ASSESSING THE PARTITIONING OF PHARMACEUTICALS AND PERSONAL CARE PRODUCTS IN SECONDARY WASTEWATER TREATMENT AND FATE TO THE RECEIVING ENVIRONMENTS

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B.Sc., Royal Roads University, 2011

A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE
in
ENVIRONMENT AND MANAGEMENT

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October 26, 2014

Abstract

Pharmaceuticals and personal care products are increasingly being detected in the environment and little is known of the effects to the environment. Thirty pharmaceuticals and personal care products were measured in liquid and solid streams in a conventional secondary wastewater treatment plant and compared to predicted partition loadings and toxicity levels. The predicted partitioned loadings derived from the model were significantly different from partitioned loadings within the treatment plant. Concentrations discharged from the treatment plant were below predicted and known toxicity levels for aquatic and terrestrial receiving environments. Together, these findings suggest the estimation model is ineffective for predicting partitioning loadings in secondary treatment, concentrations are not likely to be toxic in the marine environment around the outfall, and secondary wastewater treatment does have a positive impact on the removal of pharmaceuticals and personal care products during secondary wastewater treatment.

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Acknowledgements

This research project has been made possible with the support of many people. First, I would like to express my thanks to Christopher Lowe and the Capital Regional District for sponsoring this research. I would also like to express my thanks to the Royal Roads University School of Environment and Sustainability thesis coordinators, Dr. Audrey Dallimore and Dr. Matt Dodd, for their support and encouragement.

Funding for this project was supported by a Natural Sciences and Engineering Research Council of Canada – Canada Graduate Scholarships-Master's (NSERC-CGSM), the Capital Regional District, and the Institute of Ocean Sciences. Thank you to Dr. Michael Ikonomou for the development of the analytical processes from which the wide range of data would not have been possible. Thank you to the Capital Regional District for providing ongoing funding and data sharing for this project and to Christopher Lowe for his ongoing support.

Many thanks to my family, friends, and colleagues who have been with me, encouraged me, and kept me sane through this journey. A special thanks to my husband, Bryan Hoekstra, for his love, his patience, his understanding, and his belief in me that has kept me going throughout my studies.

Introduction

Pharmaceuticals and personal care products (PPCPs) are of emerging concern as they are increasingly being detected at low levels in aquatic and terrestrial environments and little is known of the effects to the environment. This thesis investigates the partitioning of PPCPs within secondary wastewater treatment at the Capital Regional District (CRD) Saanich Peninsula Wastewater Treatment Plant (SPWWTP) located in Sidney, BC, Canada and investigates the loadings and toxicity of the PPCPs discharged to the marine and terrestrial receiving environments.

Pharmaceuticals and Personal Care Products

There is an increasing concern over the large quantities of PPCPs being used on a daily basis. The United States Environmental Protection Agency (US EPA) defines pharmaceuticals as over-the-counter and prescription therapeutic drugs which also includes veterinary drugs. The US EPA defines personal care products as products used for personal or cosmetic reasons including soaps, fragrances, and cosmetics; many of which contain active ingredients found in the pharmaceutical classification (US Environmental Protection Agency, 2014a). It is estimated that worldwide there are approximately six million PPCPs in use, the use of pharmaceuticals is increasing 3-4% by weight per year, and approximately 170 pharmaceuticals are used in excess of one tonne per year (Ellis, 2006). PPCPs and the biological metabolites are introduced into the environment primarily through wastewater treatment effluent as a results of excretion, washing, and improper disposal (Xia, Bhandari, Das, & Pillar, 2005). Based partly on solubility and affinity for organic adsorption, PPCPs may partition to the effluent stream during wastewater treatment and be discharged into the aquatic receiving environment or partition to the sludge stream and be discharged onto the terrestrial receiving environment (Lowe, 2011). The use of octanol-water partition coefficient (K_{ow}) and water solubility as the predictors of PPCP partitioning behaviour has proven to be inadequate. It is a method that does not take into account other dependent wastewater stream factors such as biological degradation or loading concentrations and volumes (Ternes, Adriano, & Siegrist, 2004). PPCPs can degrade or combine with other components within the wastewater systems and transform into unknown or difficult to trace compounds (Xia et al., 2005). For example, triclosan contains chlorinated impurities in the form of polychlorinated dibenzodioxins and dibenzofurans, has degradation and reaction products in the form of 2,4-dichlorophenol and polychlorinated dioxins, and through methylation during wastewater treatment can transform into methyl-triclosan (Environment Canada, 2012b). PPCP metabolites have also been known to reactivate into the parent compound through the deconjugation of the excreted conjugated metabolite (Leusch et al., 2006; Sun, Deng, Huang, Shen, & Yu, 2008).

The environmental and human health impacts of these transformation, degradation, and reaction products are largely unknown (Daughton, 2003). PPCPs are continually introduced into the environment through wastewater treatment systems, replacing those either degraded or transformed. This results in pseudo-persistent chemicals; chemicals with a level of persistence that would otherwise not possess chemical stability in the environment (Daughton, 2003). Little is known of the impacts to aquatic and terrestrial environments from the continual, life-cycle, multi-generational, low-dose exposure to PPCPs (Daughton, 2003; Xia et al., 2005). Changes in ecosystems from the pseudo-persistence of PPCP may occur at such slow rates they go undetected or are attributed to natural adaptation or ecological succession (Ternes & Daughton, 1999). Studies of the impacts of chemical pollutants on human health and the environment have been primarily devoted to persistent bioaccumulative contaminants, persistent organic pollutants, and other bioaccumulative chemicals (Ellis, 2006; Sharpe, 2003).

