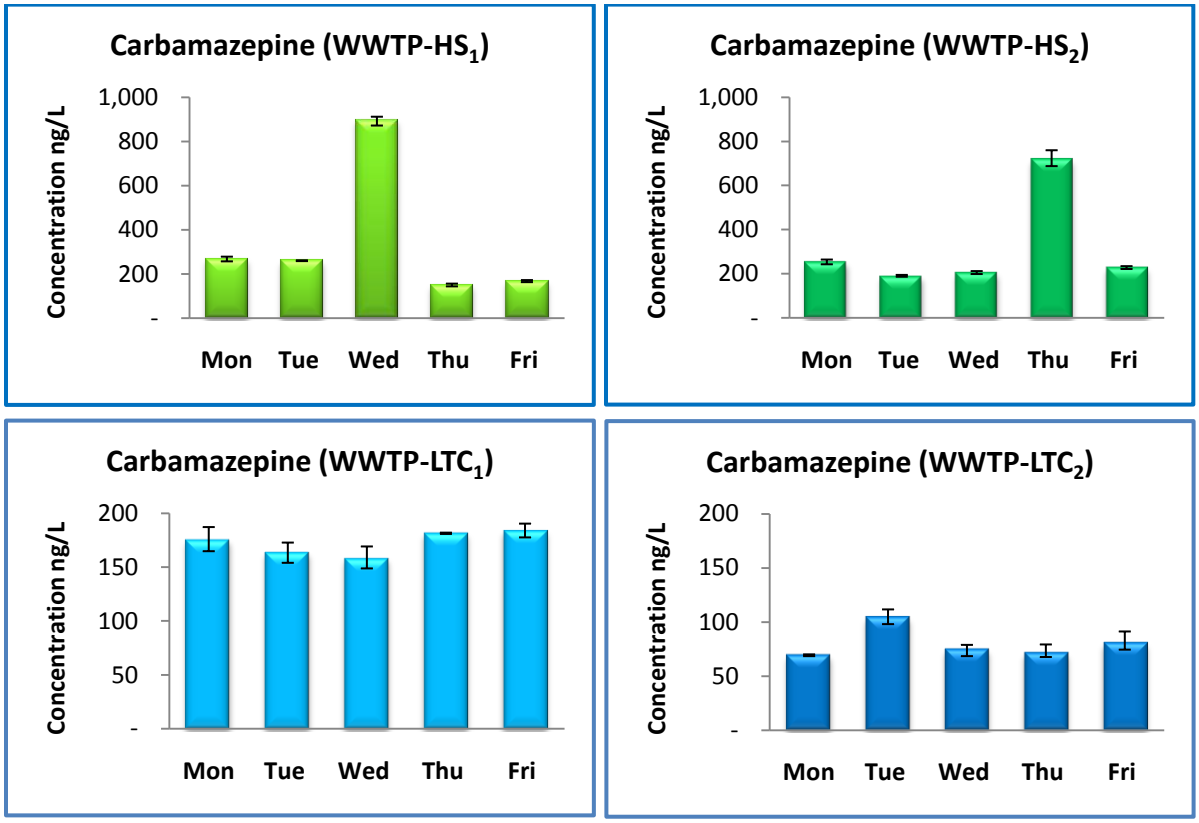


Figure 4-19 Concentration of Carbamazepine in the WWTP influents

Considerable variability in Carbamazepine concentrations in the WWTP influents was observed (Table 4-20) except for WWTP-LTC_1 (CV< 0.10). The higher variability in the WWTP-HS_1 and WWTP-HS_2 influents was due to the individual day peak concentrations (bold values Table 4-20). WWTPs that received hospital discharges (WWTP-HS_1, WWTP-HS_2) had an individual peak concentration (Wednesday and Thursday respectively in Table 4-20). These individual peaks may have been due to the disposal of un-wanted compounds.

Table 4-20 Variability in Carbamazepine concentration about the mean in WWTP influents

Facility	Concentrations (ng/L)					Mean	Sd	CV
	Mon	Tue	Wed	Thu	Fri			
WWTP-HS$_1$	269	261	**897**	151	168	349	311	0.89
WWTP-HS$_2$	255	191	207	**719**	230	321	224	0.70
WWTP-LTC$_2$	69	**104**	75	72	81	80	14	0.18
WWTP-LTC$_1$	175	164	158	181	**184**	172	11	0.06

Sd = Standard deviation
Bold numbers show the maximum concentrations measured

4.4.6 Metoprolol

The weekly maximum Metoprolol concentrations in WWTP-HS$_1$, WWTP-HS$_2$, WWTP-LTC$_1$, and WWTP-LTC$_2$ were 207, 104, 261, and 282 ng/L respectively. WWTP-HS$_2$ influent had relatively lower Metoprolol concentrations than other WWTPs investigated (Figure 4-20). No common patterns in the Metoprolol concentrations were found in the WWTP influents. WWTP-HS$_1$ and WWTP-HS$_2$ had maximum concentrations on Wednesday (Figure 4-20). The Metoprolol concentrations varied between 200-261 ng/L in WWTP-LTC$_1$ influent, with the highest concentration detected on Friday. WWTP-LTC$_2$ had its highest concentration on Monday and lowest concentration on Friday (135 ng/L).

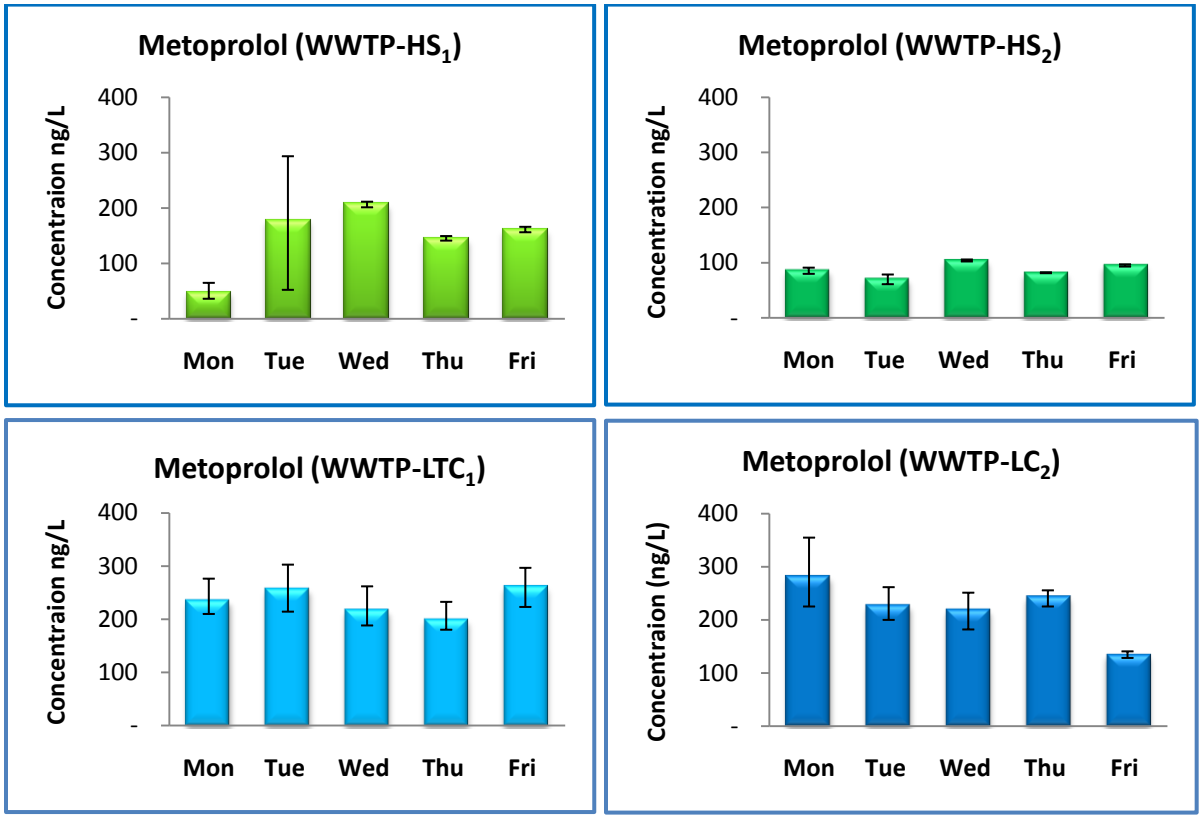


Figure 4-20 Concentrations of Metoprolol in the WWTP influents

Considerable variability (CV >0.1) in Metoprolol concentration was observed in all WWTP influents, with the highest variability in WWTP-HS_1 (CV= 0.4), and the least variability in WWTP-LTC_1 (CV=0.11).

Table 4-21 Variability in Metoprolol concentration about the mean in WWTP influents

Facility	Concentrations (ng/L)					Mean	Sd	CV
	Mon	Tue	Wed	Thu	Fri			
WWTP-HS_1	49	179	**207**	145	161	148	60	0.41
WWTP-LTC_2	**282**	227	219	244	135	221	54	0.24
WWTP-HS_2	86	71	**104**	82	95	88	13	0.14
WWTP-LTC_1	235	257	217	199	**261**	234	26	0.11

Sd = Standard deviation
Bold numbers show the maximum concentrations measured

4.4.7 Venlafaxine

Relatively higher concentrations of Venlafaxine were observed in the WWTP-HS_2 influent than in the other investigated WWTPs, with a weekly average of 494 ng/L (Figure 4-21). The weekly maximum concentrations measured in the WWTP influents were WWTP-HS_1 (632 ng/L), WWTP-HS_2 (521 ng/L), WWTP-LTC_1 (513 ng/L), and WWTP-LTC_2 (456 ng/L).

In WWTP-HS_1 and WWTP-HS_2 higher concentrations were detected on Monday (632 ng/L and 526 ng/L respectively); then, the concentrations decreased in WWTP-HS_1 over the next three days, with a minimum value on Thursday (259 ng/L), while in WWTP-HS_2, concentrations remained relatively stable over the other week days (between 467-509 ng/L Figure 4-21). The WWTP-LTC_1 influent concentrations varied between 394 to 513 ng/L. In WWTP-LTC_2, the influent Venlafaxine concentration varied between 427-456 ng/L from Monday to Thursday then dropped on Friday to 308 ng/L. The concentration patterns of Venlafaxine in all the investigated WWTP influents were similar to that of Acetaminophen, suggesting similar use patterns to Acetaminophen (Figure 4-18 and 4-21).

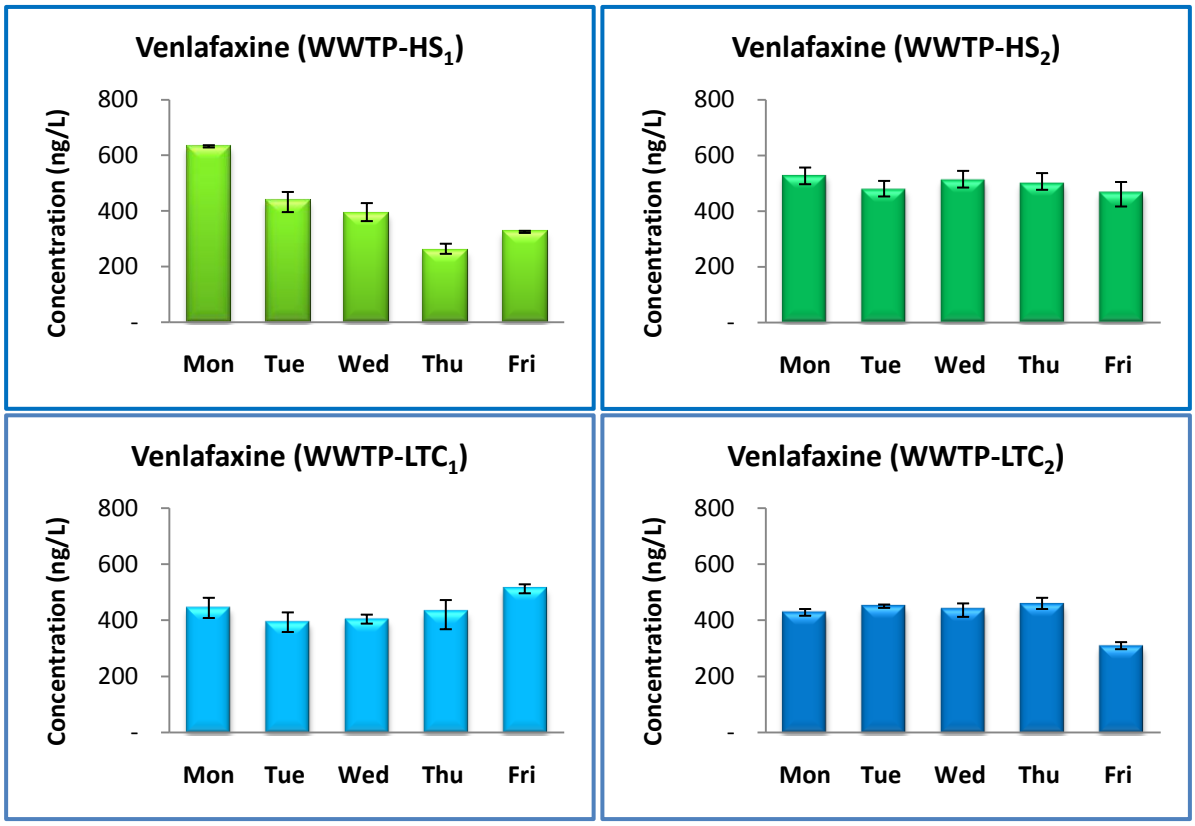


Figure 4-21: Concentrations of Venlafaxine in the WWTP influents

The variability in the Venlafaxine concentration in the order from the highest to lowest was WWTP-HS_1 (CV= 0.35), WWTP-LTC_2 (CV= 0.15) and WWTP-LTC_1 (CV=0.11). The least variability was found in WWTP-HS_2, with CV= 0.05 (Table 4-22).

Table 4-22 Variability in Venlafaxine concentration about the mean in WWTP influents

Facility	Concentrations (ng/L)					Mean	Sd	CV
	Mon	Tue	Wed	Thu	Fri			
WWTP-HS_1	**632**	439	389	259	325	409	142	0.35
WWTP-LTC_2	427	449	439	**456**	308	416	61	0.15
WWTP-LTC_1	444	394	402	432	**513**	437	47	0.11
WWTP-HS_2	**521**	476	509	499	467	494	23	0.05

Sd = Standard deviation
Bold numbers show the maximum concentrations measured

4.4.8 N-desmethylvenlafaxine

The WWTP influent concentrations of N-desmethylvenlafaxine are presented in Figure 4-23. The weekly maximum concentrations in the influents of WWTP-HS_1, WWTP-HS_2, WWTP-LTC_1, and WWTP-LTC_2 were 248, 178, 190, and 177 ng/L respectively.

Similar concentration patterns were observed in WWTP-HS_1, WWTP-HS_2, and WWTP-LTC_1, with a maximum concentration on Monday and minimum detected concentrations on Thursday (Figure 4-22). In WWTP-LTC_2, the concentration was relatively stable from Monday to Wednesday (between 143-145 ng/L), then was higher on Thursday (177 ng/L), before dropping on Friday (103 ng/L).

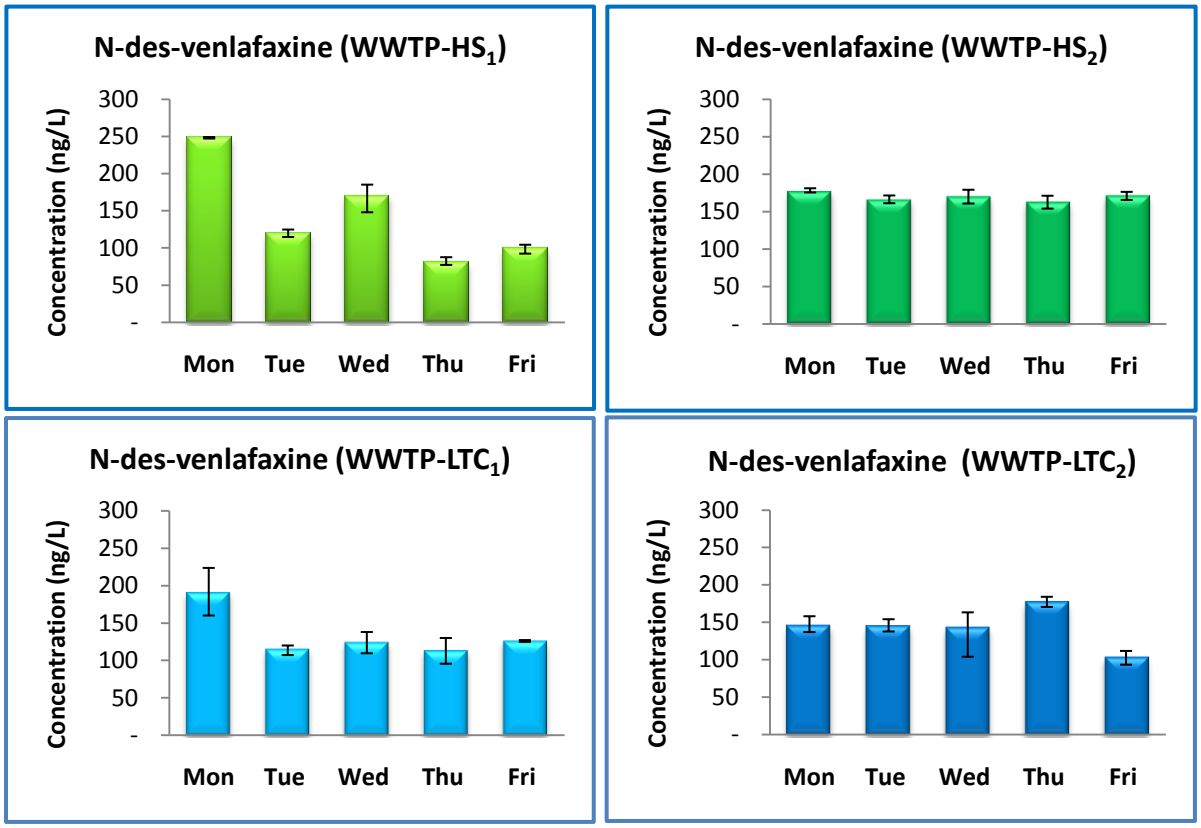


Figure 4-22 Concentration of N-desmethylvenlafaxine in the WWTP influents

Considerable variability in N-desmethylvenlafaxine concentrations existed in the WWTP influents (Table 4-23) except WWTP-HS_2 (CV = 0.03). Similar to Venlafaxine, the highest variability was observed in the WWTP-HS_1 influent (CV = 0.47).

Table 4-23 Variability in N-desmethylvenlafaxine concentration about the mean in WWTP influents

Facility	Concentrations (ng/L)					Mean	Sd	CV
	Mon	Tue	Wed	Thu	Fri			
WWTP-HS_1	248	120	170	81	99	144	67	0.47
WWTP-LTC_1	190	115	124	114	126	134	32	0.24
WWTP-LTC_2	145	145	143	177	103	143	26	0.18
WWTP-HS_2	178	165	170	162	171	169	6	0.03

Sd = Standard deviation
Bold numbers show the maximum concentrations measured

4.4.9 O-desmethylvenlafaxine

The weekly maximum concentrations detected in the WWTP influents (Figure 4- 23) were WWTP-HS_1 (6580 ng/L), WWTP-HS_2 (1623 ng/L), WWTP-LTC_1 (5493 ng/L), and WWTP-LTC_2 (1390 ng/L). Maximum O-desmethylvenlafaxine concentrations occurred on Mondays for all WWTPs except for WWTP-LTC_2. Monday concentrations in WWTP-HS_1 and WWTP-LTC_1 influents were considerably higher (6.5 and 5.5 µg/L respectively) than those for rest of the week days; during which influent concentrations varied from 0.6 to 1.8 µg/L in WWTP-HS_1 and from 2.3 to 3.2 µg/L in WWTP-LTC_1. WWTP-LTC_2 had its maximum concentration on Wednesday (1.4 µg/L).

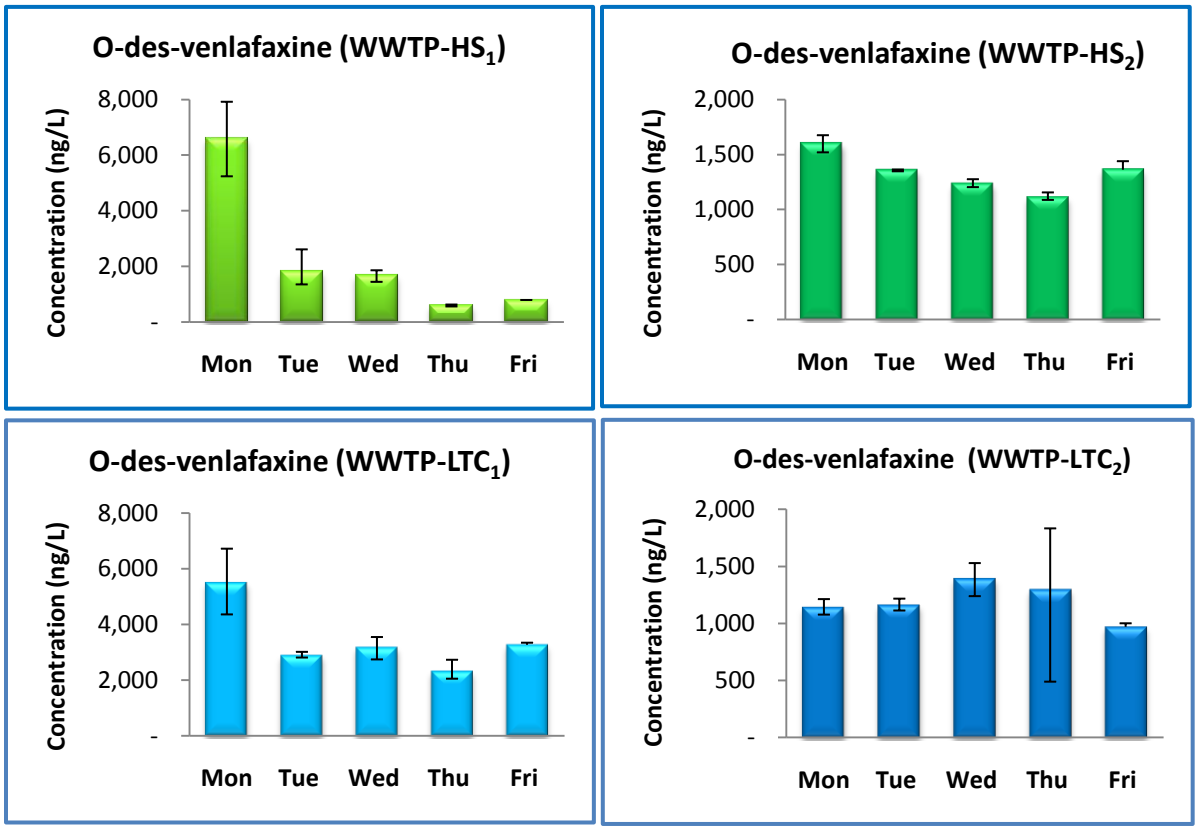


Figure 4-23 Concentration of O-desmethylvenlafaxine in the WWTP influents

The variability in the of O-desmethylvenlafaxine concentrations in WWTP-HS_1 and WWTP-LTC_1 was higher (CV=1.07 and 0.35 respectively) than the other two WWTPs, due to the individual peak concentrations of this compound on Monday, 6580 ng/L in WWTP-HS_1 and 5493 ng/L in WWTP-LTC_1 influents. WWTP-HS_2 and WWTP-LTC_2 had the similar variability, CV=0.14 and 0.13, respectively (Table 4-24).

Table 4-24 Variability in O-desmethylvenlafaxine concentration about the mean in WWTP influents

Facility	Concentrations (ng/L)					Mean	Sd	CV
	Mon	Tue	Wed	Thu	Fri			
WWTP-HS$_1$	**6580**	1816	1683	595	776	2290	2458	1.07
WWTP-LTC$_1$	**5493**	2897	3185	2328	3269	3435	1208	0.35
WWTP-HS$_2$	**1613**	1357	1233	1112	1363	1336	186	0.14
WWTP-LTC$_2$	1140	1160	**1390**	1295	969	1191	161	0.13

Bold numbers show the maximum concentrations measured

4.4.10 Relationship between the Concentrations of Venlafaxine and its Metabolites

The relationship between the Venlafaxine and its metabolites in urinary excretion for healthy adults has been reported to be 1% as N-desmethylvenlafaxine, 1-10% as Venlafaxine and 30% as the active metabolite O-desmethylvenlafaxine (Klamerus et al., 1992). The measured concentrations of these compounds at the influents of the WWTPs were overall found to be in the range of ratios 1% N-desmethylvenlafaxine, 1-4% Venlafaxine, and 7-30% O-desmethylvenlafaxine, and their observed concentration ratios are presented in Table 4-25. The relationship between Venlafaxine and its metabolites differed between the WWTP influents, but remained consistent within WWTPs over the week days within a small range. For example In WWTP-HS$_2$ (Table 4-25) the relationship, 1% N-desmethylvenlafaxine, 3% Venlafaxine and 7-9% O-desmethylvenlafaxine (1:3:7-9) was consistent from Monday to Friday. A similar relationship was observed in WWTP-LTC$_2$, with only a slight increase in the O-desmethylvenlafaxine range (1:3:7-10%) for all weekdays.

Table 4-25 Relationship between the concentration of N-desmethylvenlafaxine, Venlafaxine and O-desmethylvenlafaxine in the WWTP influents

Facility	N-desmethylvenlafaxine : Venlafaxine : O-desmethylvenlafaxine														
	Monday			Tuesday			Wednesday			Thursday			Friday		
WWTP-HS$_1$	1	3	27	1	4	15	1	2	10	1	3	7	1	3	8
WWTP-HS$_2$	1	3	9	1	3	8	1	3	7	1	3	7	1	3	8
WWTP-LTC$_1$	1	2	29	1	3	25	1	3	26	1	4	20	1	4	26
WWTP-LTC$_2$	1	3	8	1	3	8	1	3	10	1	3	7	1	3	9

Concentration of N-desmethylvenlafaxine was taken as 1 to estimate the concentrations of Venlafaxine and O-desmethylvenlafaxine relative to this value.

Comparing the size of communities that contributed PhAC loads to the WWTPs reveals that the largest (WWTP-HS_2, 171000 pop) and the smallest (WWTP-LTC2, 33000 pop) WWTPs had a similar relationship (1:3: 7-10%). WWTP-LTC_1 (80000 pop) had a relation range of 1:2-4: 20-29%, while WWTP-HS_1 (51218 pop) varied, with results similar to WWTP-LTC_1 during the first two days (1:3-4:15-27%), and similar to WWTP-HS_2 and WWTP-LTC_2 for rest of the days (1:2-3:7-10%). To illustrate these finding the data from Table 4-25, are reorganized facility-wise in Table 4-26.

Table 4-26 Relationship between Venlafaxine and its metabolites in the WWTP influents

WWTP ID	WWTP-HS_1	WWTP-HS_2	WWTP-LTC_1	WWTP-LTC_2
Size (Population served)	51,218	171,000	80,000	33,000
N-desmethylvenlafaxine	1%	1%	1%	1%
Venlafaxine	2-4%	3%	2-4%	3%
O-desmethylvenlafaxine	7-27%	7-9%	20-29%	7-10%

Table (4-26) shows a similar relationship occurred between Venlafaxine and its metabolites in the influents of the largest and the smallest WWTPs (columns with blue background). The mid-sized WWTP (WWTP-LTC_1) showed relatively higher concentrations of O-desmethylvenlafaxine (20-29%), the mid to lower WWTP size had a wide range O-desmethylvenlafaxine that accommodates both the ranges.