There are a few studies that show the impacts to aquatic and terrestrial environments from the continual, life-cycle, multi-generational, low-dose exposure to PPCPs. Studies indicate exposure to serotonin reuptake inhibitors such as antidepressants adversely affect the reproductive behaviour of aquatic organisms (Daughton, 2003; Fong, Huminski, & D'Urso, 1998; Han et al., 2010). Steroid hormones and estrogens have been observed to cause endocrine-disrupting effects such as altered gender expression and reproductive development in aquatic species (Dietrich, Ploessl, Bracher, & Laforsch, 2010; Jobling et al., 2003; Kidd et al., 2007; Lange et al., 2001; Nakada et al., 2007; Nakada, Tanishima, Shinohara, Kiri, & Takada, 2006; Quinn et al., 2004). Antibiotic resistance has been observed in wastewater treatment systems (Reinthaler et al., 2003; Zhang, Marrs, Simon, & Xi, 2009; Zwenger & Gillock, 2009) and antibiotic resistant bacterial strains and adverse effects to antimicrobials have been observed in the environment (Chee-Sanford, Aminov, Krapac, Garrigues-Jeanjean, & Mackie, 2001; Halling-Sorensen, Sengelov, Ingerslev, & Jensen, 2003; Porsbring, Blanck, Tjellstrom, & Backhaus, 2009). Existing studies do not necessarily provide clear toxicity endpoints as there can be different reported endpoints, different target organisms, and/or different receiving environments. Most research has assessed acute effects of PPCPs on freshwater aquatic species, with relatively few studies focusing on the direct effects of concentrations found discharged into the receiving environments, chronic or long-term exposures, marine and terrestrial environmental effects, or cumulative and mixture effects (Dietrich et al., 2010; Ellis, 2006; Kostich & Lazorchak, 2008;

Kummerer, 2009; Pal, Gin, Lin, & Reinhard, 2010; Quinn, Gagne, & Blaise, 2008; Richardson, 2009; Tyler & Jobling, 2008). Studies are emerging on long-term effects of estrogens in fish species (Nash et al., 2004), chronic effects of central nervous system drugs in treated wastewater (Ferrari, Paxéus, Giudice, Pollio, & Garric, 2003), effects of anti-infective agents on marine species (Hidu, 1965), effects of anti-infective agents and blood formation drugs on terrestrial species (US National Library of Medicine, 2013), and effects of secondary exposure to veterinary drugs on vultures (Swan et al., 2006); though little is known of the cumulative effects and exposure to mixtures of PPCPs in the environment (Dietrich et al., 2010; Koplin et al., 2002).

Recently, more sophisticated analytical methods and instruments such as liquid chromatography with full scan and high-resolution mass spectrometry have been developed leading to the detection of trace contaminants and more robust analysis of chemicals with low concentrations such as PPCPs (Caliman & Gavrilescu, 2009; Richardson, 2009). Lower detection limits for a wider array of chemicals has increased the attention given to the issue of what is considered emerging contaminants of concern. PPCPs have been present in the environment since their development; it is the ability to detect them at trace levels that is raising concern without the proper understanding of the impacts and effects to the environment (Daughton, 2003). With the lack of comprehensive toxicity and partitioning data for PPCPs, modeling is still used to predict the fates (US Environmental Protection Agency, 2014c) and concentrations (US Environmental Protection Agency, 2014b) of PPCPs. With a greater knowledge and understanding of the impacts of PPCPs on the environment, there comes a greater focus on removal of PPCP in wastewater treatment and the receiving environments and the development of source control measures to minimize the input of PPCPs into the environment (Daughton, 2003).

Environment Canada has conducted a Preliminary Assessment (Environment Canada, 2012a) and developed a Risk Management Scope (Environment Canada, 2012b) for triclosan, an antimicrobial found in everything from clothing to toothpaste, from cleaning products to cosmetics. After identifying triclosan as having potential bioaccumulative effects in the environment, Environment Canada is seeking voluntary removal of triclosan as a non-essential ingredient in some personal care products (Environment Canada, 2012b). Though driven predominantly by human resistance to antibiotics, Alberta Health Services started the *Do Bugs Need Drugs* community education campaign (Alberta Health Services, 2013). The campaign, now also funded by the British Columbia (BC) Ministry of Health (MoH), is aimed at reducing dependence on antibiotics. This education campaign subsequently reduces the input of antibiotics into wastewater systems and the environment.

Estimation Programs Interface (EPI) Suite

The US EPA Office of Pollution Prevention and Toxics and Syracuse Research Corporation (SRC) developed the Estimation Programs Interface (EPI) Suite of models as screening-level tools for organic chemicals (US Environmental Protection Agency, 2014c).

Sewage Treatment Plant (STPWIN) model.

The STPWIN model (sewage treatment plant model) is based on fugacity principles to predict the fate and determine the behaviour of organic chemicals within the parameters of a conventional wastewater treatment plant utilizing activated sludge secondary treatment (US Environmental Protection Agency, 2014c). STPWIN estimates the fates of a chemical during different stages of treatment as it is subject to removal by evaporation, biodegradation, sorption to sludge, and loss in the final effluent. The biodegradation rate is both the most critical variable and the most uncertain variable; a rate determined by factors such as retention times (Clara, Kreuzinger, Strenn, Gans, & Kroiss, 2005). STPWIN can utilize biodegradation half-life relationships assigned from the BIOWIN model (biodegradation model) or utilize inputs of site-specific biodegradation half-life relationships. Treatment system properties for a conventional wastewater treatment plant such as treatment tanks and tank dimensions, influent properties, and gas and liquid phase mass transfer coefficients are pre-determined as defaults. The US EPA provides a disclaimer for the use of the STPWIN model that “estimated values should not be used when experimental (measured) values are available” (US Environmental Protection Agency, 2014c), though is a valuable resource for determining the partitioned fate of organic chemicals such as PPCPs in the absence of experimental data.

Ecological Structure-Activity Relationship (ECOSAR) model.

The Ecological Structure-Activity Relationship (ECOSAR) model integrates quantitative structure activity relationship (QSAR) models to predict the ecotoxicity hazards of organic chemicals. The QSAR models utilizes predicted octanol-water partition coefficient (K_{ow}) from the KOWWIN model (log octanol-water partition coefficient model) and measured toxicity values (mmol/L) for a set of 111 known chemicals classes and applies it to the class constituents of a chemical (US Environmental Protection Agency, 2014b). ECOSAR is designed to estimate general toxicity of organic chemicals in the absence of measured data. ECOSAR provides a standard toxicity profile characterizing the potential aquatic hazards as defined by acute effects in fish (LC50, 96hr), daphnid (LC50, 48hr), and algae (EC50, 72 or 96hr) and chronic effects in fish (ChV), daphnid (ChV), and algae (ChV) for each of the chemicals class constituents. LC50 is a standard measure of toxicity that is defined as the lethal concentration of a substance that will kill half of the sample population through exposure in a specified period of time. EC50 is also a standard measure of toxicity that is defined as the median effective concentration of a substance that produces 50% of the maximum possible effect through exposure in a specified period of time. Chronic value (ChV) is determined by the geometric mean of the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC). Additional freshwater species and some terrestrial and marine species toxicity endpoints are available when measured data is made available. It is common to use the most conservative effect level when provided with results from multiple classes for a chemical. Similar to the STPWIN model, the US EPA provides a disclaimer for the use of the ECOSAR model that "estimated values should not be used when experimental (measured) values are available" (US Environmental Protection Agency, 2014c), though is a valuable starting point for determining the ecotoxicity of organic chemicals such as PPCPs in the absence of experimental data. There are databases of chemical toxicity data available such as National Centers for Coastal Ocean Science (NCCOS) Pharmaceuticals in the Environment database (National Centers for Coastal Ocean Science, 2014), US National Library of Medicine Hazardous Substances Data Bank (HSDB) (US National Library of Medicine, 2013), and US National Library of Medicine ChemID*plus* database (US National Library of Medicine, 2012). These databases are not always updated with the most current research, and as such are only useful as a preliminary source for toxicity data.

Capital Regional District

The Capital Regional District (CRD) is the regional government providing regional services which includes wastewater treatment to the 13 municipalities and three electoral areas on southern Vancouver Island and surrounding Gulf Islands in British Columba (BC), Canada. Upon the development of the CRD Sewer Use Bylaw regulating the discharge of contaminants to sanitary sewer, the CRD implemented the Regional Source Control Program (RSCP) in 1994 (Capital Regional District, 2012a). The RSCP is a pollution prevention initiative for the reduction of contaminants discharged from the industrial, commercial, and institutional (ICI) sectors into the regional sanitary sewer systems (Capital Regional District, 2012a). Source control has proven to be an effective method of reducing contaminant loading to the environment.

The CRD 2003 Core Area Liquid Waste Management Plan (LWMP) provides the guidance for the management of liquid waste for the seven municipalities within the Capital Regional District core area, which includes a proposal for the addition of secondary wastewater treatment for the core area (Capital Regional District, 2011). The CRD currently has three major wastewater treatment facilities serving the core area and the Saanich Peninsula, with outfalls discharging effluent to the marine receiving environment. Macaulay Point and Clover Point treatment facilities provide preliminary wastewater treatment for approximately 330,000 people within the core area. The preliminary treatment facilities utilize a mechanized 6 mm bar screen, and the predominantly inorganic filtered particles are disposed in the regional engineered sanitary landfill. The Macaulay Point facility outfall extends 1.7 km to a depth of 60 m into the Strait of Juan de Fuca marine waters, and discharges an average annual flow of 44,000 m^3 of sewage per day (Capital Regional District, 2011). The Clover Point facility outfall extends 1.1 km to a depth of 67 m into the Strait of Juan de Fuca marine waters, and discharges an average annual flow of 50,000 m^3 of sewage per day (Capital Regional District, 2011). The proposal for secondary wastewater treatment for the core area would replace the two preliminary treatment facilities.