Another factor contributing to the differences between Venlafaxine and its metabolites in the collected samples may be the sewer travel times for the compounds, from the major discharge points (point sources) to the influents of the WWTPs. This may define how many batches of the excreted portions reach the WWTP influent in 24hours (the elimination half-life of Venlafaxine and O-desmethylvenlafaxine is 4 and 10 hours respectively). For example the sewer travel time from HS_2 and LTC_2 to their respective WWTPs was the same (0.5 hrs), and similar relationships between Venlafaxine and its metabolites existed in their WWTPs (WWTP-HS_2 and WWTP-LTC_2). The sewer travel times were different from $HS_1$and LTC_1 to their respective WWTPs (3 and 5 hours respectively), and hence, relationships differs in them. Further research is need to identify the main sources contributing the differences in relationship between Venlafaxine and its active metabolite O-desmethylvenlafaxine, as greater difference were observed for this metabolite.

All the target PhACs were detected in all the influent samples of the investigated WWTPs. The measured concentration of antibiotic compounds ranged Sulfamethoxazole (170 - 605ng/L), Trimethoprim (100-412 ng/L), and Ciprofloxacin (40-150 ng/L). Acetaminophen was detected in highest concentrations (between 39673 and 83066 ng/L). The measured concentrations of Carbamazepine and Metoprolol were (69 -897 ng/L) and (49 -281 ng/L), respectively. The detected antidepressant concentrations were venlafaxine (258-632 ng/L), and its metabolites, N-desmethylvenlafaxine (80-248 ng/L) and O-desmethylvenlafaxine (594-6580 ng/L).

4.5 Comparison of day to day variability of target compounds in the WWTP influents.

The day to day variability in concentrations of the target PhACs in the WWTP influents were estimated as described in section 4.3 (for detailed calculations see Appendix C). The calculated coefficients of variability of all investigated PhACs for each WWTP are shown in Table-4-27, and plotted in Figure 4-24.

Table 4-27 Variability about the mean concentration of target PhACs in the WWTP influents

Target Compounds	CV			
	WWTP-HS_1	**WWTP-HS_2**	**WWTP-LTC_1**	**WWTP-LTC_2**
Sulfamethoxazole	0.51	0.30	0.08	0.28
Trimethoprim	0.32	0.15	0.17	0.28
Ciprofloxacin	0.34	0.17	0.13	0.52
Acetaminophen	0.28	0.07	0.03	0.18
Carbamazepine	0.89	0.70	0.06	0.18
Metoprolol	0.41	0.14	0.11	0.24
Venlafaxine	0.35	0.05	0.11	0.15

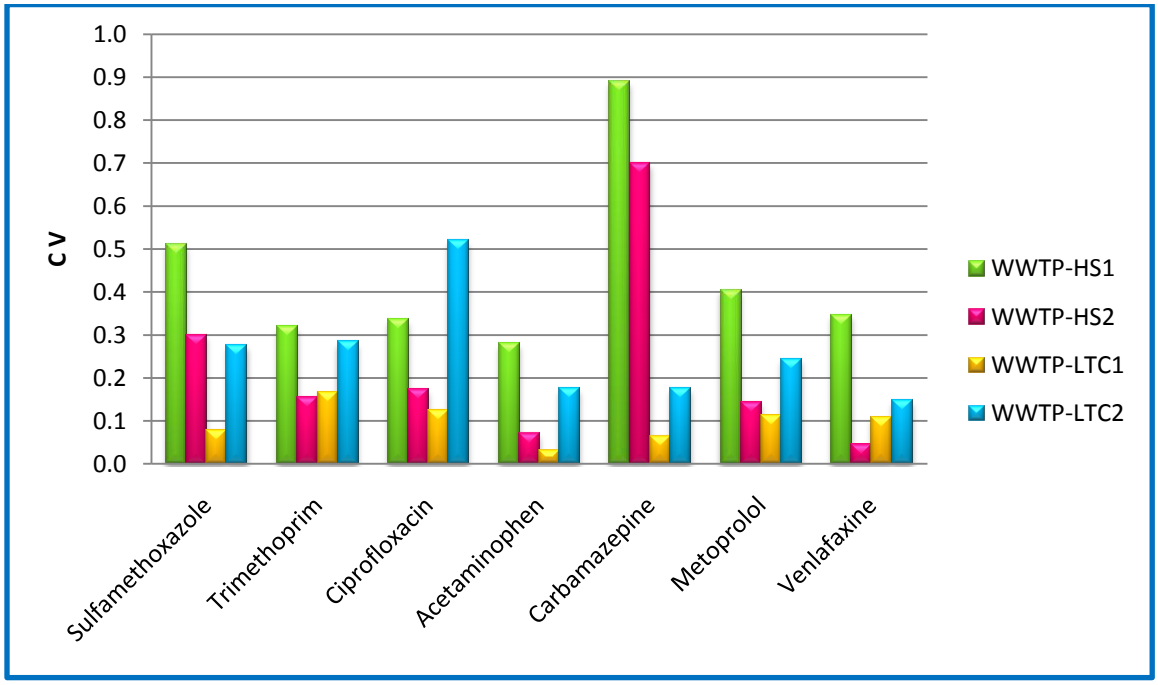


Figure 4-24 Coefficient of variation for concentration of target compounds in the WWTP influents

The day-to-day variability of the target compound concentrations in the WWTP influents (as suggested by CV values) indicates that the highest variability in the target compounds existed in the WWTP-HS_1 influent which had a relatively larger hospital (365 beds) upstream and a relatively smaller community (51218 inhabitants) contributing target compounds to this facility. The next lowest variability occured in WWTP-LTC_2, which was the smallest facility investigated (30000 inhabitants) and had a long-term-care home upstream (200 beds). The least variability existed in WWTP-LTC_1, with a larger treatment facility (80000 inhabitants), a very small hospital (68 beds) and a long-term-care home upstream. The least day-to-day variability about the mean occurred in the Acetaminophen concentrations in all treatment plant influents. These findings suggest that the variability in target compounds concentration may be affected by the size of the hospitals in relations to the size of the community that contributes to the compound loads in the WWTPs.

4.5.1 Comparison of CV values for Target Compounds in the healthcare facilities and downstream WWTPs

The CV values for the target PhACs in the investigated healthcare facility effluents and their respective downstream WWTP influents are plotted in Figures 4-25 and 4-26.

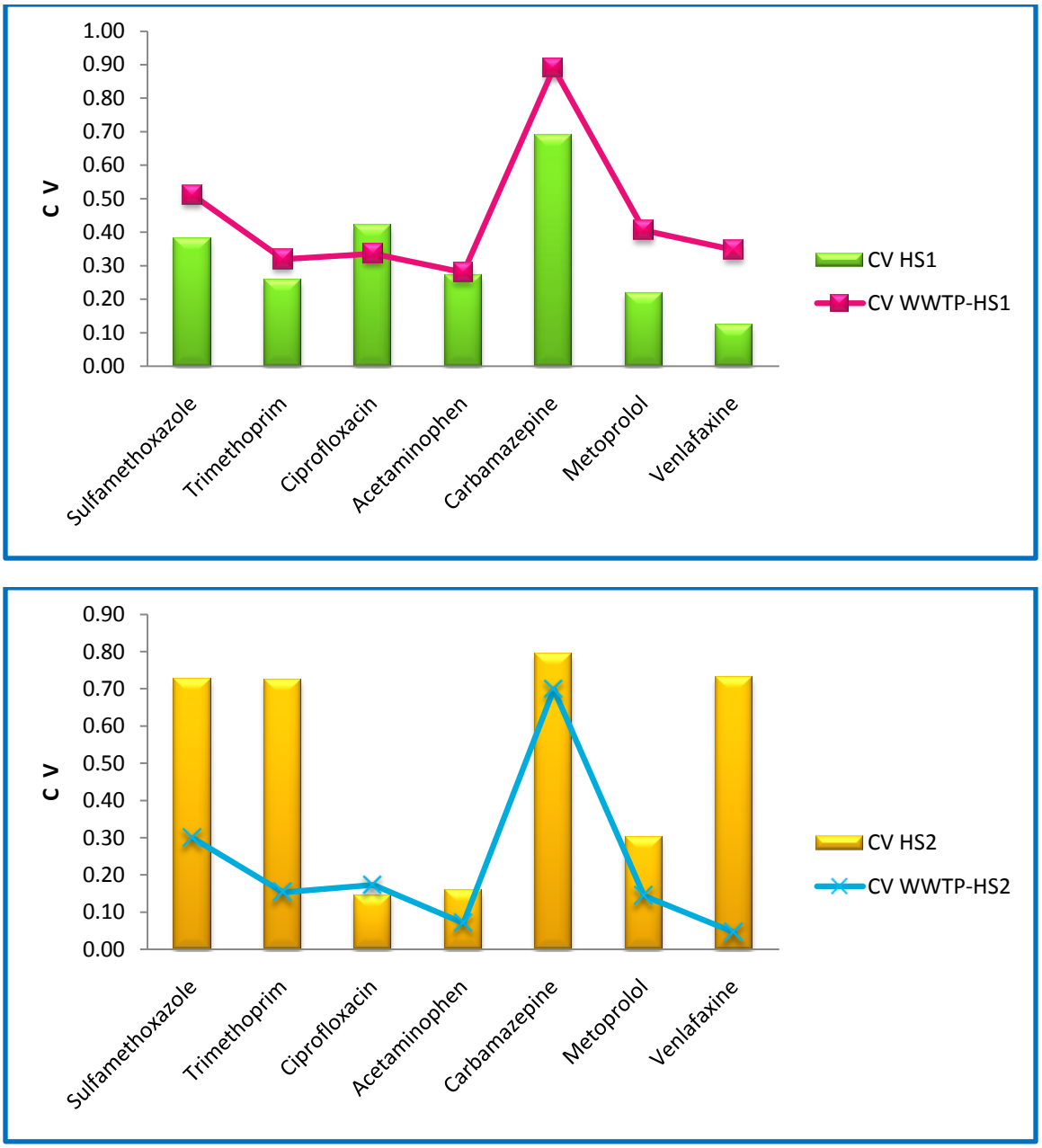


Figure 4-25 Coefficient of variation of the target PhACs in the hospital effluents and their downstream WWTP influents

The relative trends in CV for target PhACs in the hospital effluents and their downstream WWTP influents followed similar tends, especially in HS_1 (a relatively bigger facility) and its downstream WWTP-HS_1 (see the upper chart). This finding may suggest a connection between the variability of the compound concentrations in the WWTPs to that of the hospitals.

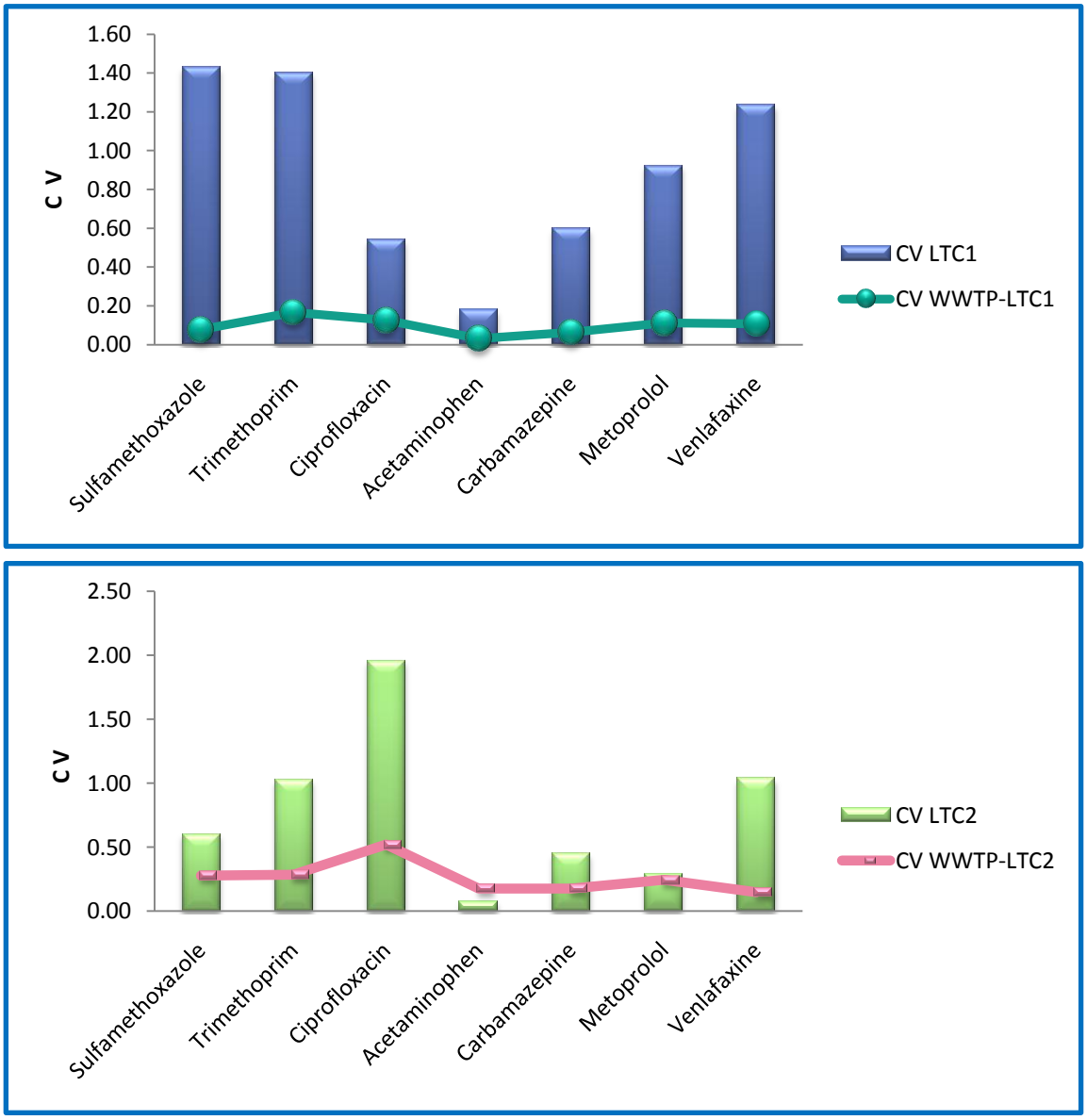


Figure 4-26 Coefficient of variation of the target PhACs in the long-term-care home effluents and their downstream WWTP influents

The variability in the compounds in the long-term-care effluent was relatively much higher than in their respective downstream WWTP influents. The trends in variability between the compounds in the long-term-care effluents and WWTP influents did not follow similar trends. Perhaps this is due to the

relatively small contribution of the long term care homes to the WWTPs. Hence, fluctuations in the LTC effluents would have insignificant effects on the variability of the WWTPs.

4.6 Comparison of Target Compound Concentrations in the WWTP influents

The investigated WWTP influent concentrations were compared using SPSS software (ANOVA) and Fisher least significant difference (LSD) method for multiple comparisons. No significant differences existed between any of the investigated WWTP influents for the concentrations of Sulfamethoxazole, Ciprofloxacin, Venlafaxine, and N-desmethylvenlafaxine. Trimethoprim concentrations were significantly different between WWTP-$HS_1$and WWTP-LTC_2, and between WWTP-LTC_1 and WWTP-LTC_2 influents. Significant differences occurred in Acetaminophen concentrations between WWTP-HS_2 and all the other WWTPs. Carbamazepine concentrations were significantly different between WWTP-HS_1 and WWTP-LTC_2. Metoprolol concentrations in WWTP influent were not significantly different between WWTP-HS_1 and WWTP-HS_2, and WWTP-LTC_1 and WWTP-LTC_2; and significant differences were observed in all other WWTP combinations. Significant differences existed in the concentrations of O-desmethylvenlafaxine in WWTP-HS_2 and WWTP-LTC_1, and between WWTP-LTC_1 and WWTP-LTC_2. No differences were found in any other WWTP combinations.

These findings suggest that in WWTP influents 1) similar concentrations of Sulfamethoxazole, Ciprofloxacin, Venlafaxine and N-desmethylvenlafaxine can be expected regardless of the size of the WWTP, existing healthcare facilities upstream, and sampling dates. This claim is supported by the finding that no significant differences in the concentration of these compounds were observed in the influents of wastewater treatment facilities of different sizes (varied between 30,000 and 171,000), with different healthcare facilities upstream (hospitals and long-term-care homes), and at different sample time/dates (July, November, February and March). 2) Trimethoprim concentrations may vary depending upon sizes of the WWTP and sizes of the upstream healthcare facilities. WWTP-LTC_2, the smallest treatment facility investigated, did not receive hospital discharges, had a lower daily average concentration in its influent (184 ng/L) than all other WWTPs (319, 281, 270 ng/L). 3) Carbamazepine concentrations seem to be affected by upstream hospital sizes, as its daily average concentration varied as 80,172, 320 and 350 ng/L for no hospitals upstream, and for 68, 263 and 365-bed hospitals respectively. Significant differences in Carbamazepine concentration only existed

between WWTP-HS_1 (a 365-bed hospital upstream) and WWTP-LTC_2 (no hospital discharges). 4) Acetaminophen concentrations may differ between WWTPs, provided that substantial differences exist in their sizes. In this study only significant differences were observed between the biggest WWTP investigated (serves 171000 population) and rest of the WWTPs (for of 80000, 51218, 30,000 inhabitants). A lower daily average concentration (~43 μg/L) of Acetaminophen was found in the biggest WWTP (WWTP-HS_2) compared to all other investigated WWTPs (average concentration varied from 61 to 67 μg/L). 5) O-desmethylvenlafaxine concentration may vary between WWTPs depending upon the size of the WWTP and the existing hospitals upstream. No significant differences were observed between the WWTPs that received hospital discharges. Lower daily average concentrations were observed in the biggest and the smallest WWTPs (1.3 and 1.2 μg/L respectively) than in other WWTPs (3.3 and 3.4 μg/L).

Chapter 5
Mass Flows of Target PhACs

Mass flows were calculated to quantify the total load of target PhACs in the investigated healthcare facility effluents and in the downstream wastewater treatment plant influents. The healthcare facility effluents were compared with each other on the basis of the mass flows of target PhACs over week days, similarly WWTP influents were compared. The per-bed contributions of PhACs to each facility's effluent load and per-capita mass contributions to the WWTP influent loads were investigated.

The wastewater flows of the healthcare facilities (HS_1, HS_2, LTC_1, and LTC_2) were calculated from the amount of water the facilities purchased during the sampling month. This data was provided by the regional municipality of the target area. Using monthly water consumption data, daily water consumption was estimated by assuming that the average water consumption during each day of the week was the same. It was further assumed that ninety percent of the water consumed within the facility was discharged to the sewers (Metcalf & Eddy, 1991). Table 5-1 shows the water consumed (m^3/day) by each facility and wastewater flows.

Table 5-1 : Daily water consumption and wastewater flows of the healthcare facilities

Target Facilities (Size)	HS_1 (365 beds)	HS_2 (263 beds)	LTC_1 (228 beds)	LTC_2 (200 beds)
Water consumption (m^3/day)	516	197	76	139
Wastewater flow (m^3/day)	464.4	177.3	68.4	125

Wastewater flow = 90% of water consumption

The water consumed by LTC_2 (200 beds) a relatively smaller facility, was considerably higher than by LTC_1 with 228 beds (Table 5-1). This difference was presumably due to the presence of private showers for the residents in the LTC_2 facility, while LTC_1 had communal showers. This finding suggests that the water consumption estimates for a healthcare facility using typical per-bed

consumption values may sometimes lead to wide differences between estimated values and actual consumption.

The daily average wastewater inflow volumes (m^3/day) of the WWTPs were provided by the treatment plant operators (Table 5-2). The per-capita wastewater contribution to the wastewater treatment facilities was calculated by dividing the inflow volume with the population served by each facility. The average per-capita wastewater generation for the areas investigated varied between 390 to 620 L/capita/day (Table 5-2).

Table 5-2 Inflows of WWTPs and per-capita wastewater contribution

Week days	WWTP-HS_1 (m^3/day)		WWTP-HS_2 (m^3/day)		WWTP-LTC_1 (m^3/day)		WWTP-LTC_2 (m^3/day)	
	Inflow	Per-capita	Inflow	Per-capita	Inflow	Per-capita	Inflow	Per-capita
Monday	22663	0.44	93784	0.55	36905	0.46	10835	0.36
Tuesday	27265	0.53	92909	0.54	36717	0.46	11103	0.37
Wednesday	26157	0.51	89543	0.52	37783	0.47	11068	0.37
Thursday	49779	0.97	93297	0.55	38162	0.48	10976	0.37
Friday	31798	0.62	87807	0.51	37839	0.47	14963	0.50
MEAN		**0.62**		**0.53**		**0.47**		**0.39**

The mass flows (g/day) of the target compounds in the healthcare facility effluents and the respective downstream WWTP influents were calculated using equation 5.1. The results are tabulated in Table 5-3. This table also shows weekly average mass flows for each compound in the facility effluents. As mentioned earlier Sulfamethoxazole was not tested on Monday samples from HS_1 effluent and WWTP-HS_1 influent and Tuesday samples from HS_1 and HS_2 effluents did not represent the full hospital effluent; therefore, mass flows for these days are not shown in Table 5-3.

$$\textit{Mass flow (g/day)} = \textit{Concentration (ng/L)} \times \textit{Wastewater Flow } (m^3/day) \times 10^6 \quad (5.1)$$

Table 5-3: Daily mass flows of target PhACs in the healthcare facility effluents and WWTP influents

Compound	Week day	Healthcare facility effluent (g/day)				WWTP influents (g/day)			
		HS_1	HS_2	LTC_1	LTC_2	WWTP-HS_1	WWTP-HS_2	WWTP-LTC_1	WWTP-LTC_2
Sulfamethoxazole	Mon		1.17	0.03	0.07		51.39	16.27	5.11
	Tue			0.16	0.01	16.5	40.51	13.88	6.00
	Wed	0.46	1.09	0.01	0.09	10.98	19.34	17.43	5.27
	Thu	0.41	1.94	0.01	0.05	8.49	36.27	15.52	3.84
	Fri	0.20		0.02	0.03	8.81	35.81	16.85	3.77
Daily average		**0.36**	**1.4**	**0.05**	**0.05**	**11.2**	**36.7**	**16.0**	**4.8**
Trimethoprim	Mon	0.24	0.88	0.05	0.03	8.82	27.74	8.34	2.16
	Tue			0.06	0.03	11.24	21.99	10.72	2.71
	Wed	0.15	1.67	0.03	0.24	8.08	19.46	9.97	2.05
	Thu	0.15	1.83	0.45	0.11	7.6	29.47	10.34	2.12
	Fri	0.16		0.05	0.04	10.5	25.03	13.37	1.50
Daily average		**0.18**	**1.5**	**0.13**	**0.09**	**9.2**	**24.7**	**10.5**	**2.1**
Ciprofloxacin	Mon	0.29	0.02	0.01	0.01	2.21	7.88	2.10	0.85
	Tue			0.02	0.01	1.11	9.16	2.81	1.67
	Wed	0.58	0.03	0.04	0.004	2.15	10.03	2.59	0.75
	Thu	0.39	0.03	0.02	0.18	5.2	12.04	3.05	0.49
	Fri	0.22		0.01	0.01	1.99	8.16	2.78	0.86
Daily average		**0.37**	**0.03**	**0.02**	**0.04**	**2.5**	**9.5**	**2.7**	**0.9**
Acetaminophen	Mon	62.29	2.35	5.87	9.10	1882.5	4,088.9	2,376.6	732.4
	Tue			5.75	11.10	2068.5	3,865.0	2,472.2	701.7
	Wed	44.15	2.83	5.39	10.67	1715.9	4,262.2	2,513.8	709.8
	Thu	46.69	2.09	7.97	10.72	1986.5	3,888.6	2,579.7	750.7
	Fri	31.83		5.33	9.87	1628.0	3,474.8	2,658.8	632.4
Daily average		**46.24**	**2.4**	**6.06**	**10.29**	**1856.3**	**3915.9**	**2520.2**	**705.4**
Carbamazepine	Mon	0.07	0.12	0.01	0.01	6.09	23.95	6.47	0.75
	Tue			0.01	0.01	7.12	17.74	6.01	1.16
	Wed	0.02	0.01	0.02	0.003	23.47	18.55	5.97	0.83
	Thu	0.02	0.08	0.04	0.004	7.52	67.05	6.92	0.79
	Fri	0.04		0.01	0.01	5.35	20.23	6.95	1.21
Daily average		**0.04**	**0.06**	**0.02**	**0.01**	**9.9**	**29.5**	**6.5**	**0.9**
Metoprolol	Mon	0.14	0.02	0.07	0.02	1.11	8.09	8.67	3.05
	Tue			0.13	0.04	4.88	6.57	9.42	2.53
	Wed	0.23	0.03	0.08	0.02	5.42	9.29	8.21	2.42
	Thu	0.18	0.03	0.34	0.03	7.22	7.66	7.61	2.67
	Fri	0.23		0.04	0.02	5.12	8.37	9.88	2.02
Daily average		**0.20**	**0.03**	**0.13**	**0.03**	**4.8**	**8.0**	**8.8**	**2.5**
Venlafaxine	Mon	0.26	0.13	0.04	0.04	14.32	48.89	16.39	4.62
	Tue			0.03	0.02	11.96	44.22	14.45	4.99
	Wed	0.29	1.58	0.16	0.01	10.18	45.61	15.20	4.86
	Thu	0.35	0.72	0.01	0.005	12.88	46.52	16.49	5.01
	Fri	0.30		0.02	0.09	10.33	40.98	19.42	4.60
Daily average		**0.30**	**0.8**	**0.05**	**0.03**	**11.9**	**45.2**	**16.4**	**4.8**

Compound	Week day	Healthcare facility effluent (g/day)				WWTP influents (g/day)			
		HS_1	HS_2	LTC_1	LTC_2	WWTP-HS_1	WWTP-HS_2	WWTP-LTC_1	WWTP-LTC_2
N-desmethylvenlafaxine	Mon	0.10	0.04	0.01	0.01	5.62	16.68	7.01	1.58
	Tue			0.02	0.004	3.26	15.37	4.23	1.61
	Wed	0.13	0.02	0.01	-	4.46	15.20	4.69	1.58
	Thu	0.16	0.04	0.00	-	4.02	15.15	4.34	1.94
	Fri	0.19		0.01	0.01	3.15	14.99	4.77	1.55
Daily average		**0.15**	**0.03**	**0.01**	**0.01**	**4.1**	**15.5**	**5.0**	**1.7**
O-desmethylvenlafaxine	Mon	1.34	0.17	0.04	0.11	149.12	151.30	202.73	12.35
	Tue			0.03	0.08	49.51	126.11	106.38	12.88
	Wed	0.29	.2	0.16	0.06	44.01	110.44	120.35	15.38
	Thu	0.35	0.45	0.01	0.09	29.60	103.75	88.84	14.21
	Fri	0.30		0.02	0.27	24.67	119.65	123.71	14.50
Daily average		**0.57**	**0.2**	**0.05**	**0.12**	**59.38**	**122.24**	**128.40**	**13.86**

Heberer et al., (2005) measured the weekly load (7 days) of 3.6 g of Carbamazepine (0.514 g/day) in a German hospital's (300 beds) effluent. This value was higher than that found in this study, for which the maximum carbamazepine load per day was 0.12 g/day on Monday in the HS_2 (263 beds) effluent (Table 5-3). This may have resulted from differences in the consumption of Carbamazepine between the facilities characterized in the two studies.