The Saanich Peninsula Wastewater Treatment Plant (SPWWTP) provides secondary wastewater treatment for approximately 35,000 people within the Saanich Peninsula. The SPWWTP utilizes activated sludge, a standard secondary treatment process based on natural bacterial populations to treat raw sewage. The preliminary screenings of approximately 19.38 tonnes per year, as well as the organic sludge of approximately 3210.06 tonnes per year produced during secondary treatment, are disposed in the regional engineered sanitary landfill (Capital Regional District, 2009, 2013). The SPWWTP outfall extends 1,580 m to a depth of 30 m into the Haro Strait marine waters, and discharges an average annual flow of 15,000 m^3 of sewage per day (Capital Regional District, 2013).

Secondary wastewater treatment produces sludge which can be treated through physical, chemical, and/or biological processes to produce biosolids. Biosolids, rich in nitrogen and phosphorous, can be used in land applications such as soil amendments and fertilizers. The presence in biosolids of heavy metals, pathogens, hydrocarbons, chemical residues, and other chemicals of concern such as pharmaceuticals and personal care products are a source of concern for land application uses. These chemicals and pathogens can be introduced into the terrestrial receiving environments and leach into the surrounding groundwater and aquatic environments (Dawson, 2011). The use of biosolids has been banned for land application uses within the

Capital Regional District (Capital Regional District, 2009). However, there is a commitment by the CRD to pursue opportunities for the beneficial use for biosolids (Capital Regional District, 2009).

The LWMP includes a requirement by the British Columbia (BC) Ministry of Environment (MoE) for the CRD to conduct and/or collaborate on studies of emerging scientific concerns, such as PCPPs in wastewater and the receiving environments (Capital Regional District, 2011). The CRD has identified 125 PPCPs within the core area wastewater stream, and PPCPs are being detected at low levels in marine surface waters surrounding the CRD sewer outfalls (Lowe, 2011). Studies conducted for the CRD have compared the PPCP loading concentrations to the effluent discharge concentrations following preliminary treatment, the wastewater treatment method currently utilized in the region's core area, finding that ibuprofen concentrations were close to freshwater chronic sub-lethal effects levels (Lowe, 2011). A risk analysis conducted for the CRD assessed the impacts of PPCPs within the CRD receiving environments following secondary wastewater treatment, identifying antimicrobials triclosan and triclocarbon as priority concerns based on toxicity, loading, and environmental persistence; and identified pain reliever acetaminophen and antibiotic clarithromycin for priority concern based on loading, soil persistence, and leaching potential (Barnard, Frias, Laloge, Rose, & Van Tongeren, 2011). Further research has been conducted for the CRD to assess source control strategies for antimicrobial triclosan and surfactant nonylphenols (Dinn, Jenmsa, & Alava, 2014).

Objectives

The objectives of this research were to:

- conduct an assessment of partitioned PPCP loadings within secondary wastewater treatment at the Saanich Peninsula Wastewater Treatment Plant; and
- conduct an assessment of partitioned PPCP concentrations in the receiving environments at the Saanich Peninsula Wastewater Treatment Plant.

A mass balance analysis will be conducted on approximately one year of wastewater sampling results for a select group of thirty PPCPs entering the SPWWTP through influent, exiting through effluent and sludge, and being removed through biodegradation or other transformative processes. A comparative assessment will be conducted for the SPWWTP effluent, sludge, and transformative loss PPCP loadings against predicted effluent, sludge, and transformative loss PPCP loadings to determine the effectiveness of estimation models for determining PPCP partitioning in wastewater systems. A comparative assessment will be conducted for the SPWWTP marine and terrestrial receiving environments PPCP concentrations against predicted toxicity values for aquatic and terrestrial species to determine priority PPCPs of concern. Finally, this research will provide a discussion on the implications of PPCPs within the CRD's wastewaters and receiving environments, recommendations for addressing PPCPs of concern, and suggestions for future research.

General Methodology

As part of an agreement between the Department of Fisheries and Oceans Canada (DFO) and the Capital Regional District (CRD), the analytical data from wastewater samples collected at the Saanich Peninsula Wastewater Treatment Plant (SPWWTP) was made available for this research by Dr. Michael Ikonomou. The methods used to collect, prepare, and analyze the wastewater samples collected at the SPWWTP and the methods used to prepare the data for assessment are outlined in the following section.

Sample Collection

Approximately 280 wastewater samples were collected at the SPWWTP bi-weekly from August, 2011 to September, 2012 for a total of 56 sample days. *Figure 1* shows the five sampling sites located within the secondary wastewater treatment processes at the SPWWTP. Wastewater samples were collected at the bar rack (Site 1, influent, preliminary screened) and effluent outfall (Site 2, after treatment by secondary clarifiers prior to discharge). Sludge samples were collected from primary sludge (Site 3, primary treatment), return activated sludge (Site 4, secondary treatment), and dewatered sludge (Site 5, mixed primary and secondary sludge after dewatering press).