5.1 Comparison of Healthcare Facility Effluents for the Mass discharges of Target PhACs

5.1.1 Hospitals (HS_1 & HS_2)

The hospital effluent mass flows were compared using analysis of variance (ANOVA) to further investigate whether the mass flows of target compounds were significantly different between these streams. A comparison between the HS_1 and HS_2 facility effluents (Table 5-4) indicated significant differences existed between these facilities in mass flows of Sulfamethoxazole, Trimethoprim, Ciprofloxacin, Acetaminophen, Metoprolol and the metabolite of Venlafaxine N-desmethylvenlafaxine (P-values highlighted red in Table 5-3).

Table 5-4 Comparison between HS_1 and HS_2 effluents for the mass flow of target compounds

Target Compounds	Facility	Daily Mass Flows (g/day)				P-value
		Mon	Wed	Thu	Fri	
Sulfamethoxazole	**HS_1**		0.46	0.41	0.20	**0.02**
	HS_2	1	1.09	2		
Trimethoprim	**HS_1**	0.24	0.15	0.15	0.16	**0.003**
	HS_2	0.88	1.67	2		
Ciprofloxacin	**HS_1**	0.29	0.58	0.39	0.22	**0.01**
	HS_2	0.02	0.03	0.03		
Acetaminophen	**HS_1**	62	44	47	32	**0.002**
	HS_2	2.35	2.83	2		
Carbamazepine	**HS_1**	0.07	0.02	0.017	0.04	**0.27**
	HS_2	0.12	0.01	0.08		
Metoprolol	**HS_1**	0.14	0.23	0.176	0.23	**0.001**
	HS_2	0.02	0.03	0.03		
Venlafaxine	**HS_1**	0.26	0.29	0.346	0.30	**0.2**
	HS_2	0.13	1.58	1		
N-desmethylvenlafaxine	**HS_1**	0.10	0.13	0.164	0.19	**0.005**
	HS_2	0.04	0.02	0.04		
O-desmethylvenlafaxine	**HS_1**	1.34	0.45	0.83	0.56	**0.08**
	HS_2	0.17	0.20	0.45		

The higher average mass flows of Ciprofloxacin (370 mg/day), Acetaminophen (46240 mg/day), Metoprolol (195 mg/day), and N-desmethylvenlafaxine (140 mg/day) in HS_1 effluent than in HS_2 Ciprofloxacin (20 mg/day), Acetaminophen (2320 mg/day), Metoprolol (20 mg/day), and N-desmethylvenlafaxine (40 mg/day) were likely due to the differences in facility sizes, HS_1 (365 beds) is relatively bigger than HS_2 (263 beds). Additionally there may be seasonal differences in consumption; drug purchase data shows relatively higher purchases of Ciprofloxacin, Acetaminophen and Metoprolol compounds in July than in October (Figure 5-1).

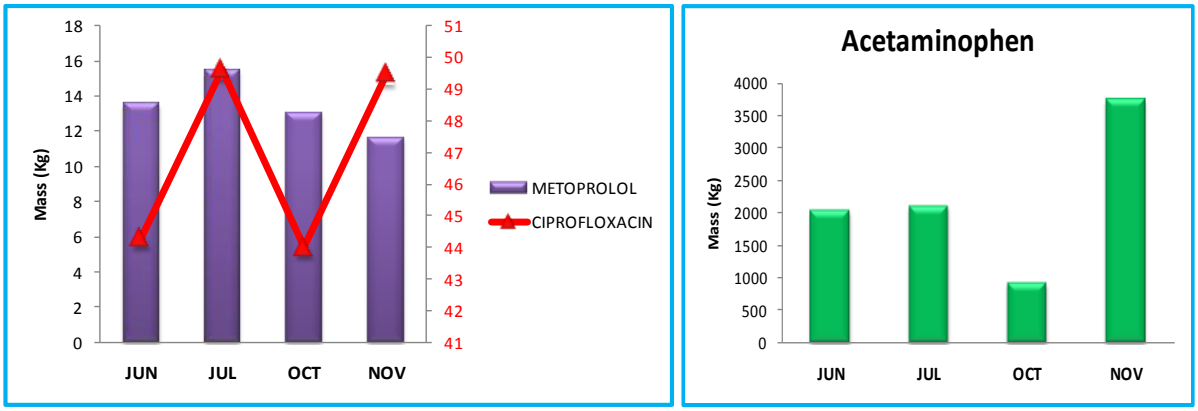


Figure 5-1 Ciprofloxacin, Metoprolol and Acetaminophen purchases (Kg) by Ontario hospitals in 2009 (IMS Canada)

The higher average daily mass flows of Sulfamethoxazole (1.4 g/day), Trimethoprim (1.5 g/day), Carbamazepine (0.1 g/day) and Venlafaxine (0.8 g/day) in HS_2 effluent, a relatively smaller facility than HS_1, may have been due to seasonal variations in pharmaceutical consumption and differences in the services provided by each facility. Higher WWTP influent Sulfamethoxazole loads in winters than in summer have been reported by (Castiglioni et al., 2006).

The elevated mass flow from the smaller hospital may have been due to differences in the services between the two hospitals. The presence of the cancer clinic in HS_2 may lead to higher mass discharges of Venlafaxine in its effluent than in HS_1's. The higher detected concentration of Venlafaxine in the cancer clinic effluent (36 μg/L) supports this assumption (Figure 4-12). These findings suggest that the information about the number of beds in a hospital is not enough data to explain the mass flows of target compounds in its effluent. The type of services available in the hospital and whether a facility is fully operational also have a direct influence.

5.1.2 Long-Term-Care Homes (LTC_1 & LTC_2)

The long-term-care home effluents were compared to identify differences between these streams for the daily mass flows of target compounds. The homes differed slightly in terms of size (LTC_1 has 228 beds and LTC_2 has 200 beds) and location. However no significant difference was detected between these streams (Table 5-5) except in the mass flow of Acetaminophen (P = 0.000), which was higher (10290 mg/day) for LTC_2 than for LTC_1 (6062 mg/day).

The similar mass flows for most of the compounds may have occurred because long-term-care homes provide similar services, the residents are relatively homogenous group (the elderly), and also the facility sizes were not substantially different. The significant differences in Acetaminophen mass flows between the homes of almost the same size is an interesting finding, potentially occurs due to different approaches to medication, but needs need further investigation for other possible causes.

Table 5-5 Comparison between LTC_1 and LTC_2 effluents for the mean mass flows of target compounds

Target Compounds	Facility	Daily Mass Flows (g/day)					P-value
		Mon	Tue	Wed	Thu	Fri	
Sulfamethoxazole	**LTC_1**	0.026	0.157	0.010	0.006	0.023	**0.83**
	LTC_2	0.074	0.013	0.090	0.049	0.033	
Trimethoprim	**LTC_1**	0.050	0.063	0.031	0.451	0.048	**0.66**
	LTC_2	0.030	0.029	0.241	0.106	0.037	
Ciprofloxacin	**LTC_1**	0.014	0.024	0.041	0.015	0.015	**0.61**
	LTC_2	0.006	0.005	0.004	0.184	0.005	
Acetaminophen	**LTC_1**	5.868	5.749	5.393	7.971	5.329	**0.000**
	LTC_2	9.103	11.10	10.67	10.72	9.870	
Carbamazepine	**LTC_1**	0.012	0.010	0.017	0.036	0.012	**0.054**
	LTC_2	0.008	0.008	0.003	0.004	0.010	
Metoprolol	**LTC_1**	0.071	0.129	0.078	0.337	0.037	**0.094**
	LTC_2	0.025	0.040	0.024	0.032	0.020	
Venlafaxine	**LTC_1**	0.037	0.028	0.156	0.007	0.017	**0.62**
	LTC_2	0.042	0.018	0.012	0.005	0.090	
N-desmethylvenlafaxine	**LTC_1**	0.009	0.018	0.006	0.003	0.009	**0.56**
	LTC_2	0.015	0.004	0.000	0.000	0.014	
O-desmethylvenlafaxine	**LTC_1**	0.089	0.087	0.156	0.022	0.479	**0.62**
	LTC_2	0.112	0.078	0.061	0.092	0.266	

Relatively higher mass flows were observed in the hospital effluents than in the long-term-care homes (Table 5-3). This difference perhaps occurred because more pharmaceutical compounds are consumed in hospitals than in long-term-care homes as all the people admitted in hospitals are presumably ill and often need drug therapy. While in long term-care homes only a fraction of the population is expected to receive pharmaceutical compounds for any given time, resulting higher mass flows in hospital effluents.

The mass flows of target compounds in long-term-care homes over the weekdays indicate that only Acetaminophen is regularly being used in long-term-care homes (Table 5-5). The mass flow of this compound varied between 9 and 11 g/day in LTC_2 effluent and in LTC_1 effluent it varied only between 5.3 and 5.8 g/day except on Thursday (7.9 g/day).

5.2 WWTP Influent Mass Flows and Per-Capita Mass Contributions

Per-capita mass contribution is often used to predict mass flows of PhACs to WWTPs, and is usually calculated using county-level prescription or sales data along with the pharmacokinetic properties of the PhACs. The estimated figures are then assumed to be uniformly distributed over the year and throughout the geographical area. To test this assumption for this study the per-capita mass contributions to each WWTP influent load were calculated. In the per-capita mass contributions only parent compounds were considered because the metabolites are generated by the breakdown of parent compounds, and once the mass of the parent compounds is known, the mass of the metabolites can be estimated using the pharmacokinetic relationship between the parent compound and its metabolites. In addition the sales and prescription data is not applicable to the metabolites, so comparison with the sales data is only possible for the parent compounds.

The per-capita mass contribution of the target PhACs to the influent load of WWTPs was calculated by dividing WWTP influent mass flows of the target PhACs by the size of the WWTPs (population served) as described in equation 5.2.

$$Per\text{-}capita\ mass\ contribution = \frac{Mass\ of\ the\ compound\ in\ the\ WWTP\ influent}{Population\ served\ by\ WWTP} \qquad (5.2)$$

Table 5-6 Per-capita mass contribution range to the WWTPs

Compound	Range	Per-Capita Mass inflow (mg/day)			
		WWTP-HS_1	WWTP-HS_2	WWTP-LTC_1	WWTP-LTC_2
Sulfamethoxazole	**Min**	0.166	0.113	0.174	0.114
	Max	0.322	0.301	0.218	0.182
Trimethoprim	**Min**	0.148	0.114	0.104	0.045
	Max	0.219	0.172	0.167	0.082
Ciprofloxacin	**Min**	0.022	0.046	0.026	0.015
	Max	0.102	0.071	0.038	0.051
Acetaminophen	**Min**	31.78	20.32	29.70	19.16
	Max	40.38	24.92	33.23	22.75
Carbamazepine	**Min**	0.104	0.104	0.075	0.023
	Max	0.458	0.392	0.087	0.037
Metoprolol	**Min**	0.022	0.038	0.095	0.061
	Max	0.141	0.054	0.123	0.092
Venlafaxine	**Min**	0.199	0.240	0.181	0.139
	Max	0.280	0.286	0.243	0.152

Table 5-6 shows the range of per-capita mass contributions to the influent load of each investigated wastewater treatment facility. The differences in the per-capita-per-day mass contributions, estimated using data for the four different facilities, were apparently caused by regional differences in PhAC consumption. Relatively higher per-capita-per-day mass contributions of Sulfamethoxazole, Trimethoprim, Ciprofloxacin, Carbamazepine and Venlafaxine were observed for WWTP-HS_1 and WWTP-HS_2 (Figure 5-3). These differences may be attributed to the presence of hospitals in these communities. The per-capita contribution of Acetaminophen varied between all the communities with the lowest value of 19-22 mg/day for the community of size 30,000 (WWTP-LTC_2) and highest value of 31-40 mg/day for the WWTP-HS_2 which serves for 51218 people (Table 5-6). These findings suggest that the assumption in the prediction models, that pharmaceutical consumption is evenly distributed over a year and throughout geographical locations may not hold true for all compounds. The per-capita values from Table 5-6 is plotted in Figure 5-3 to better visualize the range of values in investigated communities and to compare these with each other.

A comparison of these results with other WWTP influent studies showed differences in Carbamazepine per-capita contributions and comparable values for Sulfamethoxazole contributions.

Heberer et al., (2005) found the mass flow of carbamazepine in the influent of a WWTP that served 1 million people to be 3218 g/week, giving a per-capita-per-day mass contribution of 0.46 mg/day, which was slightly higher than that found in this study (max 0.32mg). A Sulfamethoxazole load of 209 mg/1000 inhabitants in the influent of a WWTP in Italy was reported in winter (January to March) and was comparable to the maximum influent loads of WWTP-LTC_1 and WWTP-LTC_2 that were sampled in February and March (208 and 182 mg/1000 inhabitants respectively) (Castiglioni et al., 2006).

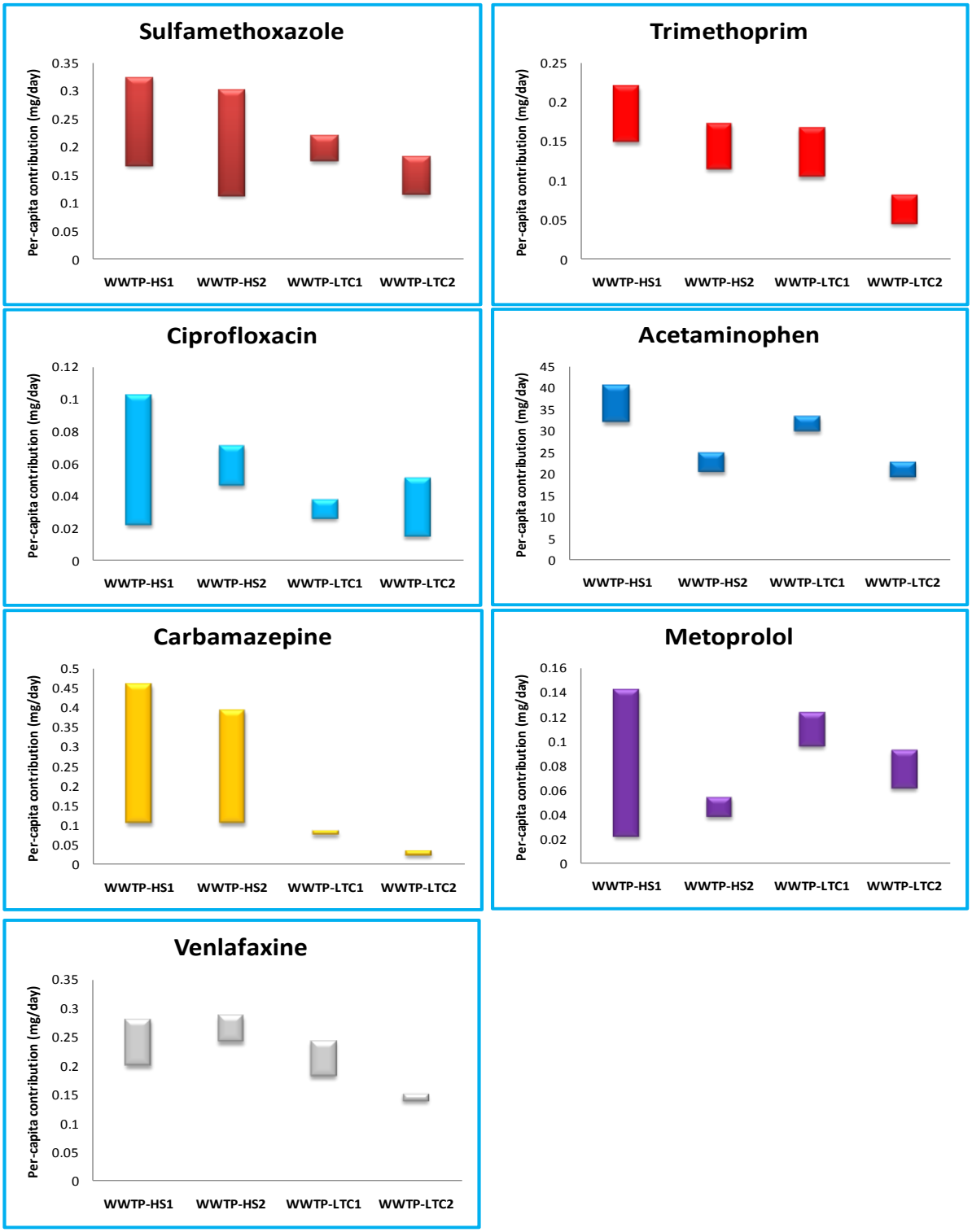


Figure 5-2 Per-capita mass contribution (Range) to WWTPs

5.2.1 Mass contribution of PhACs Per- bed to Effluents

Estimating the per-bed mass contribution of target compounds allows comparison of healthcare facilities regardless of their size. It may be used to extend the results from one facility to estimate the discharges of PhACs by the other similar type of facilities. The per-bed mass contributions of all the four investigated healthcare facility effluents were determined. To calculate mass flow per-bed-per-day, the daily mass flow of target PhACs in the effluent of each healthcare facility was divided by its number of beds, as described in Equation 5.3.

$$Per\text{-}bed\ Mass\ Contribution = \frac{Mass\ of\ the\ compound\ in\ Healthcare\ facility\ effluent}{Facility\ size\ (Number\ of\ beds)} \quad (5.3)$$

The calculated per bed mass contributions of the target PhACs to the effluent load of each facility are shown in Tables 5-7 and 5-8. The results indicate (Table-5-7) that in the case of hospitals, considerable differences existed between the facilities in the average per bed contribution of all target PhACs. These differences may have been caused by seasonal variations in pharmaceutical consumption or by differences in the services provided by the facilities. For example (Table 5-7) the mean per-bed mass contributions of Sulfamethoxazole and Trimethoprim in HS_1 were 0.99 mg/day and 0.47 mg/day, while in HS_2, these contributions were is 4 and 4.2 mg/day. The per-bed mass flow of ciprofloxacin was 10 times higher in HS_1 than in HS_2. The per-bed contribution range of Acetaminophen in HS_1 was 87-170 mg/day while in HS_2 this range was 7.7-10.7 mg/day. These findings suggest that knowing only the number of beds for a healthcare facility is not sufficient to predict the mass flow of any particular PhAC in its wastewater. The number of staffed and in-operation beds for any type and level of service may impact on the mass flow of PhACs related to that service. Since group-wise (for condition being treated/ investigated) breakdown of the number of beds for the hospitals could not be tested for in this study, this hypothesis was not tested.

Table 5-7 Per-bed mass contribution of target PhACs to each hospital effluent

Target Compounds	Facility	Mass discharge (g/day)			Number of Beds	Per Bed Mass Contribution (mg/day)		
		Min	Max	Mean		Min	Max	Mean
Sulfamethoxazole	HS_1	0.20	0.46	0.36	365	0.56	1.27	0.99
	HS_2	1.09	1.94	1.40	263	4.15	7.37	5.32
Trimethoprim	HS_1	0.15	0.24	0.17	365	0.40	0.65	0.47
	HS_2	0.88	1.83	1.46	263	3.33	6.96	5.55
Ciprofloxacin	HS_1	0.22	0.58	0.37	365	0.59	1.58	1.01
	HS_2	0.02	0.03	0.03	263	0.08	0.11	0.10
Acetaminophen	HS_1	31.83	62.29	46.24	365	87.20	170.66	126.68
	HS_2	2.09	2.83	2.42	263	7.94	10.77	9.21
Carbamazepine	HS_1	0.02	0.07	0.03	365	0.04	0.18	0.10
	HS_2	0.01	0.12	0.07	263	0.05	0.46	0.27
Metoprolol	HS_1	0.14	0.23	0.19	365	0.39	0.63	0.53
	HS_2	0.02	0.03	0.03	263	0.06	0.13	0.10
Venlafaxine	HS_1	0.26	0.35	0.30	365	0.70	0.95	0.82
	HS_2	0.13	1.58	0.81	263	0.51	6.00	3.08
N-desmethylvenlafaxine	HS_1	0.10	0.19	0.15	365	0.28	0.53	0.40
	HS_2	0.02	0.04	0.03	263	0.07	0.16	0.12
O-desmethylvenlafaxine	HS_1	0.45	1.34	0.80	365	1.23	3.66	2.18
	HS_2	0.17	0.45	0.27	263	0.64	1.71	1.04

The calculated per-bed mass contributions to the long-term-care home effluents are presented in Table 5-8. These values could be considered representative of the per-capita mass contribution by the elderly population (as each bed is assigned to one person). A comparison of these values with the per-capita contributions by the community (mixed aged group) calculated in Table 5-6, showed relatively higher mass contribution of certain compounds by the elderly population than the mixed population in the community. For example, Acetaminophen which is often used in communities had a maximum per-capita range in WWTP-HS_1 of 31-40 mg/day (Table 5-6), while the per-capita contribution by the elderly (per-bed mass contribution) was 45-55 mg/day. The Metoprolol maximum per-capita contribution range for the community was 0.1-0.2 mg/day, and its maximum contribution by the elderly varied between 0.16-1.4 mg/day (Table 5-8 in LTC_1).

Table 5-8 Per-bed mass contribution of target PhACs to each long-term-care home effluent

Target Compounds	Facility	Mass discharge (g/day)		Number of Beds	Per Bed Mass Contribution (mg/day)	
		Min	Max		Min	Max
Sulfamethoxazole	LTC_1	0.006	0.157	228	0.028	0.689
	LTC_2	0.013	0.090	200	0.063	0.448
Trimethoprim	LTC_1	0.031	0.451	228	0.136	1.976
	LTC_2	0.029	0.241	200	0.146	1.203
Ciprofloxacin	LTC_1	0.014	0.041	228	0.059	0.182
	LTC_2	0.004	0.184	200	0.020	0.919
Acetaminophen	LTC_1	5.32	7.97	228	23.37	34.95
	LTC_2	9.10	11.10	200	45.51	55.51
Carbamazepine	LTC_1	0.010	0.036	228	0.046	0.158
	LTC_2	0.003	0.010	200	0.014	0.048
Metoprolol	LTC_1	0.037	0.337	228	0.163	1.479
	LTC_2	0.020	0.040	200	0.098	0.200
Venlafaxine	LTC_1	0.007	0.156	228	0.033	0.684
	LTC_2	0.005	0.090	200	0.023	0.448
N-desmethylvenlafaxine	LTC_1	0.003	0.018	228	0.013	0.080
	LTC_2	0.000	0.015	200	0.000	0.074
O-desmethylvenlafaxine	LTC_1	0.022	0.479	228	0.097	2.101
	LTC_2	0.061	0.266	200	0.303	1.328

The per-bed contributions of the target PhACs to each healthcare facility's effluent load were compared using ANOVA. The P-values for the comparisons are presented in Table 5-9.

Table 5-9 Comparison of Healthcare facility effluent results for per bed contribution of target PhACs

Facility ID	HS_1 Vs HS_2	LTC_1 Vs LTC_2
Target compounds	**P-value**	
Sulfamethoxazole	**0.015**	**0.673**
Trimethoprim	**0.003**	**0.773**
Ciprofloxacin	**0.015**	**0.562**
Acetaminophen	**0.002**	**0.000**
Carbamazepine	**0.160**	**0.076**
Metoprolol	**0.002**	**0.106**
Venlafaxine	**0.150**	**0.736**
N-desmethylvenlafaxine	**0.010**	**0.739**
O-desmethylvenlafaxine	**0.163**	**0.765**

The highlighted P-values in Table 5-19 show the significant differences in per-bed mass contributions to each facility's effluent. More compounds showed significant differences in hospitals than in long-term-care homes. The per-bed contributions of Sulfamethoxazole, Trimethoprim Ciprofloxacin, Acetaminophen, Metoprolol, and N-desmethylvenlafaxine were significantly different between the two investigated hospitals (HS_1 and HS_2). Seasonal variations may have contributed to these differences, as HS_1 and HS_2 were sampled in July and November respectively. In the long-term-care homes, the per-bed contribution of only Acetaminophen was significantly different between the two homes. These findings may be explained by the similar services provided in the long term care homes, as compared to the hospitals.

The significant differences in the per-bed contributions of six out of nine target compounds between the two hospitals which are located in different communities, but in the same region, undermine the idea of using per-bed contributions of one facility to estimate the discharges of PhACs in the other such facility effluent.

5.2.2 Comparison with other Hospital Effluent Studies

The reported mass flows per-bed of the PhACs in the hospital effluents, in various studies shows that there are considerable variations in the per-bed mass contributions of different-sized hospitals. Table 5-10 shows the results of this study and those of others; HS_3 (Heberer et al., 2005) and HS_4 (Thomas et al., 2007). The per-bed mass flow of Carbamazepine (HS_3 in Table 5-9) was higher (1.71 mg/day) than that in this study (0.27 and 0.1 mg/day). Similarly, the per-bed contributions reported (HS_4 in Table 5-9) for Ciprofloxacin (23.91 mg/day) and Metoprolol (0.92 mg/day) were higher than that found in this study (between 0.1 and 1 mg/day). In contrast, the per-bed mass flows of Sulfamethoxazole (5.52 mg/day), Trimethoprim (5.5 mg/day), and Acetaminophen (126.8 mg/day) were higher in this study than the other two studies (0.25, 1.83, 46.6 mg/day respectively). Therefore, it would appear that the mass flows in hospital effluents are not related to facility size only, as defined by number of beds, but services provided by each facility need to be considered and further research about the causes of variability need to be investigated.

Table 5-10 Per-bed mass contributions of target PhACs to hospital effluents, comparison with other studies.

Target PhACs	Mass flows in hospital effluents (mg/bed /day)			
	263[a](This Study)	300[a] (HS_3)	365[a](This Study)	1200[a] (HS_4)
Sulfamethoxazole	5.32		0.98	0.25
Trimethoprim	5.55		0.48	1.83
Ciprofloxacin	0.10		1.01	23.91
Acetaminophen	9.21		126.68	46.66
Carbamazepine	0.27	1.71	0.10	
Metoprolol	0.10		0.53	0.92
Venlafaxine	3.08		0.82	

The average mass flows are divided with the number of hospital beds

[a] *This number represent the number of beds in hospital*

Chapter 6
Contribution of Target PhACs by Healthcare Facilities to WWTPs

Healthcare facilities, especially hospitals are suspected to be the major contributors of PhACs to WWTPs because they use considerable amounts of these compounds. To investigate this assumption the relative contributions of hospitals and long-term-care homes to their respective downstream WWTPs were studied. The mass flow of the target PhACs discharged by each investigated healthcare facility was compared with the mass flow entering the respective downstream WWTPs. The relative contributions were calculated using mass balances. An important consideration in this comparison is the sewer travel times. Sewer travel time (for this study) was defined as "the time taken by the pharmaceutical compound to travel from the sampling point at the healthcare facility (discharge point) to the sampling point at the influent of its downstream WWTP". It was appropriate to compare same day composite samples from the two points only when the sewer-travel times were in a reasonable range (< 6 hours was assumed for this study using 24-hours composite samples).

Sewer travel times were estimated using a minimum sewer velocity of 2 ft/sec (Stephenson, 1998), and sewer line lengths were estimated based on measurements taken of the roads running above them. Table 6-1 shows the estimated travel times between the investigated healthcare facilities and their respective downstream WWTPs.

A maximum sewer travel time of five hours was found between LTC_1 and WWTP-LTC_1 (Table 6-1); for the rest of the healthcare facilities, it varied from <1 to 3 hours. This finding suggests that for 24- hour composite samples, the healthcare facility effluents arrive on the same day (24 hours) in the WWTP influents.

Table 6-1 Sewer travel times from the healthcare facilities to downstream WWTPs

FROM	TO	Length of Sewer line (meters)	Wastewater flow velocity (meters/min)	Sewer Travel time (min)	Sewer Travel time (hr)
HS_1	**WWTP-HS_1**	7000	36.57	191	**3**
HS_2	**WWTP-HS_2**	1000	36.57	27	**0.5**
LTC_1	**WWTP-LTC_1**	11000	36.57	300	**5**
LTC_2	**WWTP-LTC_2**	1000	36.57	27	**0.5**

6.1 Target Compound Contributions by Healthcare Facilities to WWTPs

The relative contribution of PhACs by the healthcare facilities to their respective WWTPs' influent loads were calculated using mass balances. The mass flows of the target compounds in the healthcare facility effluents during 24 hours and the total influent loads of these compounds in the respective downstream WWTPs were compared to estimate the relative contributions by the investigated facilities. For example the contribution of Sulfamethoxazole by the HS_1 to its downstream WWTP (WWTP-HS_1) is presented in Table 6-2. This table shows that the mass of Sulfamethoxazole discharged by HS_1 on Wednesday was 0.46 g, which was calculated by multiplying column (1) and column (2) of Table 6-2 *(Results were then multiplied by 10^{-6} for unit conversions)*. Similarly the influent mass of WWTP-$HS_1$was calculated using column (4) and (5), which yielded 10.98 g for that day. Thus, the contribution of HS_1 for Sulfamethoxazole on Wednesday (0.46 g) was 4.2% of the total influent mass flow of WWTP-HS_1 (10.98 g) for that day. Likewise, the daily mass contributions of the target compounds by the healthcare facilities to their respective WWTPs were calculated and are presented in Figures 6-1 to 6-4.

Table 6-2 Sulfamethoxazole contributions by HS_1 to WWTP-HS_1

Target compound	Week day	HS_1			WWTP-HS_1			Contribution by HS_1 (7)
		Effluent Conc (ng/L) (1)	Avg WW flow (m^3/day) (2)	Mass (g/day) (3)	Influent Conc (ng/L) (4)	Avg WW flow (m^3/day) (5)	Mass (g/day) (6)	
Sulfamethoxazole	Wed	996.0	464.4	0.46	420	26157	10.98	**4.2 %**
	Thu	888.0	464.4	0.41	171	49779	8.49	**4.8 %**
	Fri	440.0	464.4	0.20	277	31798	8.81	**2.3 %**

Conc = Concentration
Avg = Average
WW = Wastewater

The contribution of the target compounds by the HS_1 facility (Figure 6-1) to the influent load of WWTP-HS_1 varied over the week days. The contribution varied from 7.5 to 26.7 % for Ciprofloxacin, 2.4 to 13% for Metoprolol, 2 to 6% for N-desmethylvenlafaxine, 3 to 5% for Sulfamethoxazole, 2 to 3% for Acetaminophen, 1 to 3% for Venlafaxine and O-desmethylvenlafaxine, 1.5 to 2.5% for Trimethoprim, and <1 to 1% for Carbamazepine. The Ciprofloxacin contribution of HS_1 was greater than 10% except for Thursday (7.5%).

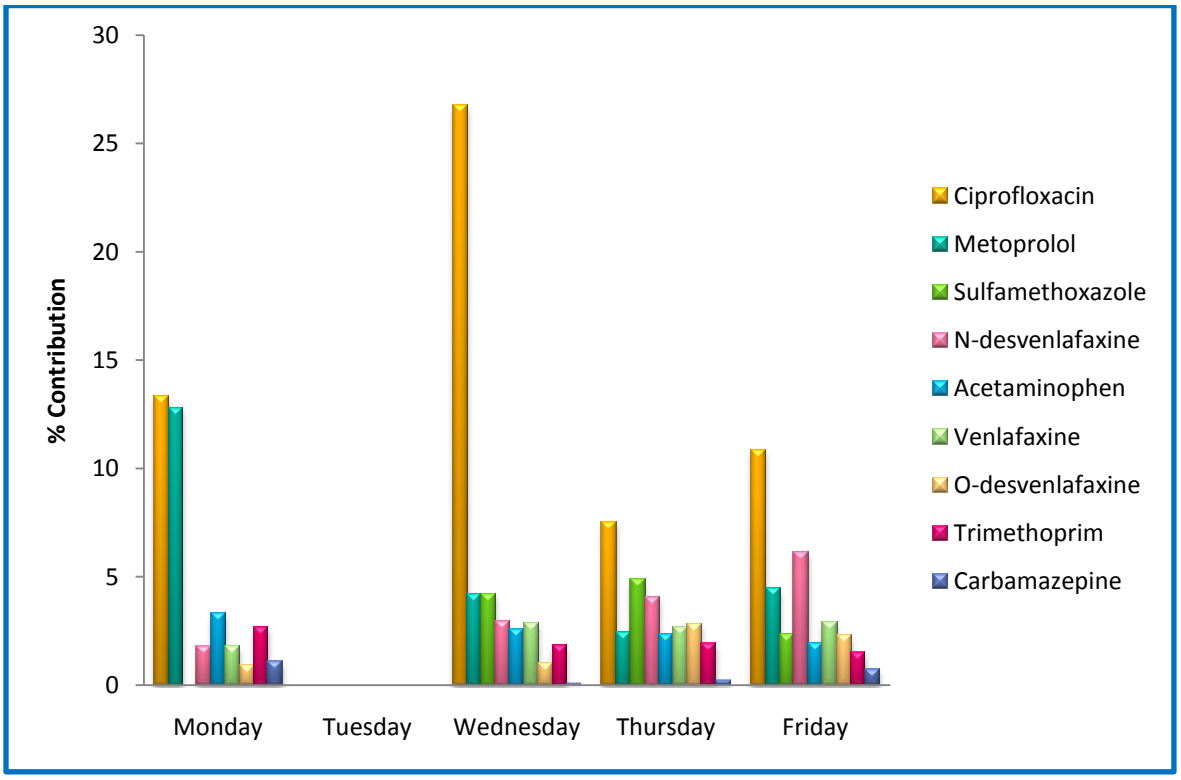


Figure 6-1 Contribution of target PhACs by the HS_1 to WWTP-HS_1

The contribution of Trimethoprim, Sulfamethoxazole and Venlafaxine by HS_2 to WWTP-HS_2 over the weekdays varied between 3 and 8.5 %; 2 and 5.6 %; and <1 and 3.4 % respectively (Figure 6-2). For the rest of the target compounds its contribution was less than 1%.

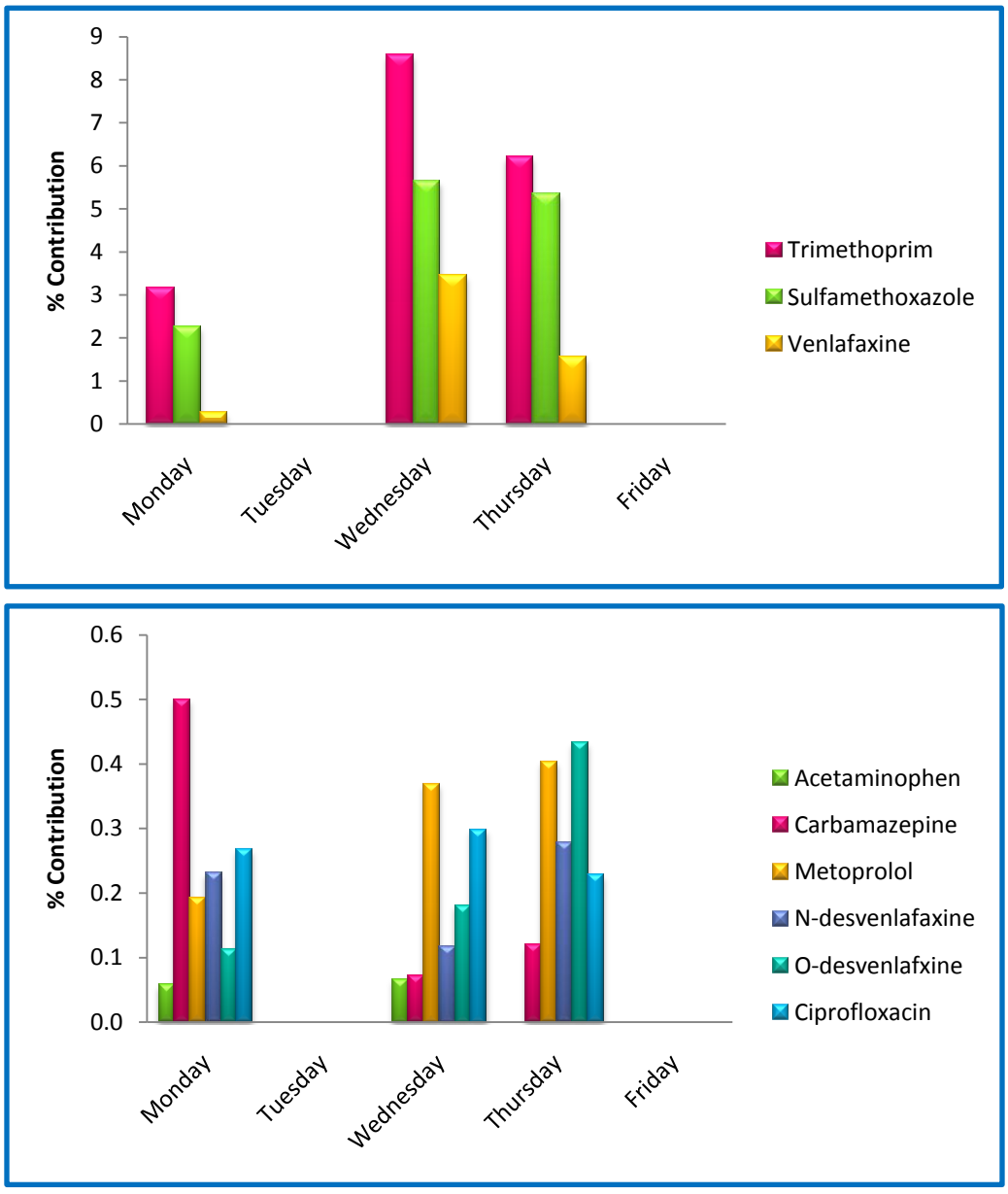


Figure 6-2 Contribution of target PhACs by HS_2 to WWTP-HS_2

The maximum contribution of the target compounds by LTC_1 facility to the WWTP-LTC_1 was 4.4% for Metoprolol (Thursday) and 4.3% of Trimethoprim (Thursday) (Figure 6-3). For the rest of the target compounds its contribution was less than 2%.

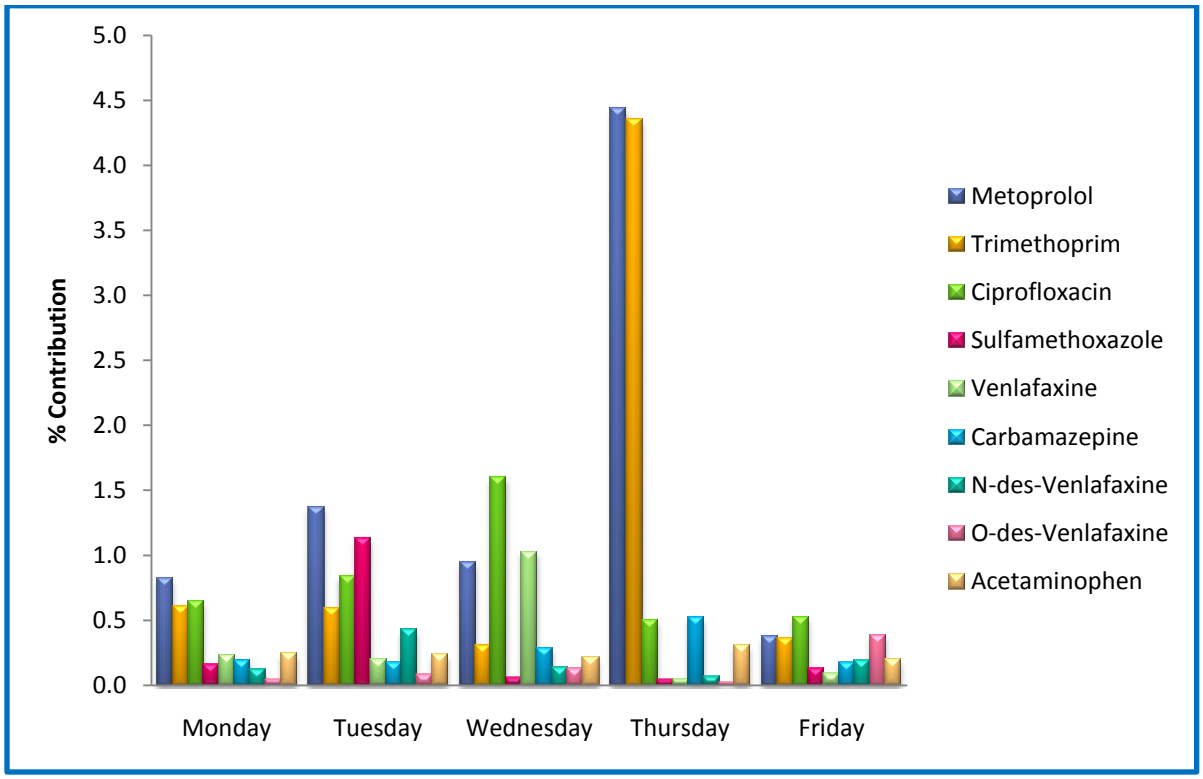


Figure 6-3 Contribution of the target PhACs by the LTC_1 to WWTP-LTC_1

The maximum contributions of the target compounds by the LTC_2 facility to WWTP-LTC_2 were 37% for Ciprofloxacin, 11.7% for Trimethoprim (Figure 6-4). For all other target compounds its contributions was less than 2%. The highest Ciprofloxacin contribution by LTC_2 was observed on Thursday (37%), and for the rest of the week days, the contribution of this compound was less than 1%.

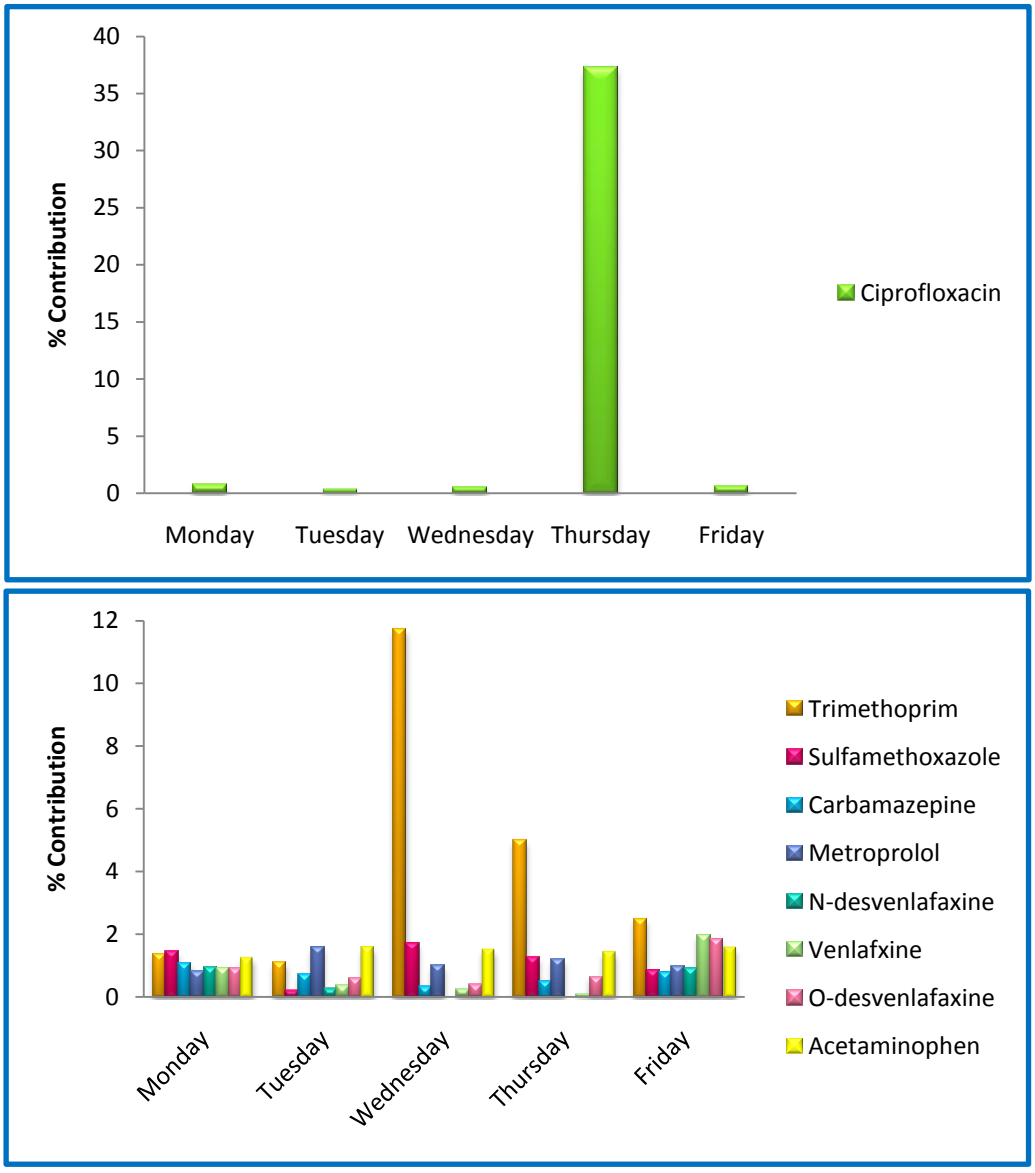


Figure 6-4 Contribution of target PhACs by LTC_2 to WWTP-LTC_2

The individual contribution peaks for certain compounds (Figures 6-1 to 6-4) reveal the importance of monitoring contributions of PhACs over a reasonable period of time. For instance the contribution of Ciprofloxacin by LTC_2 to WWTP-LTC_2 on Thursday was 37% (Figure 6-4). For the rest of the weekdays, its contribution was less than 1%. Furthermore, the contributions by the healthcare facilities varied over the weekdays, with maximum values on a particular day; for example, the contribution of Ciprofloxacin by HS_1 to WWTP-HS_1 was 13% on Monday, 26.7% on Wednesday (max), 7.5% on Thursday, and 10.8% on Friday. Therefore, in the first case the 37% contribution, which had no apparent relation/trends with other weekdays, was likely to be caused by the disposal of un-needed and expired compounds. But in the second case, the 26.7% contribution by HS_1, which showed an increasing trend from Monday to Wednesday, decreased on Thursday and started to rise on Friday, perhaps indicating therapeutic use. Only week-long sampling could identify individual spikes and trends and gives a clear picture. Shorter sampling events (one to two days) cannot identify such incidents and may provide misleading findings.

The differences between the investigated hospitals in their contribution of target PhACs to the respective downstream WWTP loads can be explained by the differences in facility sizes. Higher contributions were observed for HS_1 than HS_2. HS_1 was a relatively lager hospital (365 beds) than HS_2 (263 beds). In contrast WWTP-HS_1 (which received discharges from HS_1) was a smaller facility (serving a community of 51,218 people) than WWTP-HS_2 (171000 people). Thus, the effluent from the relatively bigger hospital (HS_1) was discharged to a relatively smaller WWTP (WWTP-HS_1) and vice-versa, which led to relatively higher contributions by HS_1 than HS_2. Similarly, higher contributions by LTC_2 than LTC_1 were probably caused by the relatively smaller community that contributed to the total pharmaceutical load of WWTP-LTC_2 than that for WWTP-LTC_1. WWTP-LTC_1 was more than double in size (80000 population) than WWTP- LTC_2 (33000 population). This suggests that, in any comparison of healthcare facilities of their contribution of PhACs to downstream WWTPs, the size of the WWTPs should be considered in addition to facility sizes.

Based on the maximum contributions by the investigated healthcare facilities to their respective WWTPs, the target PhACs could be divided into three groups. 1) compounds whose hospital contributions were below 5% (Acetaminophen, Carbamazepine, Venlafaxine and O-desmethylvenlafaxine). Four compounds out of nine were in this group. 2) Contributions between 5 and 15% (Sulfamethoxazole, Trimethoprim, Metoprolol and N-desmethylvenlafaxine), four

compounds were belong to this group. 3) Compounds whose hospital contribution exceeded 15% (Ciprofloxacin). Only one compound was found to be in this group. The average contribution of Ciprofloxacin by HS_1 to WWTP-HS_1 was 14.6%.

The weekly maximum contributions by the long-term-care homes to the WWTP loads were less than 5% for seven compounds. One compound (Trimethoprim) was between 5 to 15%, and one compound was more than 15% (Ciprofloxacin); the maximum observed contribution of Ciprofloxacin was 37% by LTC_2 to WWTP-LTC_2 (Figure 6-4).

6.2 Comparison between the Healthcare Facilities for their Contribution of PhACs to WWTPs

Unlike mass flows of the target compounds in the healthcare facility effluents, seasonal variations are not expected to affect the contribution of these compounds by the facilities to the downstream WWTPs; as seasonal differences probably affect all the possible sources contributing to the loads of WWTPs in the same way. Thus, PhAC contributions by the healthcare facilities were assumed to be a function of facility sizes and their service spectrum. Further, the facilities were located in different areas, and their effluents were discharged to different-sized WWTPs; thus, the additional factor affecting contributions may be the size of the communities that contribute PhAC loads to the same WWTPs. To study the effects of these factors, the investigated healthcare facilities were compared for their contributions to the respective WWTP influent loads using one-way ANOVA.

A comparison between HS_1 and HS_2 showed no significant differences in the contribution of Sulfamethoxazole, Carbamazepine, Metoprolol, and Venlafaxine. Significant differences were observed in Trimethoprim, Ciprofloxacin, Acetaminophen and Venlafaxine metabolites (P-values highlighted red in Table 6-2).

Table 6-3 Target compound contributions by hospitals to respective downstream WWTPs

Target Compounds	Facility	Contributions to the WWTPs (%)				P-value
		Mon	Wed	Thu	Fri	
Sulfamethoxazole	**HS_1**		4.21	4.86	2.32	**0.662**
	HS_2	2.27	5.65	5.34		
Trimethoprim	**HS_1**	2.70	1.84	1.92	1.49	**0.031**
	HS_2	3.16	8.58	6.21		
Ciprofloxacin	**HS_1**	13.3	26.7	7.53	10.8	**0.035**
	HS_2	0.27	0.30	0.23		
Acetaminophen	**HS_1**	3.31	2.57	2.35	1.95	**0.001**
	HS_2	0.06	0.07	0.05		
Carbamazepine	**HS_1**	1.10	0.07	0.22	0.75	**0.365**
	HS_2	0.50	0.07	0.12		
Metoprolol	**HS_1**	12.8	4.20	2.43	4.47	**0.095**
	HS_2	0.19	0.37	0.40		
Venlafaxine	**HS_1**	1.79	2.87	2.68	2.91	**0.380**
	HS_2	0.28	3.46	1.55		
N-desmethylvenlafaxine	**HS_1**	1.79	2.95	4.08	6.12	**0.023**
	HS_2	0.23	0.12	0.28		
O-desmethylvenlafaxine	**HS_1**	0.90	1.02	2.82	2.29	**0.044**
	HS_2	0.11	0.18	0.43		

The similar contributions of Sulfamethoxazole and Venlafaxine compounds by HS_1 and HS_2 was due to the fact that the differences in mass flows of these compounds in their effluents were compensated for by the differences in the sizes of their respective WWTPs. Higher mass flows of these compounds were observed in HS_2 effluent than in HS_1. The average mass flows of Sulfamethoxazole and Venlafaxine in HS_2 effluent were 1400 and 800 mg/day as compared to 360 and 300 mg/day in HS_1. However, the HS_2 effluent was discharged to a relatively bigger wastewater treatment facility (WWTP-HS_2) with mean influent loads of Sulfamethoxazole and Venlafaxine of 36700 and 45200 mg/day, respectively, as compared to WWTP-HS_1, with influent loads of Sulfamethoxazole (11200 mg/day) and Venlafaxine (11900 mg/day). This reversal in proportion led to similar overall contributions.

Similarly, the daily mass flow of Carbamazepine (60 mg) was higher in the HS_2 effluent than in HS_1 (40 mg). However, WWTP-HS_2 had daily average Carbamazepine load of 29500 mg, as compared to 9900 mg and in WWTP-HS_1. Thus, the overall contributions by the two hospitals to their respective WWTPs were found to be same.

In contrast, the daily mass flows of Ciprofloxacin (370 mg), Acetaminophen (46240 mg), N-desmethylvenlafaxine (150 mg) and O-desmethylvenlafaxine (570 mg) were higher in the HS_1 effluent than in HS_2 (30, 2400, 30, 200 mg respectively). The higher mass flows from HS_1 were discharged to relatively smaller a WWTP than from HS_2 leading to higher contributions by HS_1 than HS_2 to their respective WWTPs.

The contribution of Metoprolol by HS_1 and HS_2 were not statistically different, due to the higher variability in day-to-day contributions the HS_1. The contributions by HS_1 varied from 2.4 to 12.8% as compared to HS_2 (<1%). This discrepancy was probably due to the higher mass flows of Metoprolol in HS_1 and also because its effluent was discharged to a relatively smaller than WWTP compared to HS_2. In summary, the data on hospital sizes along with corresponding WWTP's size defines the relative contributions by hospitals to WWTPs.

The mass flow of Trimethoprim was higher in the HS_2 effluent than in the HS_1'effluent (1500 and 180 mg/day respectively). Although the HS_2 effluent discharged into a larger WWTP, the difference between the WWTP influent mass flows for this compound was less than the difference between the mass discharged by these facilities (WWTP-HS_2 and WWTP-HS_1 influent mass flow were 24.7 and 9.2 g/day respectively), leading to different overall contributions.

A comparison between LTC_1 and LTC_2 (Table 6-4) for their contributions to the respective WWTP influent loads showed no significant difference in the contributions of Trimethoprim, Ciprofloxacin, Metoprolol, Venlafaxine and N-desmethylvenlafaxine. Significant differences were observed in Sulfamethoxazole, Acetaminophen, Carbamazepine, and O-desmethylvenlafaxine contributions.

Table 6-4 Contribution of target PhACs by long-term-care homes to respective downstream WWTPs

Target Compounds	Facility	Contribution to the WWTPs (%)					P-value
		Mon	Tue	Wed	Thu	Fri	
Sulfamethoxazole	**LTC_1**	0.16	1.13	0.06	0.04	0.13	**0.04**
	LTC_2	1.45	0.21	1.70	1.27	0.86	
Trimethoprim	**LTC_1**	0.60	0.59	0.31	4.36	0.36	**0.18**
	LTC_2	1.38	1.08	11.71	4.98	2.48	
Ciprofloxacin	**LTC_1**	0.64	0.84	1.60	0.51	0.53	**0.36**
	LTC_2	0.73	0.30	0.52	37.27	0.62	
Acetaminophen	**LTC_1**	0.25	0.23	0.21	0.31	0.20	**000**
	LTC_2	1.24	1.58	1.50	1.43	1.56	
Carbamazepine	**LTC_1**	0.19	0.17	0.29	0.52	0.17	**0.01**
	LTC_2	1.07	0.73	0.33	0.53	0.80	
Metoprolol	**LTC_1**	0.82	1.36	0.94	4.43	0.38	**0.53**
	LTC_2	0.81	1.59	0.99	1.18	0.97	
Venlafaxine	**LTC_1**	0.23	0.20	1.03	0.05	0.09	**0.33**
	LTC_2	0.90	0.36	0.25	0.09	1.95	
N-desmethylvenlafaxine	**LTC_1**	0.12	0.43	0.14	0.07	0.19	**0.32**
	LTC_2	0.94	0.26	0.00	0.00	0.91	
O-desmethylvenlafaxine	**LTC_1**	0.04	0.08	0.13	0.02	0.39	**0.02**
	LTC_2	0.90	0.60	0.39	0.65	1.83	

Long-term-care homes provide similar services; therefore, the mass flows of the target PhACs in their effluents were not significantly different except for Acetaminophen (Table 6-4). Differences in the contributions by each home presumably depend on the size of the downstream WWTPs. Additional consideration may be other healthcare facilities upstream of the respective WWTPs. WWTP-LTC_1 has a small hospital (68 beds) upstream, thus leading to lower overall contributions from LTC_1 to WWTP-LTC_1. The highest contribution of Ciprofloxacin by LTC_2 was 37%, as compared to 1.6% by LTC_1, but since this finding was a single event, the contributions were not found to be significantly different.