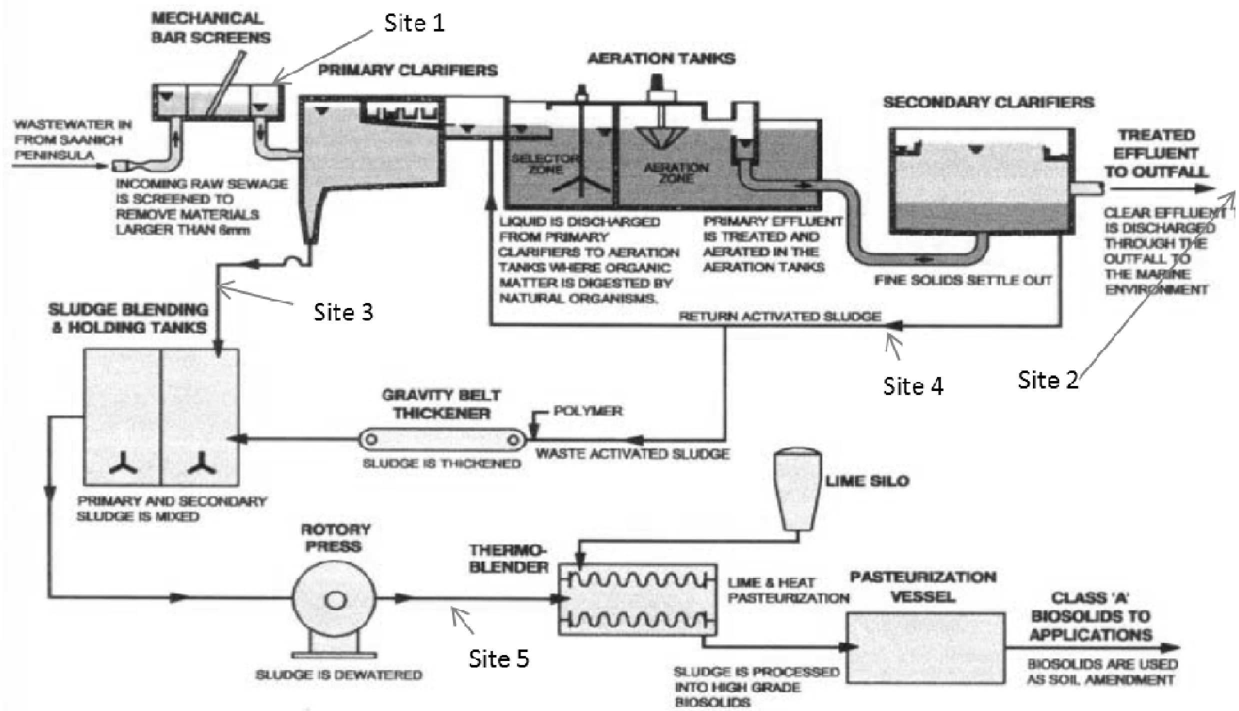

Figure 1. Secondary wastewater treatment process flow diagram with sampling site locations for Saanich Peninsula Wastewater Treatment Plant. Adapted from *Saanich Peninsula Wastewater Treatment Plant* [Brochure], by Capital Regional District, n.d., Victoria, BC: Capital Regional District. Copyright n.d. by Capital Regional District. Adapted with permission.

Grab samples from Sites 1 and 2 were collected into 4 L amber glass bottles. Grab samples from Sites 3 and 4 were collected into 500 ml amber glass bottles. Grab samples from Site 5 were collected into 125 ml amber glass bottles. All amber sample bottles were pre-rinsed with deionized water and 10% methanol in water. The samples from Sites 1 through 4 were stored at 4°C until filtration. The samples from Site 5 were stored at -20°C until filtration.

Analysis

The liquid and solid phases of each sample were prepared and analyzed separately at the Regional Dioxin Laboratory at the Institute of Ocean Sciences, Fisheries and Oceans Canada in

Sidney, BC, Canada. The methods used to prepare and analyze the samples are outlined in the following section.

Liquid samples.

Samples collected from Sites 1 through 4 were filtered the day following collection through glass fibre filters in series of 5.0 µm, 2.7 µm, then 1.2 µm. After filtration, the filtering device and fibre filters were washed with acetonitrile, approximately 5% of the volume of filtrate. The wash solution was collected, combined with the original filtrate, and mixed well. One milliliter of the filtrate was taken and placed into a 1.8 ml amber vial and stored at -20°C until extraction. The filtrate was diluted 10 times with a mixture of 50% acetonitrile and 50% .05% ammonia solution ($NH_3.H_2O$). An aliquot of 950 µL of the diluted solution was spiked with 50 µL of internal standard solution and mixed prior to instrumental analysis by liquid chromatography tandem mass spectrometry (LC/MS/MS), based on US Environmental Protection Agency (US EPA) Method 1694 (US Environmental Protection Agency, 2007).

Solid samples.

Approximately 1.0 g of sample from Site 5 or solid phase material from samples from Sites 3 and 4 was mixed with 35 ml acetonitrile for 2 hours with a horizontal shaker and 1 hour by sonication. The suspension was vortexed for 30 seconds before centrifugation at 6000 rpm for 30 minutes. 1 ml of the supernatant was pipetted into a 1.5 ml Eppendorf vial and centrifuged at 14,000 rpm for 4 minutes. 950 µL of the supernatant from the Eppendorf vial was spiked with 50 µL of internal standard solution and mixed prior to instrumental analysis by LC/MS/MS.