6.3 Comparison with other Hospital Effluent Studies

The contributions of target compounds by the investigated hospitals were compared with findings from other hospital studies (Table 6-5). Higher contributions of Sulfamethoxazole and Metoprolol (5.6 and 12.8 % respectively) were observed in this study than those reported in the other hospital studies (2.5 % for Sulfamethoxazole and 7 % for Metoprolol). In contrast, lower contributions of Trimethoprim (3 and 8.5%) and Acetaminophen (0.1 and 3.3 %) were observed in this study than in other studies (18.3 and 10.5 % of Trimethoprim and 5.8 and 9.8 % of Acetaminophen). The contribution of Carbamazepine by the hospitals investigated had a similar contribution range (0.5 and 1 %) to that reported in other hospital studies (0.5 and 1.3 %).

Table 6-5 : Comparison with other studies for maximum contributions from the hospitals to the downstream WWTPs

Target Compounds	size (beds)	WWTP size (pop served)	Number of Bed/1000 population	Max Contribution to WWTPs (%)	Reference
Sulfamethoxazole	365	51218	**7.1**	5	This study
	200	45000	4.4	2.2	(Ort et al., 2010)
	1200	440000	2.7	<1	(Thomas et al., 2007)
	263	171000	**1.5**	5.6	This study
Trimethoprim	200	45000	**4.4**	18.3	(Ort et al., 2010)
	1200	440000	2.7	10.5	(Thomas et al., 2007)
	263	171000	1.5	8.5	This study
	365	51218	**7.1**	3	This study
Carbamazepine	200	45000	4.4	1.3	(Ort et al., 2010)
	263	171000	**1.5**	0.5	This study
	300	1000000	0.3	0.5	(T. Heberer et al., 2005)
	365	51218	7.1	1	This study
Metoprolol	365	51218	**7.1**	12.8	This study
	200	45000	4.4	7	(Ort et al., 2010)
	1200	440000	2.7	<1	(Thomas et al., 2007)
	263	171000	**1.5**	0.5	This study
Acetaminophen	200	45000	4.4	9.8	(Ort et al., 2010)
	1200	440000	2.7	5.8	(Thomas et al., 2007)
	263	171000	**1.5**	0.1	This study
	365	51218	7.1	3.3	This study

The differences in contributions of target compounds by the hospitals to their respective WWTPs (Table 6-5) can be explained by, the differences in the relative sizes of the hospitals to their

community. The capacity of the hospital (number of beds per 1000 population) that relates the size of the hospital to its community size can be used to illustrate this assumption. Relatively higher contributions were observed for the hospitals with higher bed capacity. For instance, contributions of Sulfamethoxazole by hospitals with capacities of 7.1, 4.4, and 2.7 beds/1000 population to their respective WWTPs were respectively 5, 2.2, <1 %. Similarly, Trimethoprim contributions by hospitals with a capacity of 4.4, 2.7, and 1.5 beds/1000 population were 18.3, 10.5, and 8.5 % respectively. This concept held true for all compounds in most of the hospitals, as shown in Table 6-5 (green background).

The contributions of the investigated long-term-care homes followed similar patterns; a higher contribution of target compounds was observed for LTC_2 (capacity 6 beds/1000 population) than for LTC_1 (capacity of 2.8 beds/1000 population). Thus, based on the available data, it is concluded that the capacity of the hospital may be a useful indicator of relative contributions by hospitals to their local WWTPs.

In addition the services provided by the hospitals are important, as some provide more specialized types of treatment than others; thus, compounds specific to their specialty have a higher probability of being contributed by these facilities. The higher contribution (5.5 %) of Sulfamethoxazole by the hospital of lower capacity (1.5 beds/1000 population), presumably occurred because the difference in the service spectrum (Table 6-5). Similarly, lower contributions of Trimethoprim, Carbamazepine and Acetaminophen by a hospital with higher capacity (7.1 beds/1000 population) may be attributed to such differences. These findings indicate that in addition to the size of the hospital, it is also important to identify the services provided by the hospitals while comparing the results.

In this study only parent compounds were investigated, with the exception of the antidepressant Venlafaxine, therefore, the results may under estimate the contributions by healthcare facilities of certain compounds that are excreted in conjugated forms in considerable amounts. Studies have revealed that the conjugates (i.e., glucuronides) may be cleaved back to the parent compound during sewer transit (Ascenzo et al., 2003; Johnson et al., 2004). For example 93% of Acetaminophen is excreted as hydrolysable conjugates (Khan et al., 2004). Similarly, up to 50% of Sulfamethoxazole is excreted as the metabolite N4-acetylsulfamethoxazole, that has the tendency to cleave back to the original compound (Gobel et al., 2005). The conjugates in wastewater are expected to be de-

conjugated through bacterial hydrolysis, as intestinal bacterial flora (Escherichia coli) have been reported to have the ability to transform the conjugates into their parent compounds. This process may occur because these organisms (E-coli) synthesize considerable amounts of ß-glucuronidase enzyme (Baronti et al., 2000). Eldere et al. (1988) reported that conjugated steroids were mostly de-conjugated in the large intestine; similarly, Ascenzo et al. (2003) found that most of the fecal estrogens were free estrogens (de-conjugated), indicating the ability of the intestinal bacterial flora (E-coli) to transform the conjugates into the original compound (de-conjugation). The ability of fecal bacterial flora to de-conjugate glucuronide and sulfate conjugates of estrogens has also been reported by (Eldere et al., 1988; Lombardi et al., 1977; Ternes et al., 1999). Furthermore, Ascenzo et al., (2003) found that de-conjugation of estrogens, especially glucuronated conjugates, occurred during the sewer transit from a condominium building to the influent of a WWTP. Since these organisms are largely present in sewers, the de-conjugation process is also expected to occur for the pharmaceutical compound conjugates in the sewer systems. Hence, Henschel et al., (1997) have suggested that both glucuronic and sulfate conjugates of Acetaminophen may transform back to original compound in sewers. In short, measurement of conjugates of the compounds would be expected to increase the relative contributions of certain compounds by the healthcare facilities.

This study revealed that long-term-care homes may contribute significant amounts of certain compounds to WWTPs. Therefore it is important to consider discharges from these facilities in addition to hospitals to assess the overall contributions of pharmaceutical loads by the healthcare units to the WWTPs.

Chapter 7
Conclusion and Recommendations

7.1 Conclusions

Healthcare institutions are suspected to be the major contributors of pharmaceutical compound loads to the aquatic environment. Still, very few studies have exclusively measured their contributions to WWTPs. Further, these studies have only considered hospital contributions and totally ignored long term care homes, and with this approach they have largely underestimated the overall healthcare facility pharmaceutical contributions. This study has considered both types of healthcare facility and investigated their effluents for the occurrence of target pharmaceutical compounds, daily mass flows and their relative contributions to downstream WWTPs. The results are summarized as follows:

7.1.1 Frequency of Detection and Occurrence of PhACs in Healthcare Facility Effluents

1. All the nine target PhACs were detected in all samples from the hospitals and long term care home effluents except one metabolite of the antidepressant venlafaxine (N-desmethylvenlafaxine). This compound was not detected on two days in the effluent of a long-term-care home otherwise it occurred in all samples. Thus the frequency of detection of target PhACs was almost 100%.
2. The antibiotic compounds were detected in relatively higher concentrations in hospital effluents than in long-term-care homes. The highest detected concentrations in hospital effluents were of Sulfamethoxazole (10.9 μg/L), Trimethoprim (10.3 μg/L) and Ciprofloxacin (1.24 μg/L), compared to 1.3 μg/L, 6.6 μg/L, and 1.47 μg/L respectively in long-term-care home effluents.
3. Seasonal variations in drug consumption were observed to be affecting the Sulfamethoxazole and Trimethoprim concentrations in hospital effluents. For instance higher concentrations of these compounds were observed in November than in July. The average detected concentration of Sulfamethoxazole (7900 ng/L) and Trimethoprim (8200 ng/L) was in

November, compared to 775 ng/L of Sulfamethoxazole and 371 ng/L of Trimethoprim in July.

4. Individual concentration peaks of antibiotic compounds were observed in the long-term-care home effluents and are attributed to either a single therapeutic dose or disposal of unneeded and expired compounds. This finding also suggests that single-day sampling events may lead to inaccurate conclusions about the occurrence of these compounds in healthcare facility effluents. Five weekday sampling is recommended to indentify such peaks.
5. Ciprofloxacin concentrations followed similar patterns over week days in the hospital effluents, with lower concentrations on the first and last days of the week (Monday and Friday), and maximum were observed on Wednesdays.
6. Among all the detected compounds, Acetaminophen was the compound in highest concentrations in both types of healthcare facility effluents. The Acetaminophen concentration was measured in levels up to 134μg/L in hospital and 116μg/L in long-term-care home effluents.
7. A higher concentration of Venlafaxine was detected in the effluent of the hospital with a cancer clinic; up to 36 μg/L of Venlafaxine was measured in this clinic's effluent.
8. Target compound concentrations were found to vary more between hospital effluents than between long-term-care homes, probably because of the similar services provided by the long-term-care homes.
9. The day-to-day variability in the target compound concentrations was observed to be higher in long-term-care home effluents than in hospitals, except for Acetaminophen and Carbamazepine. The least variability in Acetaminophen (CV =.08) in long-term-care home effluent suggests that this compound is used regularly in these facilities.

7.1.2 Mass Flow in healthcare facility effluents and WWTP Influents.

1. The mass flows of target compounds were significantly different between the hospital effluents, except for Carbamazepine, Venlafaxine, and O-desmethylvenlafaxine. No significant differences were observed between the long-term-care facilities except for Acetaminophen.
2. The per-capita mass contribution of target compounds to the four WWTPs varied, indicating that the concentration predictions using typical per-capita mass contributions may lead to considerable underestimation of the actual values.
3. The estimated wastewater flows were 1.8 times higher in the relatively smaller long-term-care facility (200 beds); a finding that suggests that the mass flow estimates using typical average wastewater flows/bed may lead to wide variation to the actual mass flows.
4. The larger size hospitals (as defined by the number of beds) do not necessarily discharge all pharmaceutical compounds in higher amounts than do smaller hospitals. Rather, the number of staffed and in-operation beds can define the expected mass discharges of PhACs into the wastewater.

7.1.3 Contributions of PhACs by the Healthcare Facilities to WWTPs

1. Relatively higher contributions of antibiotic compounds by the investigated healthcare facilities to the influent load of WWTPs were observed. The maximum contributions of antibiotic compounds by hospitals to their respective WWTPs were Sulfamethoxazole (10%), Trimethoprim (8.5%), and Ciprofloxacin (26.7%). The long-term-care home's maximum contributions were 1.7 % for Sulfamethoxazole, 11.7 % for Trimethoprim, and 37% for Ciprofloxacin.
2. Up to 12.8% of Metoprolol contribution by hospitals and 4.4% by long-term-care homes was observed. For all the other target compounds, the contributions by the investigated healthcare facilities were less than 4%.

3. Relatively higher contributions of most of the target compounds were observed for hospitals with higher bed capacity (number of beds/1000 population). For instance, the Sulfamethoxazole contributions by hospitals with bed capacities of 7.1, 4.4 and 2.7 beds/1000 population were 5, 2.2, and <1% respectively.

4. The target compound contributions by a healthcare facility varies, depending upon the size of the facility itself, and the size of the community that contributes pharmaceutical compound loads to the same WWTP. For most of the target compounds, higher contributions were observed for large size facilities (number of beds) that discharge their effluents to WWTPs serving relatively smaller communities.

5. Since hospitals vary considerably in the services they provide, and thus the drugs they use, thus the findings of this study may not be representative of all the hospitals (in Ontario). Long-term-care homes on the other hand, do tend to provide similar services. This fact is supported by statistical findings

7.2 Recommendations

1. This study revealed that long-term-care homes can contribute significant amounts of certain PhACs to downstream WWTPs. Therefore, these facilities, in addition to hospitals must be considered in any assessment of PhAC contributions by healthcare units to downstream WWTPs in relations to other sources.
2. The direct involvement of hospitals in hospital effluent studies is recommended because the information they can provide is expected to improve interpretation of the study results. Moreover, the pharmaceutical compound consumption data for the facility will be readily available to compare with the measured concentrations.
3. Prediction models can be a useful tool in assessing emissions from healthcare facilities. Existing health-related databases contain useful information, and so an assessment of the possible use of these databases to predict discharge of pharmaceuticals from healthcare

facilities is recommended. The available databases include the IMS Canada database, which maintains comprehensive purchase data on hospitals, long term care homes, and drug stores across Canada; the Canadian MIS database (CMDB), which contains statistical information about all types of Canadian hospitals; the Discharge Abstract Database (DAD), which contains patient details including diagnoses; the Hospital Morbidity Database (DHMDB), which contains clinical information with a focus on acute care; and the Health Canada Drug Product Database (DPD) , which provides information about the drugs currently registered in Canada for human and veterinary applications. The IMS database is maintained by IMS Canada; CMDB, DAD and DHMDB are maintained by the Canadian Institute of Health Information (CIHI), and DPD is maintained by Health Canada.

4. The free chlorine concentration in hospital effluent that has been reported presumably occurs due to the use of disinfectants containing chlorine within hospitals. Certain antibiotic compounds (Ciprofloxacin and Sulfamethoxazole) are highly susceptible to chlorine oxidation. Their oxidation products have also been well studied by some researchers. A study of hospital wastewater on the occurrence of these compounds after use of various disinfectants is recommended. Oxidation of these compounds by use of suitable disinfectants containing appropriate doses of chlorine may be a good onsite treatment option. The possibility of halogenated by-products would be an important consideration for such a study.

5. The significance of healthcare facilities as potential point sources of PhACs, needs to be determined to allow the evaluation of long-term risks. To do so, requires understanding of trends in the health sector (hospitals and long term care homes) in terms of number of staffed beds and average inpatient days. A literature review shows that these trends have been both negative and positive over the years. For instance the average inpatient days decreased from 13 in 1978-79 to around 10 in 2002-2003, while the ambulatory care visits increased during this period. Such an assessment for both hospitals and long-term-care homes is recommended.

6. Examination of the top 50 drug prescriptions in recent years shows that the use of certain compounds is increasing, while some other are decreasing and some show similar usage (IMS Canada). Further, therapeutic doses of these compounds vary widely, so it is possible that a large mass of a relatively less-prescribed compound will be discharged instead of a highly prescribed one because of the differences in the therapeutic dose. Thus, developing a priority

list of compounds based on their consumption tends and therapeutic dose is recommended for future studies.

7. Due to time constraints the conjugates of the target compounds were not measured in this study, as the method development and validation was expected to take a longer time than the project duration. It is recommended that the conjugated form of target compounds be measure, as there is evidence that these compounds, especially glucuronides, have a tendency to revert to the original compounds during sewer transit. Measuring conjugates will provide more accurate assessment of the contributions by healthcare facilities to downstream WWTPs.
8. The biodegradation and sorption properties of biofilm in sewers have been reported. It is recommended that such a process be considered while comparing discharges from healthcare facilities and the influent concentrations of downstream WWTPs.

Appendix A

Chain of Custody forms

CHAIN OF CUSTODY FORM | University of Waterloo

Sampling Site: LTC_2 **Dated:** ----------/----------/ **2010**

Location: _____

a) Sampling information

Sample point	Facility effluent
Sample type	24 hours Composite
Sampling interval & volume	_____
Volume of the sample collected	_____
Volume of sample transported	_____
Sampling container type	Wide mouth amber glass bottles
No of sampling bottles	_____

b) Sample collected by

Name: ----------

Signature: ----------

c) Sample Received/transported by

Name: ----------

Signature: ----------

d) Sample Transportation

From:_____ To: Department of Civil & Environmental Engineering University of Waterloo

Date & Time of arrival : ----------/----------/2010, ----------am/pm

Transportation container Cooler

The samples are collected for a research project only that is being conducted within the Department of Civil and Environmental Engineering at the University of Waterloo
200 university Ave, Road West Waterloo Ontario N2L 3G1
Phone: 519-888-4567

Sampling information

Sampling Site: LTC_2_____

Dated: ----------/----------/2010

Activity description	Status
Take sampler out of manhole	☐
Check the machine whether any sample is missed	☐
Mixing the collected sample in the container	☐
Transferring the sample into sample bottles 02 bottles	☐
Flushing remaining sample back into the manhole	☐
Rinse the sampler container with DI water.	☐
Flush the sampler suction tubing with DI water.	☐
Replace the ICE packs in the sampler	☐
Reassemble & reprogram the machine for next day.	☐
Reposition it in the manhole	☐
Transfer the sampling bottles in the cold box.	☐
Transfer used ice packs to the car for next day	

Notes:

Number of samples missed: ----------

Time interval of missed samples: ----------

Time at sampler taken out, from manhole: ----------

Time the sampler positioned back into manhole: ----------

Sampling interval: ----------

Volume of the Sample collected in each interval: ----------

Total Volume of the sample collected: ----------

Any other comments:

Prepared by (Name & Signature): _____

Appendix B

Preliminary list of compounds (IMS databse,2008)

Preliminary list of compounds of in extended units (number of tablets, capsules etc)

Therapeutic class	Compound	Ontario Hospital purchases
Anti-Infective		
	CIPROFLOXACIN	43,778,610
	MOXIFLOXACIN	22,292,690
	LEVOFLOXACIN	7,439,350
	NYSTATIN	4,013,199
	AMOXICILLIN	3,558,440
	CEPHALEXIN	3,371,750
	LINEZOLID	2,056,500
	FLUCONAZOLE	1,892,911
	CLINDAMYCIN	2,056,254
	TRIMETHOPRIM	1,781,215
	SULFAMETHOXAZOLE	1,737,815
	PENICILLIN V	1,290,800
	ISONIAZID	2,259,900
	ERYTHROMYCIN	436,647
	METRONIDAZOLE	1,756,855
Anesthetics		
	PROPOFOL	30,123,410
	SEVOFLURANE	6,931,500
Psychotherapeutics		
	CLOZAPINE	8,313,300
	MIDAZOLAM	5,703,735
	LORAZEPAM	4,368,757
Cardiovasculars		
	METOPROLOL	9,965,140
	NITROGLYCERIN	3,177,090
	RAMIPRIL	2,532,468
	DILTIAZEM	2,401,165

Therapeutic class	Compound	Ontario Hospital purchases
Analgesics		
	ACETAMINOPHEN	32,613,040
	ACETYLSALICYLIC ACID	8,073,365
	FENTANYL	7,120,697
	MORPHINE	5,212,095
Neuro disorder		
	VALPROIC ACID	3,903,720
	PHENYTOIN	3,178,288
	GABAPENTIN	2,687,900
	CARBAMAZEPINE	1,850,000
Hemostatic modifier		
	WARFARIN	5,667,450
Oncology (Cytostatic agents)		
	FLUOROURACIL	3,521,920
	CISPLATIN	2,077,050
	RITUXIMAB	970,280
	GEMCITABINE	1,012,648
Contraceptives		
	ETHINYLESTRADIOL	4,669,831
	LEVONORGESTREL	1,670,612
Antihistamines		
	DIPHENHYDRAMINE	2,421,908
Thyroid Therapy		
	LEVOTHYROXINE	2,268,102
Hormones		
	PREDNISONE	3,285,200
	HYDROCORTISONE	2,523,210
	DEXAMETHASONE	2,126,963
Anti diabetic		
	METFORMIN	4,636,220
Lipid Regulators		
	ATORVASTATIN	2,949,360

Appendix C

Comparison of CV values for healthcare facility effluents

Table B1 Comparison between CVs

Facilities	Target compound	v_1	n_1	v_1	n_2	Absolute Diff	Standard Error	Conclusion
HS_1 & HS_2	Sulfamethoxazole	0.38	3	0.34	3	0.04	0.578	No significant difference
	Trimethoprim	0.26	4	0.35	3	0.09	0.437	No significant difference
	Ciprofloxacin	0.42	4	0.17	3	0.25	0.421	No significant difference
	Acetaminophen	0.27	4	0.16	3	0.11	0.297	No significant difference
	Carbamazepine	0.69	4	0.76	3	0.07	1.015	No significant difference
	Metoprolol	0.22	4	0.37	3	0.15	0.437	No significant difference
	Venlafaxine	0.12	4	0.89	3	0.77	0.940	No significant difference
	N-des-venlafaxine	0.27	4	0.4	3	0.13	0.486	No significant difference
	O-des-venlafaxine	0.5	4	0.56	3	0.06	0.743	No significant difference
LTC_1 & LTC_2	Sulfamethoxazole	1.43	5	0.6	5	0.83	1.131	No significant difference
	Trimethoprim	1.4	5	1.03	5	0.37	1.267	No significant difference
	Ciprofloxacin	0.54	5	1.96	5	1.42	1.483	No significant difference
	Acetaminophen	0.18	5	0.08	5	0.1	0.144	No significant difference
	Carbamazepine	0.6	5	0.45	5	0.15	0.547	No significant difference
	Metoprolol	0.92	5	0.29	5	0.63	0.703	No significant difference
	Venlafaxine	1.24	5	1.04	5	0.2	1.180	No significant difference
	N-des-venlafaxine	0.62	5	1.11	5	0.49	0.927	No significant difference
	O-des-venlafaxine	1.08	5	0.68	5	0.4	0.931	No significant difference

Appendix D

Calculation of CV for WWTP influent concentrations

WWTP-HS_1								
Target Compounds	**Day-1**	**Day-2**	**Day-3**	**Day-4**	**Day-5**	**Mean**	**sd**	**CV**
Sulfamethoxazole		605	420	171	277	368	188	0.51
Trimethoprim	389	412	309	153	330	319	102	0.32
Ciprofloxacin	97	41	82	105	63	77	26	0.34
Acetaminophen	83067	75867	65600	39907	51200	63128	17660	0.28
Carbamazepine	269	261	897	151	168	349	311	0.89
Metoprolol	49	179	207	145	161	148	60	0.41
Venlafaxine	632	439	389	259	325	409	142	0.35
N-desmethylvenlafaxine	248	120	170	81	99	144	67	0.47
O-desmethylvenlafaxine	6580	1816	1683	595	776	2290	2458	1.07

WWTP-HS_2								
Target Compounds	**Day-1**	**Day-2**	**Day-3**	**Day-4**	**Day-5**	**Mean**	**sd**	**CV**
Sulfamethoxazole	548	436	216	389	408	399	120	0.30
Trimethoprim	296	237	217	316	285	270	41	0.15
Ciprofloxacin	84	91	112	130	103	104	18	0.17
Acetaminophen	43600	41600	47600	41680	39573	42811	3033	0.07
Carbamazepine	255	191	207	719	230	321	224	0.70
Metoprolol	86	71	104	82	95	88	13	0.14
Venlafaxine	521	476	509	499	467	494	23	0.05
N-desmethylvenlafaxine	178	165	170	162	171	169	6	0.03
O-desmethylvenlafaxine	1613	1357	1233	1112	1363	1336	186	0.14

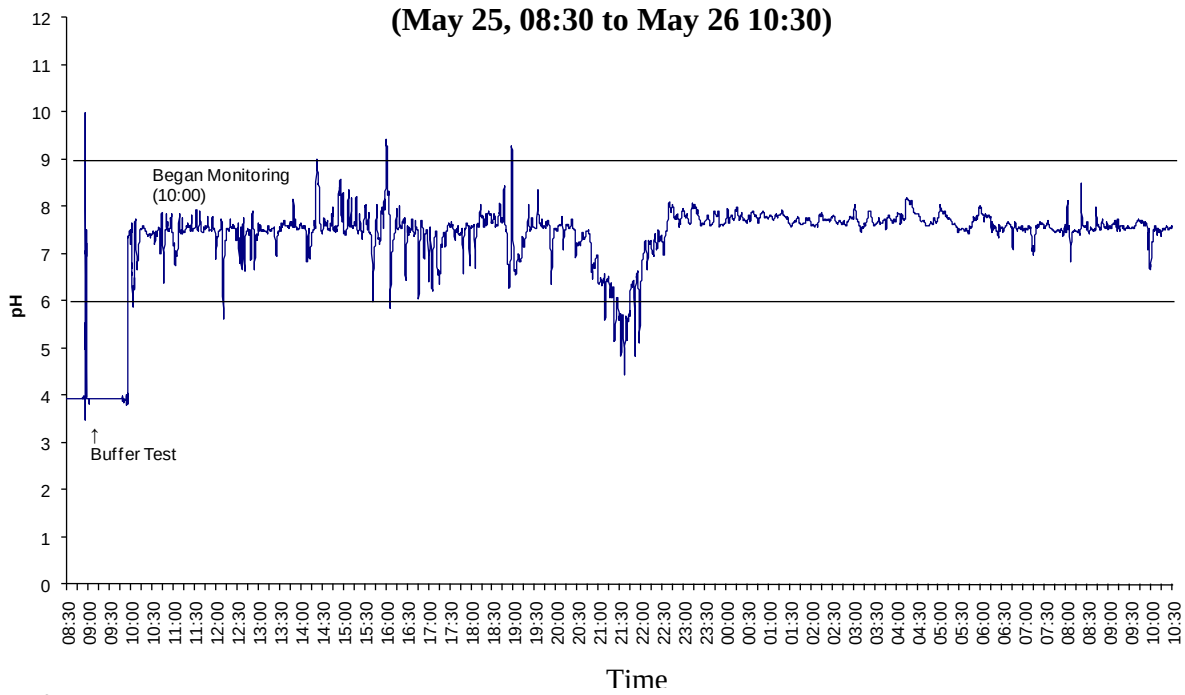
WWTP-LTC$_1$								
Target Compounds	**Day-1**	**Day-2**	**Day-3**	**Day-4**	**Day-5**	**Mean**	**sd**	**CV**
Sulfamethoxazole	441	378	461	407	445	426	34	0.08
Trimethoprim	226	292	264	271	353	281	47	0.17
Ciprofloxacin	57	76	69	80	74	71	9	0.13
Acetaminophen	64400	67333	66533	67600	70267	67227	2113	0.03
Carbamazepine	175	164	158	181	184	172	11	0.06
Metoprolol	235	257	217	199	261	234	26	0.11
Venlafaxine	444	394	402	432	513	437	47	0.11
N-desmethylvenlafaxine	190	115	124	114	126	134	32	0.24
O-desmethylvenlafaxine	5493	2897	3185	2328	3269	3435	1208	0.35

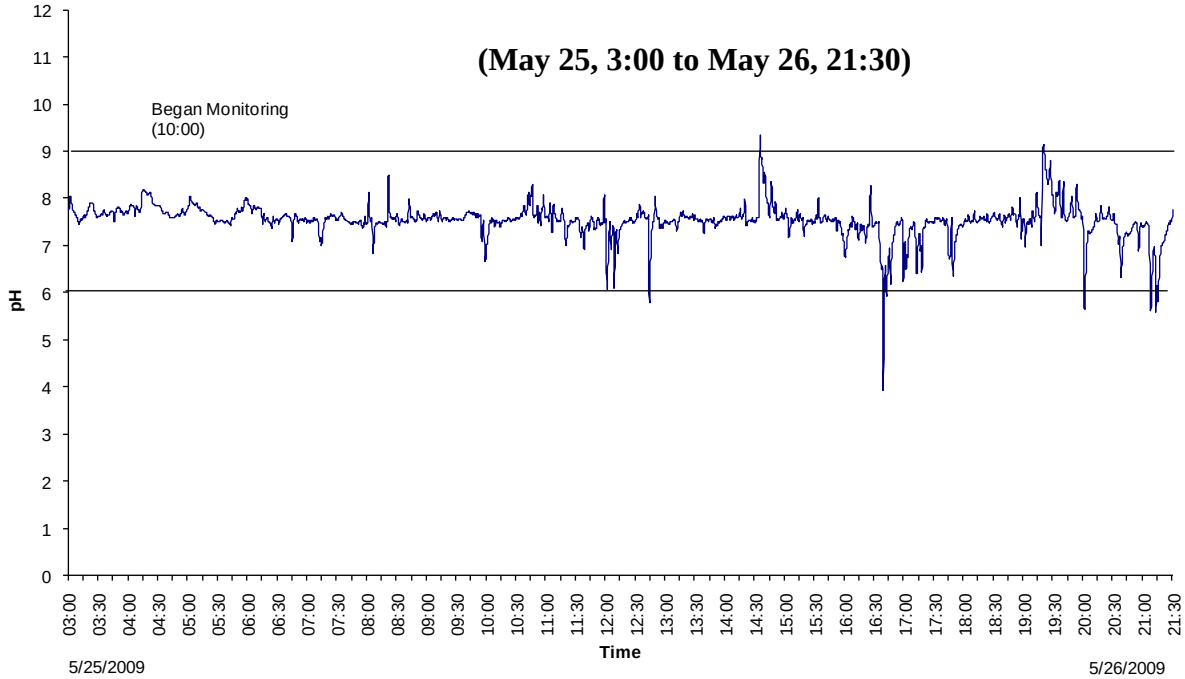
WWTP-LTC$_2$								
Target Compounds	**Day-1**	**Day-2**	**Day-3**	**Day-4**	**Day-5**	**Mean**	**sd**	**CV**
Sulfamethoxazole	472	540	476	349	252	418	115	0.28
Trimethoprim	200	244	186	194	100	185	52	0.28
Ciprofloxacin	78	151	68	45	57	80	41	0.52
Acetaminophen	67600	63200	64133	68400	42267	61120	10769	0.18
Carbamazepine	69	104	75	72	81	80	14	0.18
Metoprolol	282	227	219	244	135	221	54	0.24
Venlafaxine	427	449	439	456	308	416	61	0.15
N-desmethylvenlafaxine	145	145	143	177	103	143	26	0.18
O-desmethylvenlafaxine	1140	1160	1390	1295	969	1191	161	0.13

Appendix E

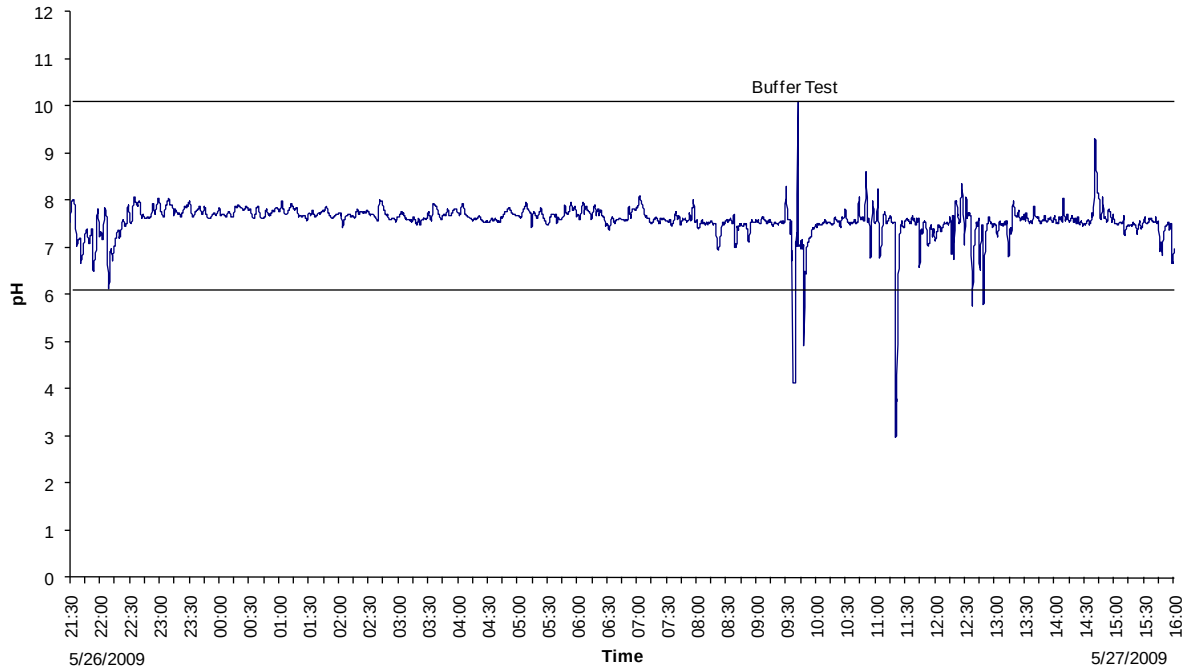
Healthcare facility effluent pH

D1) HS_1

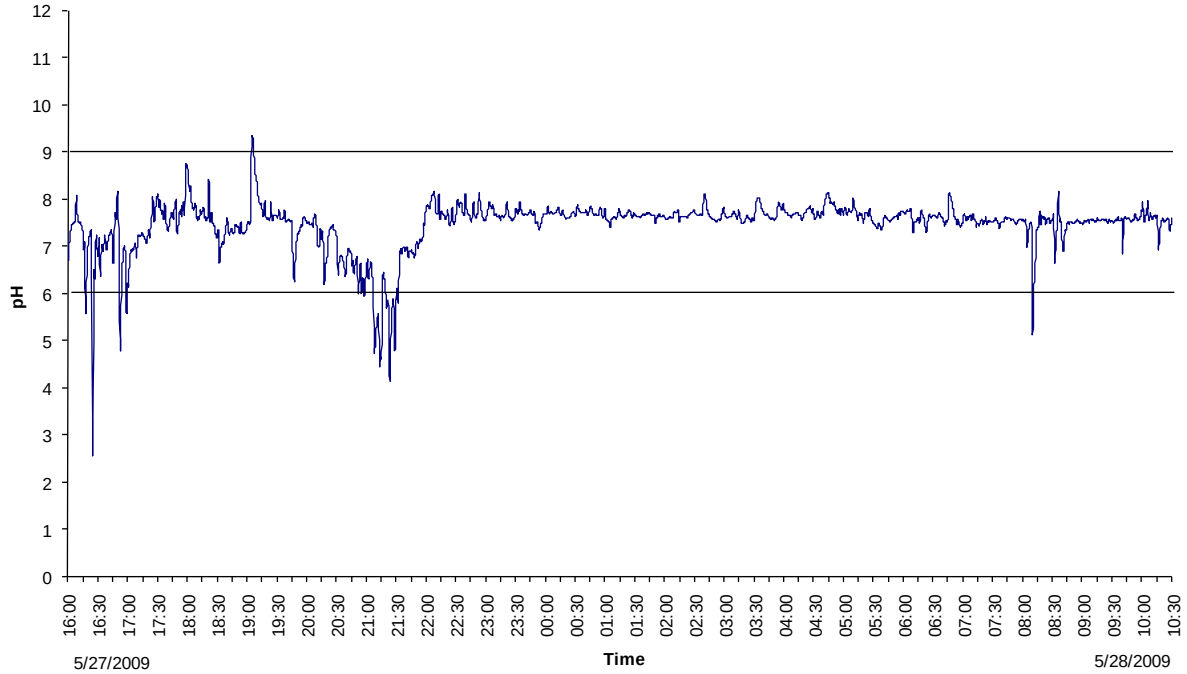




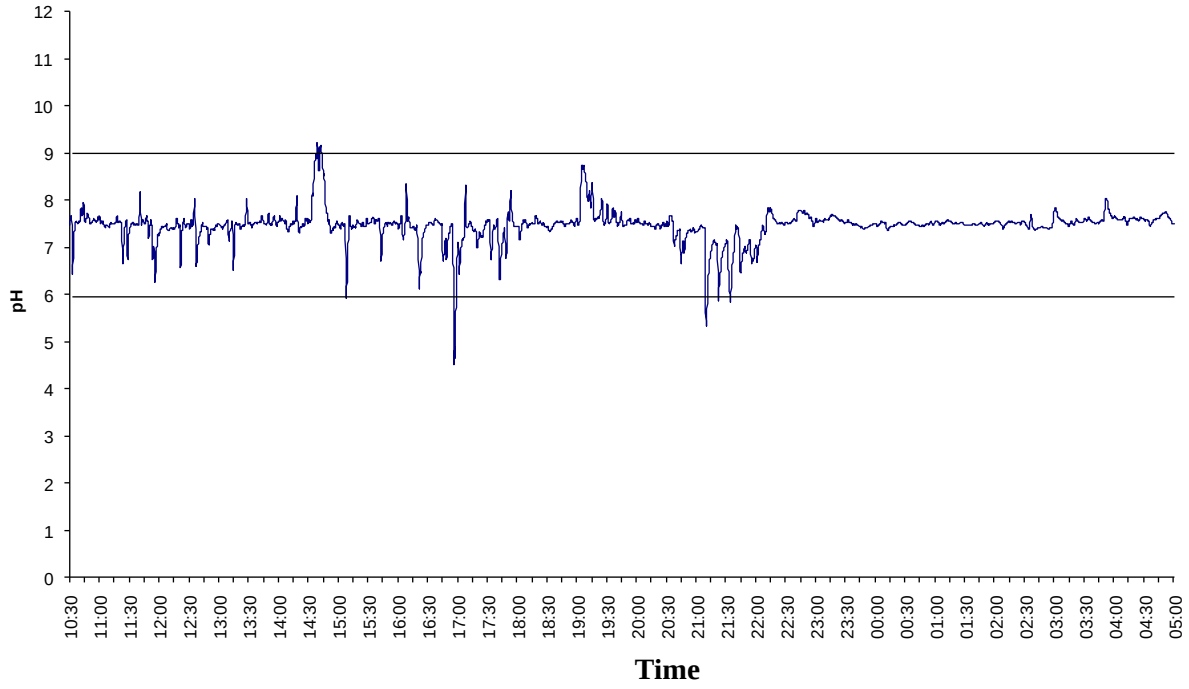
(May 26, 21:30 to May 27, 16:00)



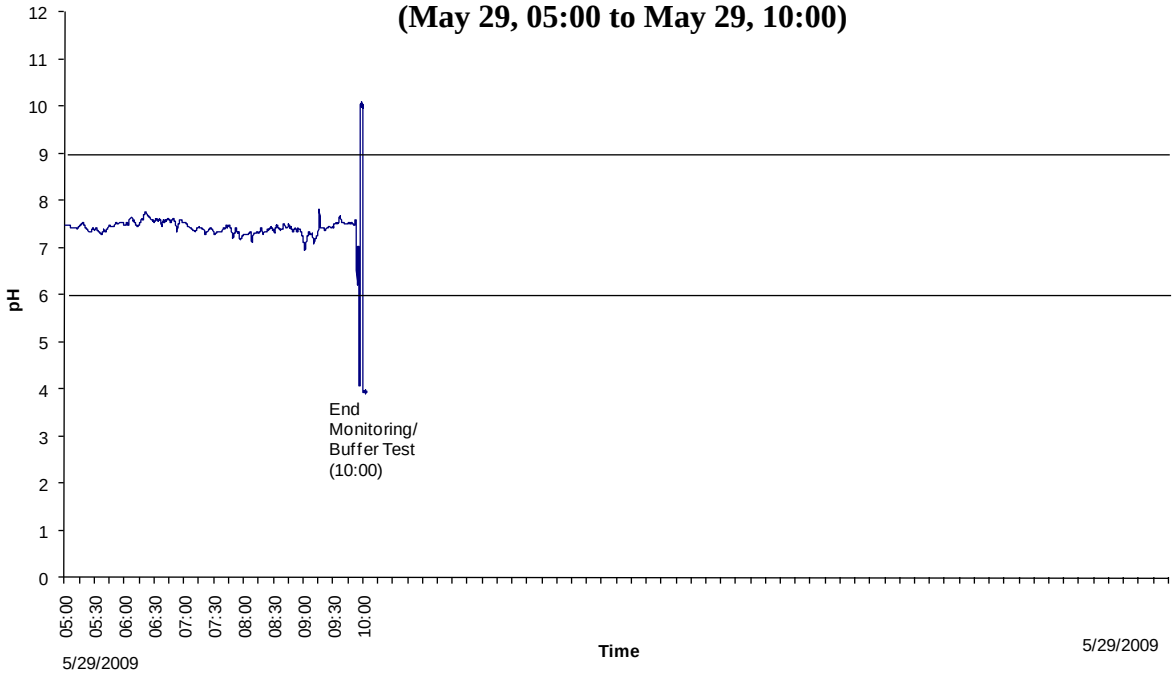
(May 27, 16:00 to May 28, 10:30)



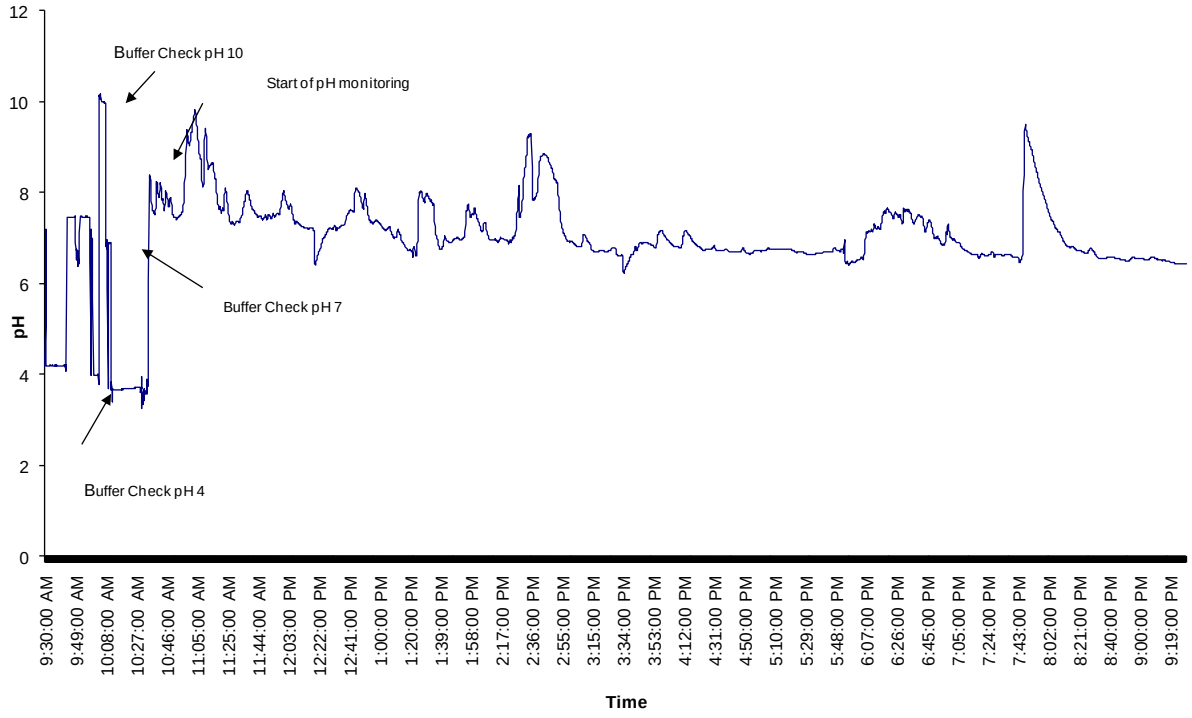
(May 29, 10:30 to May 29, 05:00)



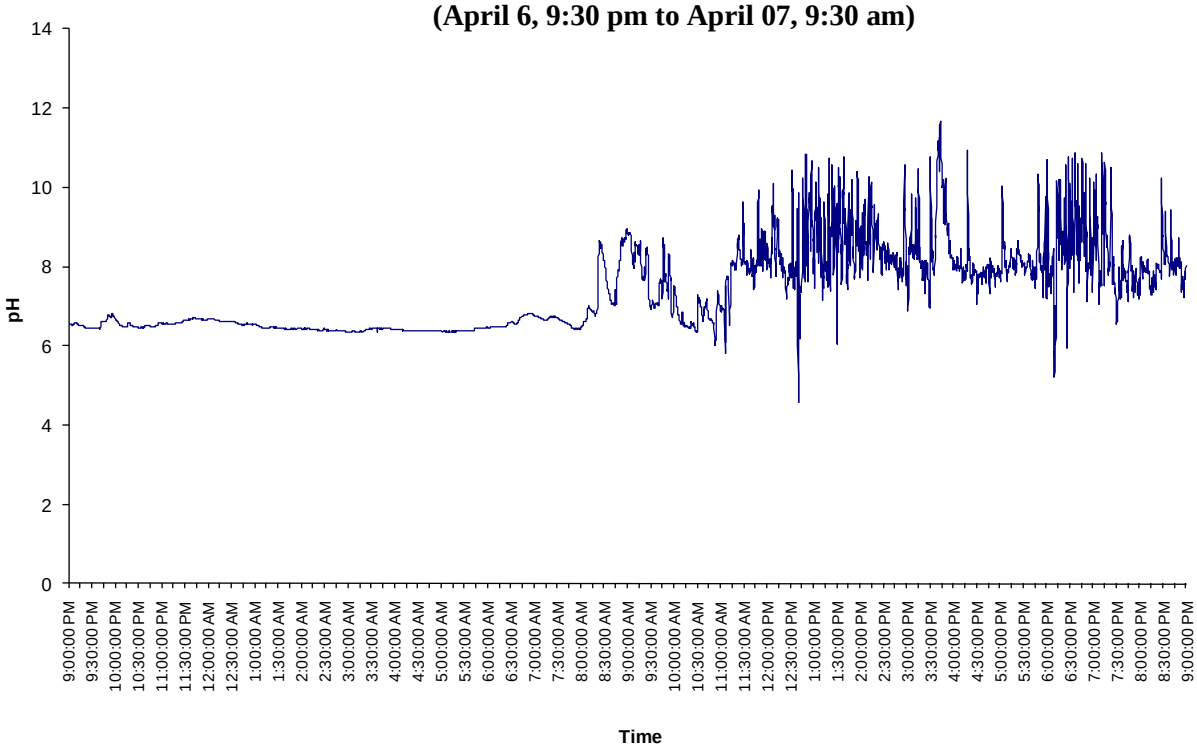
(May 29, 05:00 to May 29, 10:00)



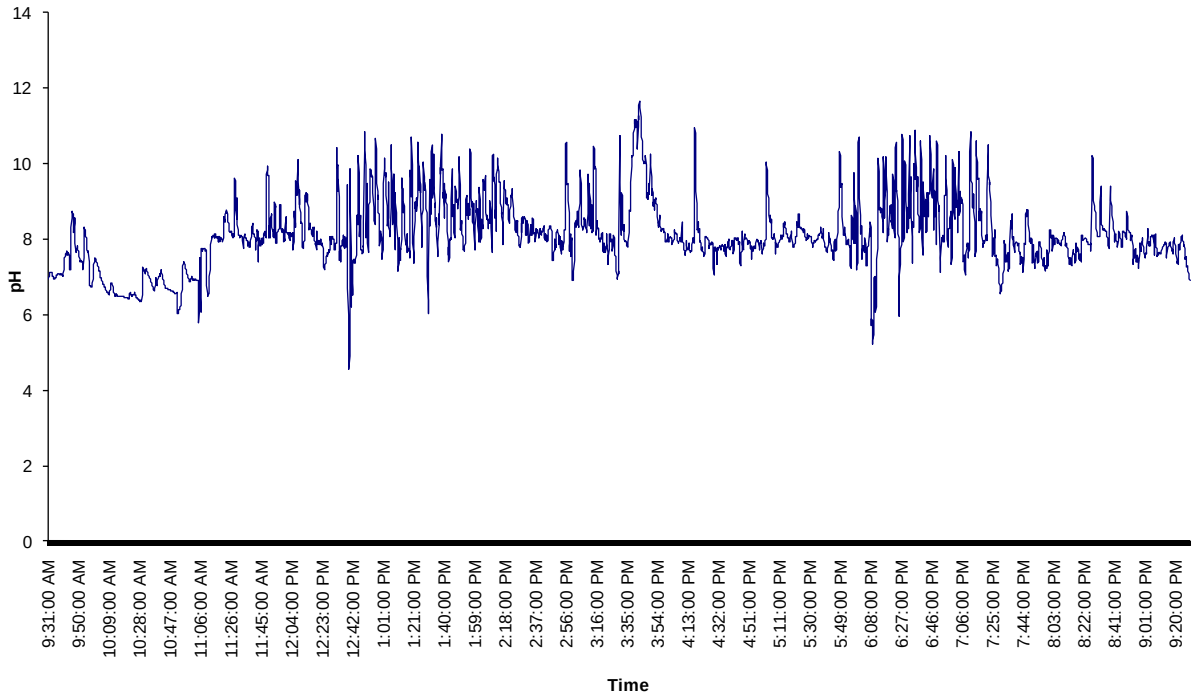
D-2) LTC_1 **(April 6, 9:30 am 9:30 pm)**



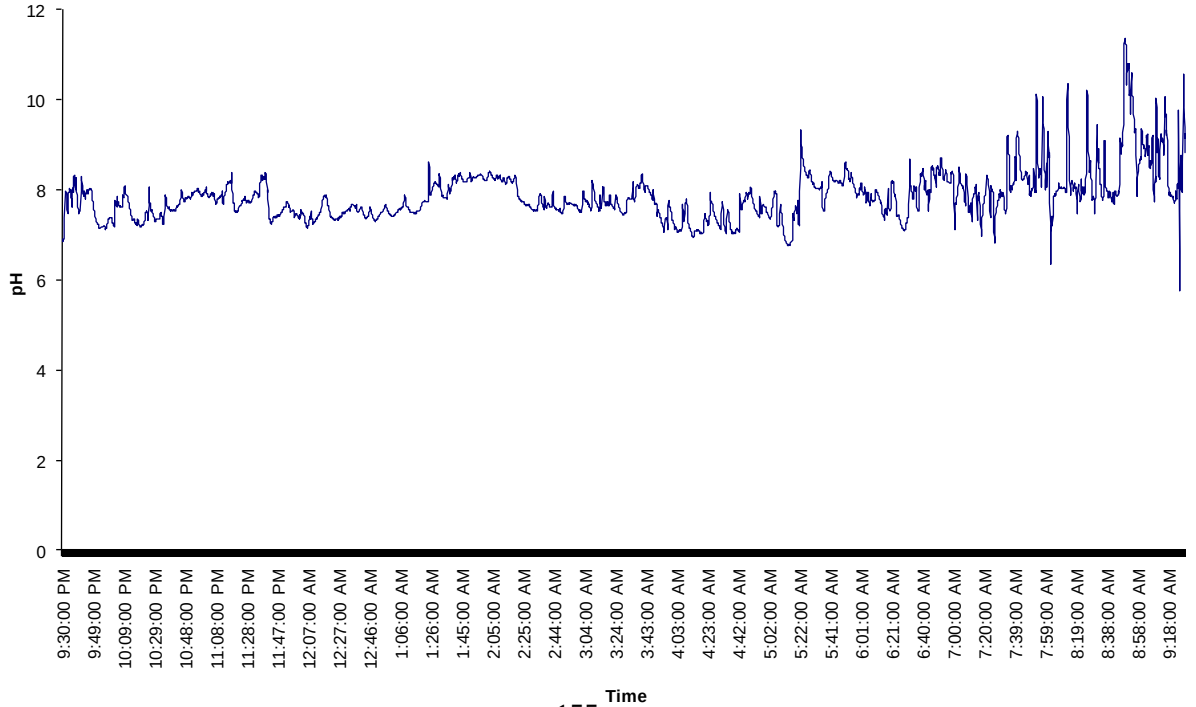
(April 6, 9:30 pm to April 07, 9:30 am)



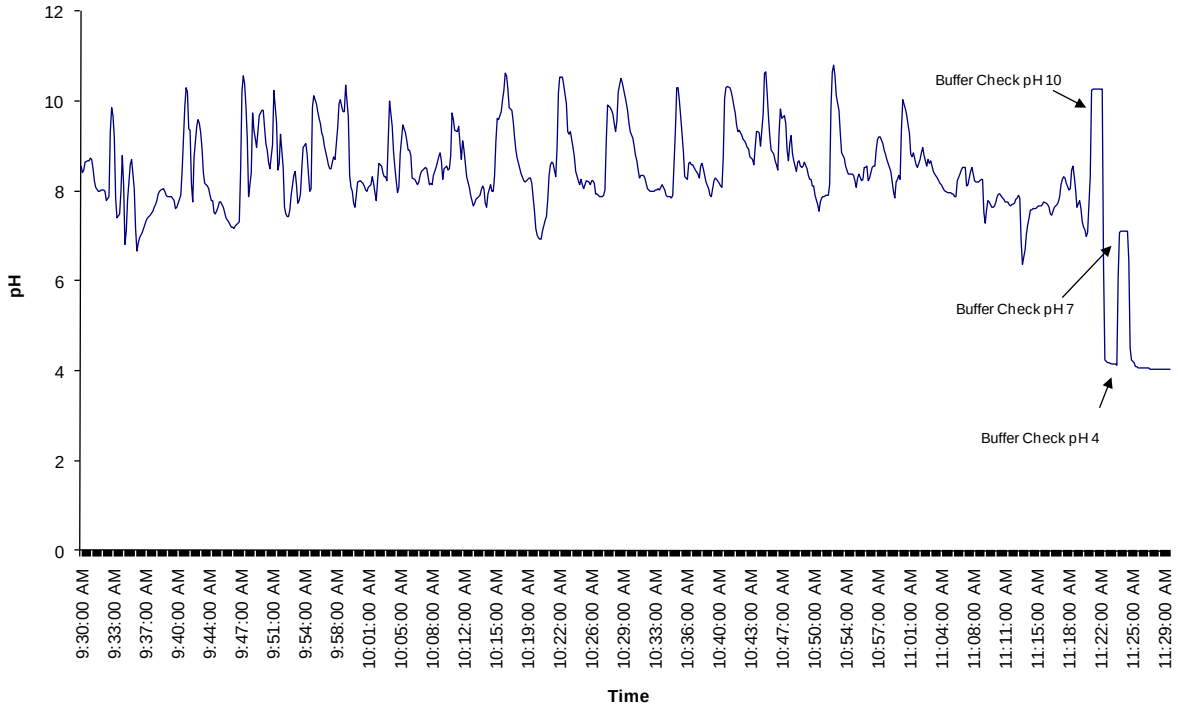
(April 7, 9:30 pm - 9:30 am)



(April 7, 9:30 pm - April 8, 9:30 am)



(April 8, 9:30 am - 11:30 am)



Appendix F

Measured concentrations of target compounds in all samples

HS_1 effluent and WWTP-HS_1 influents (R_1, R_2, R_3 are the triplicates analyzed for each sample)