Due to the trace level of solid material in Site 1, all solid phase material from Site 1 was used for extraction by adding 100 ml acetonitrile and sonicated for 1 hour. 1 ml of the supernatant was pipetted into a 1.5 ml Eppendorf vial and centrifuged at 14,000 rpm for 4 minutes. 950 µL of the supernatant from the Eppendorf vial was spiked with 50 µL of internal standard solution and mixed prior to instrumental analysis by liquid chromatography tandem mass spectrometry (LC/MS/MS), based on US EPA Method 1694 (US Environmental Protection Agency, 2007).

Data Preparation

The daily data from Sites 1, 2, and 5 for the concentrations of 30 pharmaceuticals and personal care products (PPCPs) in liquid and solid phases were matched, resulting in 25 days of complete data. Data reported as less than limit of quantitation (<LOQ) was given a value of ½ the reported LOQ. Data reported as less than limit of detection (<LOD) was given a value of ½ the reported LOD. Data reported as not detected (ND) was given a value of zero. These proxy values were determined based on previous use of data from this source (C. Lowe, personal communication, April 10, 2014).

The solid phase PPCP concentration data for Site 1 was proportionally adjusted with daily total suspended solids (TSS) data from the SPWWTP provided by the Capital Regional District (2012b, 2013), and added to the liquid phase PPCP concentration data for total influent PPCP concentrations. The total influent PPCP concentrations were calculated using the following equation:

$$C_{x,inf} = \frac{\left(S_{x,inf}\ x\left(\frac{T_{inf}}{1000}\right)\right) + \left(L_{x,inf}\ x\ 1000\right)}{1000}$$

where:

$C_{x,inf}$ is the total concentration of *X* (PPCP) in influent due to the sum of *X* concentration in influent solid phase adjusted for TSS and *X* concentration in influent liquid phase (µg/L);

$S_{x,inf}$ is the concentration of X in influent solids (ng/g);

T_{inf} is the concentration of TSS in influent (mg/L);

$L_{x,inf}$ is the concentration of *X* in influent liquids (ng/ml).

The Site 2 liquid phase concentrations constitute the total concentrations for effluent reported in µg/L. The Site 5 solid phase concentrations constitute the total concentrations for sludge reported in µg/kg.

The data from Sites 3 and 4 were not used for this research as there was no accompanying liquid phase data from the respective wastewater treatment plant processes to allow for a more detailed mass balance analysis. Only select solid samples could be analyzed resulting in incomplete matched data sets with the liquid phase data for mass balance analysis.

Statistical Assessment

The LOD and descriptive statistics: n, min, max, median, mean, standard deviation (SD), and relative standard deviation percent (RSD%) for total influent concentrations are presented in *Appendix A*, for total effluent concentrations are presented in *Appendix B*, and for total sludge concentrations are presented in *Appendix C*.

The reported LOQ values for liquid and solids were substantially different and not included for assessment of combined totals.

Assessment of Partitioned PPCP Loadings

This assessment compares the partitioned loadings of pharmaceuticals and personal care products (PPCPs) in influent, effluent, and sludge at the Saanich Peninsula Wastewater Treatment Plant (SPWWTP) to the predicted partitioned loadings of PPCPs in influent, effluent, and sludge during conventional activate sludge secondary wastewater treatment acquired from the US EPA Estimation Programs Interface (EPI) Suite.

Methodology

The methods used to prepare and assess the data for the comparison of percent partitioned loadings from the SPWWTP to the predicted percent partitioned loadings from the US EPA EPI Suite are outlined in the following section.

Data preparation.

The influent mean concentration data for each of the 30 PPCPs was calculated with the 2011 and 2012 averaged annual influent volume data from the SPWWTP Supervisory Control and Data Acquisition (SCADA) system provided by the CRD, to determine the average annual PPCP loadings partitioned from influent.

The effluent mean concentration data for each of the 30 PPCPs was calculated with the 2011 and 2012 averaged annual effluent volume data from the SPWWTP provided by the Capital Regional District (2012b, 2013), to determine the average annual PPCP loadings partitioned to effluent.

The sludge mean concentration data for each of the 30 PPCPs was calculated with the 2011 and 2012 averaged annual sludge volume data from the SPWWTP SCADA system provided by the CRD, to determine the average annual PPCP loadings partitioned to sludge.

Influent loadings were taken as 100% loading and divided into effluent and sludge loadings to proportionally calculate the respective effluent and sludge percent loadings. The remainder was calculated and classified as *other removed* using the following mass balance equation:

$$P_{x,oth} = \left(\frac{L_{x,inf} - (L_{x,eff} + L_{x,slu})}{L_{x,inf}}\right) x\ 100$$

where:

$P_{x,oth}$ is the percent loading of X (PPCPs) lost due to the sum of effluent and sludge annual loadings of X (%);

$L_{x,inf}$ is the loading of X in influent (g/year);

$L_{x,eff}$ is the loading of X in effluent (g/year);

$L_{x,slu}$ is the loading of X in sludge (g/year).

Predicted percent removal in secondary wastewater treatment for total sludge adsorption, total biodegradation, and removal to air were acquired from the US EPA EPI Suite. The STPWIN model (sewage treatment plant model) was set to utilize biodegradation half-life relationships assigned from the BIOWIN model (biodegradation model) and default treatment system properties for a conventional wastewater treatment plant using activated sludge secondary treatment (US Environmental Protection Agency, 2014c).

For the purposes of qualitative assessment, groupings were applied to the PPCPs based on the American Hospital Formulary Standard first tier classification (AHFS 1) of the AHFS Pharmacologic-Therapeutic Classification system developed by the American Society of Health-System Pharmacists and provided to the CRD by the Ministry of Health (2014). The AHFS classification provides groupings of drugs with similar pharmacological, therapeutic, or chemical characteristics.

Statistical assessments.

Three statistical methods were used to assess the differences between the calculated percent loadings to effluent, sludge, and *other removed* in the SPWWTP and the predicted percent loadings to effluent, sludge, *other removed* (biodegradation and removal to air) in secondary wastewater treatment acquired from the US EPA EPI Suite. All statistical tests were conducted using a statistical significance level of α=.05.

The data sets of mean and predicted PPCP percent loadings to effluent, sludge, and *other removed* were assessed separately for normality using the non-parametric Anderson-Darling test for distribution.

The data sets of mean and predicted PPCP percent loadings for effluent, sludge, and *other removed* were assessed for correlation using the non-parametric Spearman's rank correlation coefficient test for correlation.

The data sets of mean and predicted PPCP percent loadings for effluent, sludge, and *other removed* were assessed for mean ranked differences using the non-parametric Wilcoxon paired signed-rank test for distribution equality.

Results

The mean PPCP daily loadings (mg/day) and mean PPCP annual loadings (g/year) in the SPWWTP are presented in *Appendix D.* The mean PPCP percent loadings to effluent, sludge, and *other removed* in the SPWWTP and the predicted PPCP percent loadings to effluent, sludge, and *other removed* in secondary wastewater treatment acquired from the US EPA EPI Suite, group by AHFS 1 classification, are presented in *Appendix E*. Clarithromycin (111.90%), lincomycin (103.49%), oxytetracycline (102.89%), trimethoprim (122.26%), albuterol (101.95%), warfarin (104.19%), carbamazepine (133.46%), and fluoxetine (125.52%) effluent mean percent loadings exceeded influent mean percent loadings (100%). Triclosan (176.05%) sludge mean percent loadings exceeded influent mean percent loadings (100%). There appears to be no apparent distribution pattern between pharmaceuticals AHFS 1 groups for the occurrences of effluent and sludge mean percent loadings exceeding influent mean percent loadings (see *Appendix E*).

The mean percent loadings to effluent and sludge and the predicted percent loadings to effluent, sludge, and *other removed* did not meet the assumption of normal distribution ($p < .05$); the mean percent loadings to *other removed* meet the assumption of normal distribution ($A^2 = .59$, $p = .1146$) (see *Appendix F*).

The mean and predicted percent loadings for effluent and *other removed* meet the assumption of independence ($df = 28$, $p > .05$); the mean and predicted percent loadings for sludge did not meet the assumption of independence ($df = 28$, $p = .0454$) (see *Appendix G*). Statistically there is a correlation between mean and predicted percent loadings for sludge. The individual variations between mean and predicted percent loadings for sludge can be seen in *Figure 3*.

The mean and predicted percent loadings for effluent, sludge, and *other removed* meet the assumption of distribution equality ($n = 30$, $p > .05$) (see *Appendix H*). This distribution can be seen in *Figure 2*, *Figure 3* and *Figure 4*. The negative values represented in the mean percent loadings to *other removed* (see *Appendix E*) are a result of the calculated mass balance remainder determined by effluent and sludge percent loadings exceeding influent percent loadings, and can be seen in *Figure 4*. Statistically the mean and predicted loadings data sets are distributed equally; the data sets for effluent, sludge, and *other removed* calculated loadings are the same (symmetric around the mean) as the data sets for the predicted loadings data sets, respectively. The individual variations between mean and predicted percent loadings can be seen in *Figure 2*, *Figure 3* and *Figure 4*.