Target Compounds	Week days	HS_1 (ng/L)				WWTP-HS_1 (ng/L)			
		R_1	R_2	R_3	Avg	R_1	R_2	R_3	Avg
Sulfamethoxazole	**Tue**	3772	3392	3664	3609	696	664	456	605
	Wed	1068	1020	900	996	432	496	331	420
	Thu	900	916	848	888	159	171	182	171
	Fri	428	436	456	440	240	303	288	277
Trimethoprim	**Mon**	524	492	520	512	397	384	386	389
	Tue	540	544	568	551	408	432	397	412
	Wed	360	304	297	321	268	298	360	309
	Thu	366	268	308	314	106	207	145	153
	Fri	444	188	376	336	305	320	365	330
Ciprofloxacin	**Mon**	691	611	594	632	107	111	74	97
	Tue	382	408	575	455	42	ND	40	41
	Wed	1240	1220	1,260	1240	86	62	99	82
	Thu	873	846	811	843	ND	108	101	105
	Fri	464	459	472	465	84	ND	41	63
Acetaminophen	**Mon**	143200	122000	137200	134133	88800	81200	79200	83067
	Tue	115200	116400	111600	114400	74400	75600	77600	75867
	Wed	96800	95600	92800	95067	65200	64800	66800	65600
	Thu	110000	99200	92400	100533	40800	37720	41200	39907
	Fri	72400	66400	66800	68533	52400	52800	48400	51200
Carbamazepine	**Mon**	143	142	146	144	270	258	279	269
	Tue	41	48	42	44	262	262	259	261
	Wed	34	34	35	34	872	908	912	897
	Thu	39	33	34	36	152	144	157	151
	Fri	90	82	87	86	164	168	173	168
Metoprolol	**Mon**	356	352	210	306	36	65	46	49
	Tue	586	550	602	579	293	191	52	179
	Wed	524	466	480	490	201	209	211	207
	Thu	341	344	450	378	141	149	145	145
	Fri	475	557	447	493	161	156	166	161
Venlafaxine	**Mon**	512	528	616	552	628	636	-	632
	Tue	344	321	378	348	468	452	396	439
	Wed	636	612	640	629	363	377	428	389
	Thu	736	740	756	744	282	246	249	259
	Fri	688	608	644	647	326	321	328	325

Target Compounds	Week days	HS_1 (ng/L)				WWTP-HS_1 (ng/L)			
		R_1	R_2	R_3	Avg	R_1	R_2	R_3	Avg
N-des-venlafaxine	**Mon**	128	139	383	217	247	249	-	248
	Tue	177	232	149	186	125	115	120	120
	Wed	290	285	275	283	148	178	185	170
	Thu	340	340	380	353	88	77	78	81
	Fri	424	404	420	416	101	104	92	99
O-de-venlafaxine	**Mon**	2540	2692	3408	2880	7920	5240	-	6580
	Tue	2252	3172	1456	2293	1484	1352	2612	1816
	Wed	1140	952	812	968	1,444	1860	1744	1683
	Thu	1788	1744	1860	1797	596	560	628	595
	Fri	1368	1176	1104	1216	776	760	792	776

Detected target compound concentrations in HS_2 effluent and WWTP-HS_2 influent

Target Compounds	Week days	HS_2 (ng/L)				WWTP-HS_2 (ng/L)			
		R_1	R_2	R_3	Avg	R_1	R_2	R_3	Avg
Sulfamethoxazole	**Mon**	6560	6560	6600	6573	544	536	564	548
	Tue	424	416	374	405	440	436	432	436
	Wed	6280	6520	5680	6160	206	221	220	216
	Thu	11080	10520	11200	10933	420	379	367	389
	Fri	325	338	318	327	420	432	372	408
Trimethoprim	**Mon**	5200	4760	4880	4947	307	275	306	296
	Tue	302	351	314	322	225	249	236	237
	Wed	9200	9520	9520	9413	220	234	198	217
	Thu	10480	10080	10400	10320	314	277	356	316
	Fri	440	428	408	425	276	295	284	285
Ciprofloxacin	**Mon**	105	103	149	119	81	84	88	84
	Tue	156	149	155	153	81	75	116	91
	Wed	182	156	168	169	120	128	88	112
	Thu	168	164	134	155	131	113	145	130
	Fri	149	139	139	142	111	122	75	103

Target Compounds	Week days	HS_2 (ng/L)				WWTP-HS_2 (ng/L)			
		R_1	R_2	R_3	Avg	R_1	R_2	R_3	Avg
Acetaminophen	**Mon**	13280	14040	12440	13253	40000	46800	44000	43600
	Tue	11600	14320	14200	13373	41600	40800	42400	41600
	Wed	15760	15440	16720	15973	46800	48800	47200	47600
	Thu	12440	11600	11280	11773	47200	42000	35840	41680
	Fri	10960	11960	11280	11400	39720	40400	38600	39573
Carbamazepine	**Mon**	752	652	624	676	243	258	265	255
	Tue	632	608	644	628	191	195	187	191
	Wed	80	74	74	76	210	199	212	207
	Thu	596	360	400	452	708	688	760	719
	Fri	175	189	150	171	222	234	235	230
Metoprolol	**Mon**	88	84	91	88	91	80	88	86
	Tue	348	327	337	337	61	72	79	71
	Wed	196	190	195	193	106	103	102	104
	Thu	181	165	178	175	83	82	81	82
	Fri	166	152	163	160	93	97	96	95
Venlafaxine	**Mon**	808	692	784	761	496	556	512	521
	Tue	38400	34920	34640	35987	468	452	508	476
	Wed	8840	8880	8960	8893	544	484	500	509
	Thu	4400	3696	4080	4059	484	476	536	499
	Fri	4640	4240	4840	4573	416	480	504	467
N-des-venlafaxine	**Mon**	222	203	226	217	176	177	181	178
	Tue	291	318	294	301	172	161	164	165
	Wed	114	92	94	100	179	169	161	170
	Thu	241	236	236	238	162	154	171	162
	Fri	452	476	444	457	170	176	166	171
O-de-venlafaxine	**Mon**	1012	908	948	956	1,520	1676	1644	1613
	Tue	7840	5520	5960	6440	1348	1364	1360	1357
	Wed	1152	1072	1156	1127	1276	1220	1204	1233
	Thu	2476	2496	2632	2535	1092	1088	1156	1112
	Fri	1792	1616	1964	1791	1384	1440	1264	1363

Detected target compound concentrations in LTC_1 effluent and WWTP-LTC_1 influent

Target Compounds	Week days	LTC_1 (ng/L)				WWTP-LTC_1 (ng/L)			
		R_1	R_2	R_3	Avg	R_1	R_2	R_3	Avg
Sulfamethoxazole	**Mon**	364	404	366	378	394	440	488	441
	Tue	2188	2328	2360	2292	358	391	386	378
	Wed	154	147	141	147	460	468	456	461
	Thu	78	100	107	95	351	476	393	407
	Fri	303	324	363	330	460	448	428	445
Trimethoprim	**Mon**	712	744	752	736	230	209	238	226
	Tue	948	888	936	924	313	293	270	292
	Wed	472	420	460	451	275	284	233	264
	Thu	7000	6320	6400	6573	296	261	256	271
	Fri	696	756	652	701	408	370	282	353
Ciprofloxacin	**Mon**	189	146	256	197	55	65	50	57
	Tue	304	363	369	345	70	82	78	76
	Wed	644	583	586	604	77	58	71	69
	Thu	258	211	204	224	84	73	83	80
	Fri	203	242	196	214	68	81	72	74
Acetaminophen	**Mon**	93200	89600	74000	85600	65200	64800	63200	64400
	Tue	84000	92000	75600	83867	66800	68400	66800	67333
	Wed	65200	89600	81200	78667	68400	65200	66000	66533
	Thu	121200	102000	125600	116267	68000	65200	69600	67600
	Fri	78800	78000	76400	77733	70800	68000	72000	70267
Carbamazepine	**Mon**	186	185	174	182	187	174	165	175
	Tue	158	142	158	153	154	173	164	164
	Wed	254	254	247	252	156	169	149	158
	Thu	516	524	540	527	182	181	181	181
	Fri	177	177	174	176	190	183	178	184
Metoprolol	**Mon**	888	940	1272	1033	219	276	210	235
	Tue	1952	1648	2024	1875	302	254	214	257
	Wed	936	1284	1172	1131	262	202	188	217
	Thu	4480	4880	5400	4920	232	186	180	199
	Fri	424	624	576	541	264	223	296	261

Target Compounds	Week days	LTC_1 (ng/L)				WWTP-LTC_1 (ng/L)			
		R_1	R_2	R_3	Avg	R_1	R_2	R_3	Avg
Venlafaxine	**Mon**	536	524	580	547	444	480	408	444
	Tue	408	408	420	412	358	428	395	394
	Wed	2320	2236	2268	2275	420	388	399	402
	Thu	107	111	107	108	456	368	472	432
	Fri	225	244	261	243	516	496	528	513
N-des-venlafaxine	**Mon**	121	122	134	126	224	186	160	190
	Tue	260	266	272	266	118	107	120	115
	Wed	95	92	96	95	138	124	110	124
	Thu	47	42	44	44	130	96	115	114
	Fri	136	142	121	133	126	125	127	126
O-des-venlafaxine	**Mon**	1248	1232	1424	1301	5400	6720	4360	5493
	Tue	1292	1276	1260	1276	2864	3016	2812	2897
	Wed	2336	2356	2124	2272	3548	3264	2744	3185
	Thu	330	327	311	323	2052	2200	2732	2328
	Fri	7960	6160	6840	6987	3140	3344	3324	3269

Detected target compound concentrations in LTC_2 effluent and WWTP-LTC_2 influent

Target Compounds	Week days	LTC_2 (ng/L)				WWTP-LTC_2 (ng/L)			
		R_1	R_2	R_3	Avg	R_1	R_2	R_3	Avg
Sulfamethoxazole	**Mon**	592	-	-	592	448	492	476	472
	Tue	105	108	87	100	524	500	596	540
	Wed	672	752	724	716	468	460	500	476
	Thu	381	448	342	391	337	354	357	349
	Fri	254	230	296	260	266	238	253	252
Trimethoprim	**Mon**	238	-	-	238	233	150	216	200
	Tue	240	228	232	234	182	274	275	244
	Wed	1920	1924	1928	1924	200	181	176	186
	Thu	864	880	796	847	206	170	205	194
	Fri	306	307	280	298	88	90	122	100
Ciprofloxacin	**Mon**	49	-	-	49	75	72	88	78
	Tue	48	37	36	40	121	178	153	151
	Wed	26	27	41	31	54	56	95	68

Target Compounds	Week days	LTC_2 (ng/L)				WWTP-LTC_2 (ng/L)			
		R_1	R_2	R_3	Avg	R_1	R_2	R_3	Avg
Ciprofloxacin	**Thu**	1630	1240	1540	1470	38	44	53	45
	Fri	55	41	31	42	73	48	51	57
Acetaminophen	**Mon**	72800	-	-	72800	65200	71200	66400	67600
	Tue	88000	86400	92000	88800	62000	62000	65600	63200
	Wed	82800	81200	92000	85333	65600	66400	60400	64133
	Thu	83600	86400	87200	85733	70000	69600	65600	68400
	Fri	80400	80000	76400	78933	42800	41600	42400	42267
Carbamazepine	**Mon**	64	-	-	64	70	70	68	69
	Tue	66	67	69	67	98	103	112	104
	Wed	21	23	22	22	77	79	68	75
	Thu	35	33	31	33	68	68	79	72
	Fri	76	76	78	77	74	76	91	81
Metoprolol	**Mon**	198	-	-	198	266	225	354	282
	Tue	313	312	337	321	222	261	200	227
	Wed	168	198	211	192	182	225	251	219
	Thu	246	262	250	253	255	225	251	244
	Fri	179	147	143	157	128	140	136	135
Venlafaxine	**Mon**	334	-	-	334	424	416	440	427
	Tue	136	150	146	144	444	448	456	449
	Wed	99	93	99	97	460	412	444	439
	Thu	37	37	38	38	480	448	440	456
	Fri	712	696	740	716	297	322	304	308
N-des-venlafaxine	**Mon**	119	-	-	119	142	137	158	145
	Tue	34	31	35	33	143	154	138	145
	Wed	0	0	0	0	163	104	161	143
	Thu	0	0	0	0	175	170	184	177
	Fri	116	113	108	112	106	112	93	103
O-de-venlafaxine	**Mon**	892	-	-	892	1212	1132	1076	1140
	Tue	580	640	644	621	1152	1112	1216	1160
	Wed	476	476	504	485	1528	1238	1404	1390
	Thu	716	776	716	736	488	1564	1832	1295
	Fri	2008	2160	2204	2124	920	988	1000	969

Bibliography

Alder, A. C., McArdell, C. S., Golet, E. M., Kohler, P. E., Molnar, E., Pham Thi, N. A., Siegrist, H., Suter, M. J. F., & Giger, W. (2004). Environmental exposure of antibiotics in wastewaters, sewage sludges and surface waters in switzerland. In K. Kalus (Ed.), *Pharmaceuticals in the environment;sources, fate, effects and risks* (2nd ed., pp. 55-66) Springer.

Alder, A. C., Bruchet, A., Calrballa, M., Clara, M., Joss, A., Loffler, D., McArdell, C. S., Miksch, K., Omil, F., Tuhkanen, T., & Ternes, T. A. (2006). Consumption and occurrence. In *Human pharmaceuticals, hormones and fragrances : The challenge of micropollutants in urban water management* (pp. TML PUBLIC "-//W3C//DTD HTML 4.0 Transitional//EN" "http://www.w3.org/TR/REC-html40/loose.dtd">). London : Seattle: Iwa.

Alexy, R., Kumpel, T., & Kümmerer, K. (2004). Assessment of degradation of 18 antibiotics in the closed bottle test. *Chemosphere, 57*(6, pp. 505-512), November. doi:10.1016/j.chemosphere.2004.06.024

Ascenzo, G., Di Corcia, A., Gentili, A., Mancini, R., Mastropasqua, R., Nazzari, M., & Samperi, R. (2003). Fate of natural estrogen conjugates in municipal sewage transport and treatment facilities. *Science of the Total Environment, 302*(1-3), 199-209.

Askarian, M., Vakili, M., & Kabir, G. (2004). Results of a hospital waste survey in private hospitals in fars province, iran. *Waste Management, 24*(4), 347-352.

Avisar, D., Lester, Y.,2), & Ronen, D.,4). (2009). Sulfamethoxazole contamination of a deep phreatic aquifer. *Science of the Total Environment, 407*(14), 4278-4282. doi:10.1016/j.scitotenv.2009.03.032

Barnes, K. K., Kolpin, D. W., Furlong, E. T., Zaugg, S. D., Meyer, M. T., & Barber, L. B. (2008). A national reconnaissance of pharmaceuticals and other organic wastewater contaminants in the united states — I) groundwater. *Science of the Total Environment, 402*(2-3), 192-200. doi:10.1016/j.scitotenv.2008.04.028

Baronti, C., Curini, R., & D'Ascenzo, G. (2000). Monitoring natural and synthetic estrogens at activated sludge sewage treatment plants and in a receiving river water. *Environmental Science & Technology, 34*(24), 5059-5066.

Batt, A. L., Snow, D. D., & Aga, D. S. (2006). Occurrence of sulfonamide antimicrobials in private water wells in washington county, idaho, USA. *Chemosphere, 64*(11), 1963-1971. doi:10.1016/j.chemosphere.2006.01.029

Bendz, D., Paxeus, N., Ginn, T. R., & Loge, F. J. (2005). Occurrence and fate of pharmaceutically active compounds in the environment, a case study: Hoje river in sweden. *Journal of Hazardous Materials, 133*(3), 195.

Benotti, M. J., Trenholm, R. A., & Vanderford, B. J. (2009). Pharmaceuticals and endocrine disrupting compounds in U.S. drinking water. *Environmental Science & Technology, 43*(3), 597-603.

Berto, J., Rochenbach, G. C., Barreiros, M. A. B., Corrêa, A. X. R., Peluso-Silva, S., & Radetski, C. M. (2009). Physico-chemical, microbiological and ecotoxicological evaluation of a septic tank/Fenton reaction combination for the treatment of hospital wastewaters. *Ecotoxicology and Environmental Safety, 72*(4, pp. 1076-1081), May. doi:10.1016/j.ecoenv.2008.12.002

Boillot, C., Bazin, C., Tissot-Guerraz, F., Droguet, J., Perraud, M., Cetre, J., Trepo, D., & Perrodin, Y. (2008). Daily physicochemical, microbiological and ecotoxicological fluctuations of a hospital effluent according to technical and care activities. *Science of the Total Environment, 403*(1-3), 113-129. doi:10.1016/j.scitotenv.2008.04.037

Bonomo, R. A., & Salata, R. A. (. (2002). Managing infections in the elderly: The challenge of long-term care facilities. *Clinical Microbiology Newsletter, 24*(24, pp. 183-186), December.

Brown, K. D., Kulis, J., Thomson, B., Chapman, T. H., & Mawhinney, D. B. (2006). Occurrence of antibiotics in hospital, residential, and dairy effluent, municipal wastewater, and the rio grande in new mexico. *Science of the Total Environment, 366*(2-3), 772-783. doi:10.1016/j.scitotenv.2005.10.007

Carballa, M., Omil, F., & Lema, J. M. (2008). Comparison of predicted and measured concentrations of selected pharmaceuticals, fragrances and hormones in spanish sewage. *Chemosphere, 72*(8), 1118-1123. doi:10.1016/j.chemosphere.2008.04.034

Castiglioni, S., Fanelli, R., & Bagnati, R. (2006). Removal of pharmaceuticals in sewage treatment plants in italy. *Environmental Science & Technology, 40*(1), 357-363.

Chander, Y., Gupta, S. C., Goyal, S. M., & Kumar, K. (2007). Antibiotics: Has the magic gone? *Journal of the Science of Food and Agriculture, 87*(5, pp. 739-742), April. doi:10.1002/jsfa.2764

Chang, X., Meyer, M. T., Liu, X., Zhao, Q., Chen, H., Chen, J., Qiu, Z., Yang, L., Cao, J., & Shu, W. (2010). Determination of antibiotics in sewage from hospitals, nursery and slaughter house, wastewater treatment plant and source water in chongqing region of three gorge reservoir in china. *Environmental Pollution (Barking, Essex : 1987), 158*(5), 1444-1450.

Chiron, S.Occurrence and distribution of selected antibiotics in a small mediterranean stream (arc river, southern france).

Chitnis, V. (., Chitnis, S. (., Vaidya, K. (., Ravikant, S. (., Patil, S. (., & Chitnis, D. S. (. (2004). Bacterial population changes in hospital effluent treatment plant in central india. *Water Research, 38*(2, pp. 441-447), January. doi:10.1016/j.watres.2003.09.038

Choi, K., Kim, S., & Kim, S. (2008). Removal of antibiotics by coagulation and granular activated carbon filtration. *Journal of Hazardous Materials, 151*(1), 38-43. doi:10.1016/j.jhazmat.2007.05.059

Christen, V., Hickmann, S., Rechenberg, B., & Fent, K. (2010). Highly active human pharmaceuticals in aquatic systems: A concept for their identification based on their mode of action. *Aquatic Toxicology (Amsterdam, Netherlands), 96*(3), 167-181.

Christiansen, C. (2005). X-ray contrast media--an overview. *Toxicology, 209*(2), 185-187.

CIHI. (2000). *Development of national indicators and a reporting system for continuing care (phase one indictor definitions and data sources)*Canadian Institute of Health Information.

CIHI. (2006). *Canadian institute of health information. facility-based continuing care in canada, 2004-2005*. Ottawa, ON:

CIHI. (2009). *Canadian institute for health information, canadian MIS database, hospital financial performance indicators 1999–2000 to 2007–2008* Ottawa, ON:

Corcoran, J., Winter, M. J., & Tyler, C. R. (2010). Pharmaceuticals in the aquatic environment: A critical review of the evidence for health effects in fish. *Critical Reviews in.Toxicology*, *40*(4), 287-304.

Correia, M. A. (2007). Drug biotransformation. In G. K. Bertram (Ed.), *Basic and clinical pharmacology* (). New York : London: New York : McGraw-Hill Medical ; London : McGraw-Hill distributor.

Crnich, C., Safdar, N., Robinson, J., & Zimmerman, D. (2007). Longitudinal trends in antibiotic resistance in US nursing homes, 2000-2004.

Cunningham, V. L. (2008). Special characteristics of pharmaceuticals related to environmental fate. In K. Kümmerer (Ed.), *Pharmaceuticals in the environment: Sources, fate, effects and risks* (pp. 23-34)

Dangerous pollutants (xenobiotics) in urban water cycle (2008). In Hlavinek P. (Ed.), . Dordrecht: Dordrecht : Springer : Published in cooperation with NATO Public Diplomacy Division.

Daughton, C. G. (2001). PPCPs in the environment:Future research-begining with the end always in mind. In K. Kummerer (Ed.), *Pharmaceuticals in the environment : Sources, fate, effects and risks* (). Berlin ; New York: Berlin ; New York : Springer.

Daughton, C. G. (2004). PPCPs in the environment:Future research-beginning with the end always in mind. In K. Kalus (Ed.), *Pharmaceuticals in the environment: Sources, fate, effects and risks* (2nd ed., pp. 463-503). Berlin: Springer.

Daughton, C. G. (2007). Pharmaceuticals in the environment: Sources and their manag (Eds.), *Comprehensive analytical chemistry* (pp. 1-58) Elsevier.

Diaz, L. F., Eggerth, L. L., Enkhtsetseg, S., & Savage, G. M. (2008). Characteristics of healthcare wastes. *Waste Management (New York, N.Y.), 28*(7), 1219-1226.

-

(Eds.), *Analysis, fate and removal of pharmaceuticals in the water cycle* (pp. 61-93). Amsterdam ; Boston: Amsterdam ; Boston : Elsevier.

Diwan, V., Tamhankar, A. J., Aggarwal, M., Sen, S., Khandal, R. K., & Lundborg, C. S. (2009). Detection of antibiotics in hospital effluents in india. *Current Science, V 97*(12), 1752-1755.

Dmaia Barcelo, M. P. (Ed.). (2008). *The handbook of environmental chemistry*

Dodd, M. C. & Huang, C. (2004). Transformation of the antibacterial agent sulfamethoxazole in reactions with chlorine: kinetics, mechanisms, and pathways. *Environmental Science & Technology, 38*(21), 5607-5615.

Dodd, M. C., Shah, A. D., & Gunten, U. v. (2005). Interactions of fluoroquinolone antibacterial agents with aqueous chlorine: Reaction kinetics, mechanisms, and transformation pathways. *Environmental Science & Technology, 39*(18), 7065-7076.

Drews, J. E. (2007). Removal of pharma

(Eds.), *Analysis, fate and removal of pharmaceuticals in the water cycle* (pp. 61-93). Amsterdam ; Boston: Amsterdam ; Boston : Elsevier.

Duong, H. A., Ha Pham, N., Nguyen, H. T., Hoang, T. T., Pham, H. V., Ca Pham, V., & Berg, M. (2008). Occurrence, fate and antibiotic resistance of fluoroquinolone antibacterials in hospital wastewaters in hanoi, vietnam. *Chemosphere, 72*(6), 968.

Eldere, J., Robben, J., & De Pauw, G. (1988). Isolation and identification of intestinal steroid-desulfating bacteria from rats and humans. *Applied and Environmental Microbiology, 54*, 2112-2117.

Emmanuel, E., Keck, G., Blanchard, J., Vermande, P., & Perrodin, Y. (2004). Toxicological effects of disinfections using sodium hypochlorite on aquatic organisms and its contribution to AOX

formation in hospital wastewater. *Environment International, 30*(7, pp. 891-900), September. doi:10.1016/j.envint.2004.02.004

Emmanuel, E., Perrodin, Y., Keck, G., Blanchard, J. -., & Vermande, P. (2005). Ecotoxicological risk assessment of hospital wastewater: A proposed framework for raw effluents discharging into urban sewer network. *Journal of Hazardous Materials, 117*(1, pp. 1-11), January. doi:10.1016/j.jhazmat.2004.08.032

Emmanuel, E., Pierre, M. G.,3), & Perrodin, Y. (2009). Groundwater contamination by microbiological and chemical substances released from hospital wastewater: Health risk assessment for drinking water consumers. *Environment International, 35*(4, pp. 718-726), May. doi:10.1016/j.envint.2009.01.011

F.D.A. (2004). *US food and drug administration.* Retrieved April, 2010 http://www.fda.gov/cder/drugsatfda/glossary.htm#Fu

Feldmann, D. F., Zuehlke, S., & Heberer, T. (2008). Occurrence, fate and assessment of polar metamizole (dipyrone) residues in hospital and municipal wastewater. *Chemosphere, 71*(9, pp. 1754-1764), April. doi:10.1016/j.chemosphere.2007.11.032

Felizzola, J., & Chiron, S. (2009). Occurrence and distribution of selected antibiotics in a small mediterranean stream (arc river, southern france). *Journal of Hydrology (Amsterdam), 364*(1-2), 50-57. doi:10.1016/j.jhydrol.2008.10.006

Fluit, A., van der Bruggen, J., Aarestrup, F., Verhoef, J., & Jansen, W. (2006). Priorities for antibiotic resistance surveillance in europe. *Clinical Microbiology and Infection, 12*(5), 410-417. doi:10.1111/j.1469-0691.2006.01406.x

Gartiser, S., Brinker, L., Erbe, T., Kümmerer, K., & Willmund, R. (1996). Contamination of hospital wastewater with hazardous compounds as defined by 7a WHG.

Gautam, A., Kumar, S., & Sabumon, P. (2007). Preliminary study of physico-chemical treatment options for hospital wastewater. *Journal of Environmental Management, 83*(3), 298-306. doi:10.1016/j.jenvman.2006.03.009

Gibson, G. G., & Skett, P. (2001). *Introduction to drug metabolism* (3rd ed ed.). Cheltenham, England: Nelson Thornes.

Gilman, A. G. (2009). *Goodman & gilman's the pharmacological basis of therapeutics*. New York: New York : McGraw-Hill.

Giuliani, F., Koller, T., Würgler, F. E. (., & Widmer, R. M. (1996). Detection of genotoxic activity in native hospital waste water by the umuC test. *Mutation Research/Genetic Toxicology, 368*(1, pp. 49-57), May. doi:10.1016/S0165-1218(96)90039-7

Glassmeyer, S. T., Kolpin, D. W., Furlong, E. T., & Focazio, M. J. (2008). Environmental presence and persistence of pharmaceuticals an overview. In D. S. Aga (Ed.), *Fate of pharmaceuticals in the environment and in water treatment systems* (pp. 3-51). Boca Raton: CRC Press.

Gobel, A., Thomsen, A., McArdell, C. S. (., Joss, A., & Giger, W. (2005). Occurrence and sorption behavior of sulfonamides, macrolides, and trimethoprim in activated sludge treatment. *Environmental Science & Technology, 39*(11, pp. 3981-3989), June.

Golet, E. M., Alder, A. C., & Giger, W. (2002). Environmental exposure and risk assessment of fluoroquinolone antibacterial agents in wastewater and river water of the glatt valley watershed, switzerland. *Environmental Science & Technology, 36*(17), 3645-3651.

Gomez, M. J., Aguera, A., Mezcua, M., Hurtado, J., Mocholi, F., & Fernandez-Alba, A. R. (2007). Simultaneous analysis of neutral and acidic pharmaceuticals as well as related compounds by gas chromatography-tandem mass spectrometry in wastewater. *Talanta, 73*(2, pp. 314-320), September. doi:10.1016/j.talanta.2007.03.053

Gomez, M. J. (., Fernández-Alba, A. R. (., Petrović, M. (., & Barceló, D. (. (2006). Determination of pharmaceuticals of various therapeutic classes by solid-phase extraction and liquid chromatography-tandem mass spectrometry analysis in hospital effluent wastewaters. *Journal of Chromatography A, 1114*(2), 224-233. doi:10.1016/j.chroma.2006.02.038

Gomez, M., Malato, O., Ferrer, I., Agueera, A., & Fernandez-Alba, A. (2007). Solid-phase extraction followed by liquid chromatography-time-of-flight-mass spectrometry to evaluate

pharmaceuticals in effluents. A pilot monitoring study. *Journal of Environmental Monitoring, 9*(7), 718-729. doi:10.1039/b702844j

Goossens, H., Ferech, M., Coenen, S., Stephens, P., & European Surveillance Antimicrobia. (2007). Comparison of outpatient systemic antibacterial use in 2004 in the united states and 27 european countries. *CLINICAL INFECTIOUS DISEASES, 44*, 1091.

Goossens, H., Ferech, M., Stichele, R. V., & Elseviers, M. (2005). Outpatient antibiotic use in europe and association with resistance: A cross-national database study. *The Lancet, 365*(9459), 579.

Hardy, J., Reymond, E., & Charles, M. (2005). Acetaminophen in cancer pain. *Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology, 23*(7), 1586; author reply 186-7.

Hartmann, A., Golet, E., Gartiser, S., Alder, A., Koller, T., & Widmer, R. (1999). Primary DNA damage but not mutagenicity correlates with ciprofloxacin concentrations in german hospital wastewaters.

Hartmann, A., Alder, A. C., Koller, T., & Widmer, R. M. (1998). Identification of fluoroquinolone antibiotics as the main source of umuC genotoxicity in native hospital wastewater. *Environmental Toxicology and Chemistry, 17*(3), 377.

Hartmann, T., Lange, R., Schweinfurth, H., Tschampel, M., & Rehmann, I. (2002). Investigations into the environmental fate and effects of iopromide (ultravist), a widely used iodinated X-ray contrast medium.

Hartmann, T., Kümmerer, K., & Schecker, J. (1996). Trace analysis of the antineoplastics ifosfamide and cyclophosphamide in sewage water by two-step solid-phase extraction and gas chromatography-mass spectrometry. *Journal of Chromatography A, 726*(1-2), 179-184. doi:10.1016/0021-9673(95)01063-7

Health Canada. (2010). *Drug product database (DPD)*

Heberer, T. (2002a). Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: A review of recent research data. *Toxicology Letters, 131*(1-2), 5-17.

Heberer, T. (2002b). Tracking persistent pharmaceutical residues from municipal sewage to drinking water. *Journal of Hydrology, 266*(3-4, pp. 175-189), September. doi:10.1016/S0022-1694(02)00165-8

Heberer, T., & Feldmann, D. (2005). Contribution of effluents from hospitals and private households to the total loads of diclofenac and carbamazepine in municipal sewage effluents - modeling versus measurements (vol 122, pg 211, 2005).

Henschel, K. -., Wenzel, A., Diedrich, M., & Fliedner, A. (1997). Environmental hazard assessment of pharmaceuticals. *Regulatory Toxicology and Pharmacology, 25*(3, pp. 220-225), June. doi:10.1006/rtph.1997.1102

Hessel, H., Radon, K., Pethran, A., Maisch, B., Gröbmair, S., Sautter, I., & Fruhmann, G. (2001). The genotoxic risk of hospital, pharmacy and medical personnel occupationally exposed to cytostatic drugs — evaluation by the micronucleus assay. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 497*(1-2, pp. 101-109), October.

Hirsch, R., Ternes, T., Haberer, K., & Kratz, K. (1999). Occurrence of antibiotics in the aquatic environment. *Science of the Total Environment, 225*(1-2), 109-118.

Hongxia,Li, Helm, P. A., & Metcalfe, C. D. (2010). Sampling in the great lakes for pharmaceuticals, personal care products, and endocrine-disrupting substances using the passive polar organic chemical integrative sampler. *Environmental Toxicology and Chemistry / SETAC, April 01, 29*(4), 751-762.

Huang, C., Renew, J., & Smeby, K.Assessment of potential antibiotic contaminants in water and preliminary occurrence analysis. Baltimore, MD (USA).

Human pharmaceuticals, hormones and fragrances : The challenge of micropollutants in urban water management (2006). . London : Seattle: Iwa.

Jambhekar, S. S., & Breen, P. J. (2009). *Basic pharmacokinetics*. UK, USA: Pharmaceutical Press.

Jjemba, P. K. (2008). In ebrary I. (Ed.), *Pharma-ecology the occurrence and fate of pharmaceuticals and personal care products in the environment*. Hoboken, N.J.: Hoboken, N.J. : Wiley.

Jobling, S. (., Nolan, M. (., Tyler, C. R. (., Brighty, G. (., & Sumpter, J. P. (. (1998). Widespread sexual disruption in wild fish. *Environmental Science & Technology, 32*(17), 2498-2506.

Johnson, A. C. (., & Williams, R. J. (. (2004). A model to estimate influent and effluent concentrations of estradiol, estrone, and ethinylestradiol at sewage treatment works. *Environmental Science & Technology, 38*(13, pp. 3649-3658), July.

Jones, O., Voulvoulis, N., & Lester, J. (2002). Aquatic environmental assessment of the top 25 english prescription pharmaceuticals.

Khan, S. J. & Ongerth, J. E. (2004). Modelling of pharmaceutical residues in australian sewage by quantities of use and fugacity calculations. *Chemosphere, 54*(3, pp. 355-367), January. doi:10.1016/j.chemosphere.2003.07.001

King, R. (2009). Biotransformation in drug metabolism. In A. F. Nassar, P. F. Hollenberg & J. Scatina (Eds.), *Drug metabolism handbook : Concepts and applications* (). Hoboken, N.J.: Hoboken, N.J. : Wiley.

Klamerus, K. J., Maloney, K., Rudolph, R. L., Sisenwine, S. F., Jusko, W. J., Chiang, S. T., American College of Clinical Pharmacology, United States, & Etats-unis (Funder/Sponsor). (1992). Introduction of a composite parameter to the pharmacokinetics of venlafaxine and its active O-desmethyl metabolite. *American College of Clinical Pharmacology. Annual Meeting, , 32*(8) 716-724.

Kolpin, D. W., Furlong, E. T., Meyer, M. T., Thurman, E., Zaugg, S. D., Barber, L. B., & Buxton, H. T. (2002). Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: A naional reconnaissance. *Environmental Science and Technology, 36*(6), 1202.

Kramer, D., Allgaier, A., Fejtkova, S., Mergl, R., & Hegerl, U. (2009). Depression in nursing homes: Prevalence, recognition, and treatment. *International Journal of Psychiatry.in.Medicine, 39*(4), 345-358.

Kümmerer, K. (2001a). Drugs in the environment: Emission of drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources – a review. *Chemosphere, 45*(6-7), 957-969.

Kümmerer, K. (2001b). *Pharmaceuticals in the environment : Sources, fate, effects and risks*. Berlin ; New York: Springer.

Kümmerer, K. (2004a). Emissions from medical care units. In K. Kümmerer (Ed.), *Pharmaceuticals in the environment;sources, fate, effects and risks* (2nd ed., pp. 27-44) Springer-Verlag Berlin-Heidelberg.

Kümmerer, K. (2004b). Resistance in the environment.

Kümmerer, K. (2008a). Antibiotics in the environment. In K. Kalus (Ed.), *Pharmaceuticals in the environment: Sources, fate, effects and risks* (3rd ed., pp. 75-93) Springer.

Kümmerer, K. (2008b). Pharmaceuticals in the environmen- A brief summary. In K. Kalus (Ed.), *Pharmaceuticals in the environment: Sources, fate, effects and risks* (3rd ed., pp. 3-21) Springer.

Kümmerer, K. (2009a). Antibiotics in the aquatic environment – A review – part I. *Chemosphere, 75*(4, pp. 417-434), April. doi:10.1016/j.chemosphere.2008.11.086

Kümmerer, K. (2009b). Antibiotics in the aquatic environment – A review – part II. *Chemosphere, 75*(4, pp. 435-441), April. doi:10.1016/j.chemosphere.2008.12.006

Kümmerer, K., & Al-Ahmad, A. (. (1997). Biodegradability of the Anti-tumour agents 5-Fluorouracil, cytarabine, and gemcitabine: Impact of the chemical structure and synergistic toxicity with hospital effluent. *Acta Hydrochimica Et Hydrobiologica, 25*(4), 166-172. doi:10.1002/aheh.19970250402

Kümmerer, K., Erbe, T. Gartiser, S. , & Brinker, L. (1998). AOX-emissions from hospitals into municipal waste water. *Chemosphere, 36*(11, pp. 2437-2445), May. doi:10.1016/S0045-6535(97)10200-4

Kümmerer, K., & Henninger, a. (2003). Promoting resistance by the emission of antibiotics from hospitals and households into effluent. *Clinical Microbiology and Infection, 9*, 1203-1214.

Kümmerer, K., Steger-Hartmann, T., & Meyer, M. (1997). Biodegradability of the anti-tumour agent ifosfamide and its occurrence in hospital effluents and communal sewage. *Water Research, 31*(11, pp. 2705-2710), November. doi:10.1016/S0043-1354(97)00121-8

Langford, K., & Thomas, K. (2009). Determination of pharmaceutical compounds in hospital effluents and their contribution to wastewater treatment works. *Environment International, 35*(5; SI), 766-770.

Lau, D. T., Briesacher, B. A., Mercaldo, N. D., Halpern, L., Osterberg, E. C., Jarzebowski, M., McKoy, J. M., & Mazor, K. (2008). Older patients' perceptions of medication importance and worth: An exploratory pilot study. *Drugs & Aging, 25*(12), 1061-1075. doi:10.2165/0002512-200825120-00007

Lauridsen, F., Birkved, M., Hansen, L., Luetzhoeft, H., & Halling-Soerensen, B. (2000). Environmental risk assessment of human pharmaceuticals in denmark after normal therapeutic use. *Chemosphere, 40*(7), 783-793.

Lersch, C., Schmelz, R., Eckel, F., Erdmann, J., Mayr, M., Schulte-Frohlinde, E., Quasthoff, S., Grosskreutz, J., & Adelsberger, H. (2002). Prevention of oxaliplatin-induced peripheral sensory neuropathy by carbamazepine in patients with advanced colorectal cancer. *Clinical Colorectal Cancer., 2*(1), 54-58.

Lin, A. Y., Yu, T., & Lin, C. (2008). Pharmaceutical contamination in residential, industrial, and agricultural waste streams: Risk to aqueous environments in taiwan. *Chemosphere, 74*(1, pp. 131-141), December. doi:10.1016/j.chemosphere.2008.08.027

Lin, A., & Tsai, Y. (2009). Occurrence of pharmaceuticals in taiwan's surface waters: Impact of waste streams from hospitals and pharmaceutical production facilities. *Science of the Total Environment, 407*(12), 3793-3802. doi:10.1016/j.scitotenv.2009.03.009

Lindberg, R., Jarnheimer, P. -., Olsen, B., Johansson, M., & Tysklind, M. (2004). Determination of antibiotic substances in hospital sewage water using solid phase extraction and liquid chromatography/mass spectrometry and group analogue internal standards. *Chemosphere, 57*(10), 1479-1488. doi:10.1016/j.chemosphere.2004.09.015

Lissemore, L., Hao, C., Yang, P., Sibley, P. K., Mabury, S., & Solomon, K. R. (2006). An exposure assessment for selected pharmaceuticals within a watershed in southern ontario. *Chemosphere, 64*(5, pp. 717-729), July. doi:10.1016/j.chemosphere.2005.11.015

Llewellyn-Jones, R. H., & Snowdon, J. (2007). Depression in nursing homes: Ensuring adequate treatment. *CNS Drugs, 21*(8), 627-640. doi:10.2165/00023210-200721080-00002

Loffler, D., & Ternes, T. A. (. (2003). Analytical method for the determination of the aminoglycoside gentamicin in hospital wastewater via liquid chromatography–electrospray-tandem mass spectrometry. *Journal of Chromatography A, 1000*(1-2, pp. 583-588), June. doi:10.1016/S0021-9673(03)00059-1

Lombardi, P., Goldin, B., Boutin, E., & Gorbach, S. L. (1977). Metabolism of androgens and estrogens by human feacal microorganisms. *Journal of Steroid Biochemistry, 9*(8), 795-801.

Loos, R., Gawlik, B. M., Locoro, G., Rimaviciute, E., Contini, S., & Bidoglio, G. (2009). EU-wide survey of polar organic persistent pollutants in european river waters. *Environmental Pollution (Barking, Essex : 1987), 157*(2), 561-568.

Mahnik, S. N., Rizovski, B., Fuerhacker, M., & Mader, R. M. (2006). Development of an analytical method for the determination of anthracyclines in hospital effluents. *Chemosphere, 65*(8, pp. 1419-1425), November. doi:10.1016/j.chemosphere.2006.03.069

Mahnik, S., Rizovski, B., Fuerhacker, M., & Mader, R. (2004). Determination of 5-fluorouracil in hospital effluents.

Martins, A., Vasconcelos, T., Henriques, D., Frank, C., Konig, A., & Kümmerer, K. (2008). Concentration of ciprofloxacin in brazilian hospital effluent and preliminary risk aassessment: A case study.

Merck & Co (Ed.). (2004). *The merck manual of diagnosis and therapy*. Rahway, N.J.: Rahway, N.J. : Merck & Co. etc.

Metcalf & Eddy. (1991). In Metcalf & Eddy (Ed.), *Wastewater engineering : Treatment, disposal, and reuse*. New York ; Toronto; New York: New York ; Toronto : McGraw-Hill.

Miao, X., Bishay, F., Chen, M., & Metcalfe, C. D. (. (2004). Occurrence of antimicrobials in the final effluents of wastewater treatment plants in canada. *Environmental Science & Technology, 38*(13, pp. 3533-3541), July.

Miao, X., Bishay, F., Chen, M., & Metcalfe, C. D. (. (2004). Occurrence of antimicrobials in the final effluents of wastewater treatment plants in canada. *Environmental Science & Technology, 38*(13, pp. 3533-3541), July.

Ministry of Health and Long-term care, Ontario.*Hospitals.* Retrieved June /15, 2010, from http://www.health.gov.on.ca/english/public/contact/hosp/hosp_mn.html

Mody, L. (2007). Optimizing antimicrobial use in nursing homes: No longer optional. *Journal of the American Geriatrics Society, 55*(8, pp. 1301-1302), August. doi:10.1111/j.1532-5415.2007.01253.x

Mody, L., Maheshwari, S., Galecki, A., Kauffman, C. A. (., & Bradley, S. F. (. (2007). Indwelling device use and antibiotic resistance in nursing homes: Identifying a high-risk group. *Journal of the American Geriatrics Society, 55*(12, pp. 1921-1926), December. doi:10.1111/j.1532-5415.2007.01468.x

Mohee, R. (2005). Medical wastes characterisation in healthcare institutions in mauritius. *Waste Management, 25*(6 SPEC. ISS.), 575-581. doi:10.1016/j.wasman.2004.10.003

Mohle, E., Kempter, C., Kern, A., & Metzger, J. (1999). Examination of the degradation of drugs in municipal sewage plants using liquid chromatography-electrospray mass spectrometry.

Mompelat, S., Le Bot, B., & Thomas, O. (2009). Occurrence and fate of pharmaceutical products and by-products, from resource to drinking water. *Environment International, 35*(5), 803-814. doi:10.1016/j.envint.2008.10.008

Monette, J., Miller, M. A., Monette, M., Laurier, C., Boivin, J., Sourial, N., Le Cruguel, J., Vandal, A., & Cotton-Montpetit, M. (2007). Effect of an educational intervention on optimizing antibiotic prescribing in long-term care facilities. *Journal of the American Geriatrics Society, 55*(8), 1301-1302. doi:10.1111/j.1532-5415.2007.01250.x

Moro, M., Mongardi, M., & Marchi, M. (2007). Healthcare-related infections outside the hospital: A new frontier for infection control.

Mylotte, J. M. (. (1996). Measuring antibiotic use in a long-term care facility. *American Journal of Infection Control, 24*(3, pp. 174-179), June. doi:10.1016/S0196-6553(96)90009-7

Nadendla, R. R. (2005). *Principles of organic medicinal chemistry*

Nemathaga, F., Maringa, S., & Chimuka, L. (2008). Hospital solid waste management practices in limpopo province, south africa: A case study of two hospitals. *Waste Management (New York, N.Y.), 28*(7), 1236-1245.

Nicolle, L., Bentley, D., Garibaldi, R., Neuhaus, E., Smith, P., & SHEA Long-Term-Care Comm. (2000). Antimicrobial use in long-term-care facilities.

Oaks, J. L., Gilbert, M., Virani, M. Z., Watson, R. T., Meteyer, C. U., Rideout, B. A., Shivaprasad, H. L., Ahmed, S., Chaudhry, M. J. I., Arshad, M., Mahmood, S., Ali, A., & Khan, A. A. (2004). Diclofenac residues as the cause of vulture population decline in pakistan. Nature, 427(6975), 630-633. doi:10.1038/nature02317

Oda, Y., Nakammura, S., Oki, I., Kato, T., & Shinagawa, H. (1985). Evaluation of the new system (umu -test) for the detection of environmental mutagens and carcinogens. *Mutation Research, 147*(5), 219-229.

Ohlsen, K., Ternes, T., Werner, G., Wallner, U., Loffler, D., Ziebuhr, W., Witte, W., & Hacker, J. (2003). Impact of antibiotics on conjugational resistance gene transfer in staphylococcus aureus in sewage.

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.Research, 44*(2), 605-615.

Pal, K. S. (1998). *Statistics for geoscientists, techniques and applications*

Pauwels, B., Noppe, H., De Brabander, H., & Verstraete, W. (2008). Comparison of steroid hormone concentrations in domestic and hospital wastewater treatment plants. *Journal of Environmental Engineering, 134*(11), 933-936. doi:10.1061/(ASCE)0733-9372(2008)134:11(933)

Perez, S., & Barcelo, D. (2007). Fate and occurrence of X-ray contrast media in the environment.

Petrovic, M., Radjenvoic, J., Postigo, C., Kuster, M., Farre, M., de Alda, L., & Barcelo, D. (2008). Emerging contaminants in wastewaters: Sources and occurrence. In *Emerging contaminants from industrial and municipal waste, occurrence analysis and effects* (pp. 1-35) Springer.

Putschew, A., Wischnack, S., & Jekel, M. (2000). Occurrence of triiodinated X-ray contrast agents in the aquatic environment. *Science of the Total Environment, 255*(1-3), 129-134.

Rabiet, M., Togola, A., & Brissaud, F. (2006). Consequences of treated water recycling as regards pharmaceuticals and drugs in surface and ground waters of a medium-sized mediterranean catchment. *Environmental Science & Technology, 40*(17), 5282-5288.

Reddersen, K., Heberer, T., & Dunnbier, U. (2002). Identification and significance of phenazone drugs and their metabolites in ground- and drinking water. *CHEMOSPHERE, 49*(6), 539-544.

Reed, G. F., Lynn, F., & Meade, B. D. (2002). Use of coefficient of variation in assessing variability of quantitative assays. *Clinical and Diagnostic Laboratory Immunology, 9*(6), 1235-1239.

Rowland, M., & Tozer, T. N. (1995). In Tozer T. N. (Ed.), *Clinical pharmacokinetics : Concepts and applications*. Baltimore: Baltimore : Williams & Wilkins.

Sabourin, L., Beck, A., Duenk, P., Kleywegt, S., Lapen, D., Li, H., Metcalfe, C., Payne, M., & Topp, E. (2009). Runoff of pharmaceuticals and personal care products following application of dewatered municipal biosolids to an agricultural field. *Science of the Total Environment, 407*(16), 4596-4604. doi:10.1016/j.scitotenv.2009.04.027

Santos, L. H. M. L. M., Araújo, A. N., Fachini, A., Pena, A., Delerue-Matos, C., & Montenegro, M. C. B. S. M. (2010). Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. *Journal of Hazardous Materials, 175*(1-3), 45-95.

Schuster, A., Hadrich, C., & Kümmerer, K. (2008). Flows of active pharmaceutical ingredients originating from health care practices on a local, regional, and nationwide level in germany--is hospital effluent treatment an effective approach for risk reduction? *Water, Air, & Soil Pollution: Focus, 8*(5-6), 457-471. doi:10.1007/s11267-008-9183-9

Schwartz, T., Kohnen, W., Jansen, B., & Obst, U. (2003). Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms.

Seifrtova, M., Pena, A., Lino, C., & Solich, P. (2008). Determination of fluoroquinolone antibiotics in hospital and municipal wastewaters in coimbra by liquid chromatography with a monolithic column and fluorescence detection.

Skov, T., Lynge, E., Maarup, B., Olsen, J. c., n(3), R&oz.cansls, th, M., & Winthereik, H. (1990). Risks for physicians handling antineoplastic drugs. *The Lancet, 336*(8728, pp. 1446-1446), December. doi:10.1016/0140-6736(90)93148-I

Smith, D. A., Waterbeemd, H. V. D., & Walker, D. K. (2001). *Pharmacokinetics and metabolism in drug design* Wiley-VCH Verlag GmbH.

Stackelberg, P. E., Furlong, E. T., Meyer, M. T. , Zaugg, S. D. , Henderson, A. K. , & Reissman, D. B. (. (2004). Persistence of pharmaceutical compounds and other organic wastewater contaminants in a conventional drinking-water-treatment plant. *Science of the Total Environment, 329*(1-3), 99-113. doi:10.1016/j.scitotenv.2004.03.015

Stackelberg, P. E., Gibs, J., Furlong, E. T., Meyer, M. T., Zaugg, S. D., & Lippincott, R. L. (2007). Efficiency of conventional drinking-water-treatment processes in removal of pharmaceuticals and other organic compounds. *Science of the Total Environment, 377*(2-3), 255-272. doi:10.1016/j.scitotenv.2007.01.095

Steger-Hartmann, T., Kümmerer, K., & Hartmann, A. (1997). Biological degradation of cyclophosphamide and its occurrence in sewage water. *Ecotoxicology and Environmental Safety, 36*(2, pp. 174-179), March. doi:10.1006/eesa.1996.1506

Stephen, J. C., & Johan, H. B. (2004). Metabolic changes of drugs and related organic compounds. In J. M. Beale (Ed.), *Wilson and gisvold's textbook of organic medicinal and pharmaceutical chemistry* (). Philadelphia: Philadelphia : Lippincott Williams & Wilkins.

Stephenson, R. L., 1939-. (1998). In Blackburn J. W. (Ed.), *The industrial wastewater systems handbook*. Boca Raton, FL: Boca Raton, FL : CRC Press.

Suarez, S., Lema, J. M., & Omil, F. (2009). Pre-treatment of hospital wastewater by coagulation-flocculation and flotation. *Bioresource Technology, 100*(7), 2138-2146.

Sweetman, S. C., & Martindale, W. (2007). *Martindale : The complete drug reference* (35th ed .). London ; Chicago: Pharmaceutical Press.

Tasmuth, T., Härtel, B., & Kalso, E. (2002). Venlafaxine in neuropathic pain following treatment of breast cancer. *European Journal of Pain, 6*(1), 17-24. doi:10.1053/eujp.2001.0266

Ternes, T. A., Kreckel, P. , & Mueller, J. (1999). Behaviour and occurrence of estrogens in municipal sewage treatment plants — II. aerobic batch experiments with activated sludge. *The Science of the Total Environment, 225*(1-2, pp. 91-99), January. doi:10.1016/S0048-9697(98)00335-0

Ternes, T. (2001). Analytical methods for the determination of pharmaceuticals in aqueous environmental samples.

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

Ternes, T. A., & Hirsch, R. (2000). Occurrence and behavior of X-ray contrast media in sewage facilities and the aquatic environment. *Environmental Science & Technology, 34*(13), 2741-2748.

Ternes, T. A., Giger, W., & Joss, A. (2006). Introduction. In *Human pharmaceuticals, hormones and fragrances : The challenge of micropollutants in urban water management* (pp. 1-13). London : Seattle: Iwa.

Thomas, K. V., Dye, C., Schlabach, M., & Langford, K. H. (2007). Source to sink tracking of selected human pharmaceuticals from two oslo city hospitals and a wastewater treatment works. *Journal of Environmental Monitoring, 9*(12), 1410.

Tropsha, A. (2000). Recent trends in computer-aided drug discovery. *Current Opinion in Drug Discovery & Development,* (3), 310-313.

Troy, S. M. (., Dilea, C., Martin, P. T. (., Leister, C. A. (., Fruncillo, R. J. (., & Chiang, S. T. (. (1997). Pharmacokinetics of once-daily venlafaxine extended release in healthy volunteers. *Current Therapeutic Research, 58*(8, pp. 504-514), August.

Vasconcelos, T., Kümmerer, K., Henriques, D., & Martins, A. (2009). Ciprofloxacin in hospital effluent: Degradation by ozone and photoprocesses. *Journal of Hazardous Materials, 169*(1-3), 1154-1158. doi:10.1016/j.jhazmat.2009.03.143

Vromen, M., van der Ven, A., Knols, A., & Stobberingh, E. (1999). Antimicrobial resistance patterns in urinary isolates from nursing home residents. fifteen years of data reviewed.

Warren, J., Palumbo, F., Fitterman, L., & Speedie, S. (1991). Incidence and characteristics of antibiotic use in aged nursing-home patients.

Wasik, A., Debska, J., & Namiesnik, J. (2007). Analytical techniques in studies of the environmental fate of pharmaceuticals and personal-care products. *Trends in Analytical Chemistry, 26*(6), 557-568.

Watkinson, A., Murby, E., Kolpin, D., & Costanzo, S. (2009). The occurrence of antibiotics in an urban watershed: From wastewater to drinking water. *Science of the Total Environment, 407*(8), 2711-2723. doi:10.1016/j.scitotenv.2008.11.059

Weissbrodt, D., Kovalova, L., & Ort, C. (2009). Mass flows of X-ray contrast media and cytostatics in hospital wastewater. *Environmental Science & Technology, 43*(13), 105-108.

Wezel, A., & Jager, T. (2002). Comparison of two screening level risk assessment approaches for six disinfectants and pharmaceuticals.

WHO.*The anatomical therapeutic chemical classification system with defined daily doses (ATC/DDD).*, April, 2010, from http://www.who.int/classifications/atcddd/en/

Wise, R. (2002). Antimicrobial resistance: Priorities for action.

Wishart, D., Knox, C., Guo, A., Cheng, D., Shrivastava, S., Tzur, D., Gautam, B. & Hassanali, M.*DrugBank: A knowledgebase for drugs, drug actions and drug targets.* Retrieved Jun/23, 2010, from http://www.drugbank.ca

Yalkowsky, S. H. (. H., 1942-. (2003). In He Y. (Ed.), *Handbook of aqueous solubility data*. Boca Raton, Fla.: Boca Raton, Fla. : CRC Press.

Yamamoto, H., Nakamura, Y., Moriguchi, S., Nakamura, Y., Honda, Y., Tamura, I., Hirata, Y., Hayashi, A., & Sekizawa, J. (2009). Persistence and partitioning of eight selected pharmaceuticals in the aquatic environment: Laboratory photolysis, biodegradation, and sorption experiments. *Water Research, 43*(2), 351-362. doi:10.1016/j.watres.2008.10.039

Zhang, Y., Geißen, S., & Gal, C. (2008). Carbamazepine and diclofenac: Removal in wastewater treatment plants and occurrence in water bodies. *Chemosphere, 73*(8, pp. 1151-1161), November. doi:10.1016/j.chemosphere.2