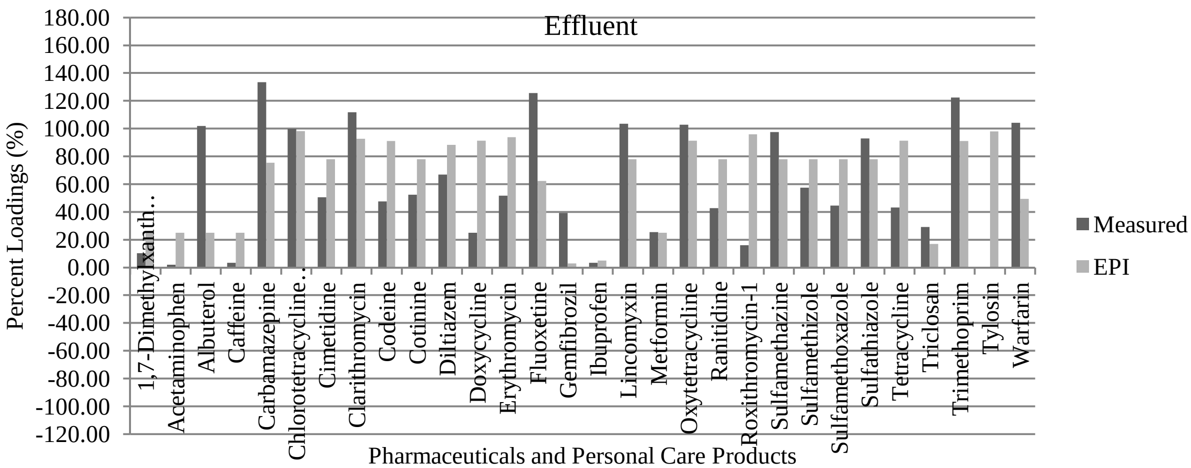

Figure 2. Measured mean percent loadings to effluent in the Saanich Peninsula Wastewater Treatment Plant with US EPA Estimation Program Interface (EPI) Suite predicted percent loadings to effluent in secondary wastewater treatment.

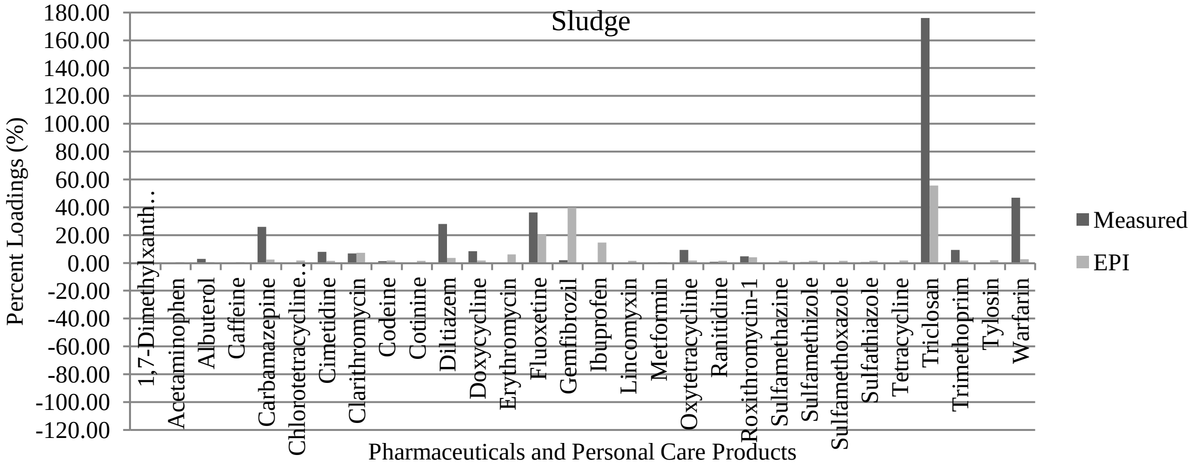

Figure 3. Measured mean percent loadings to sludge in the Saanich Peninsula Wastewater Treatment Plant with US EPA Estimation Program Interface (EPI) Suite predicted percent loadings to sludge in secondary wastewater treatment.

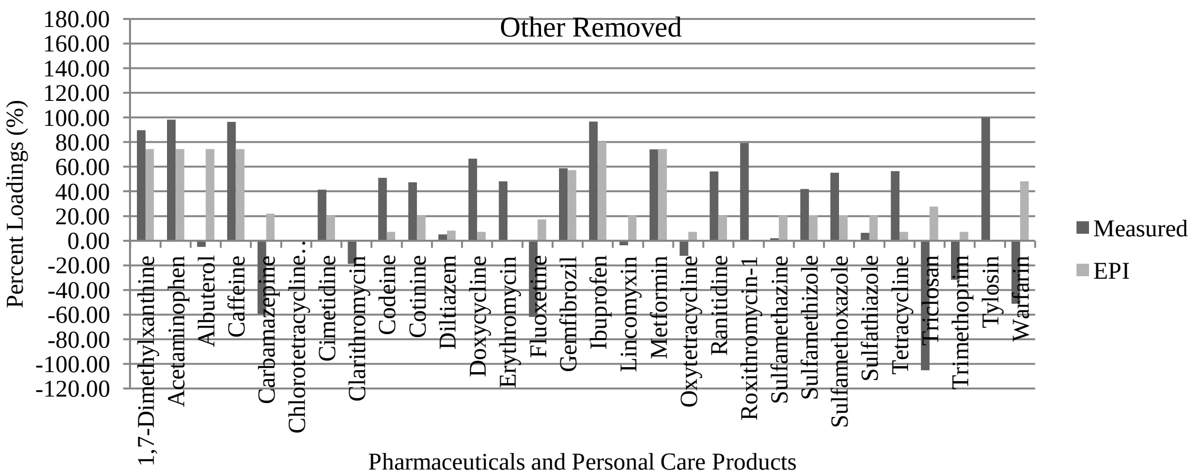

Figure 4. Calculated mean percent loadings to *other removed* in the Saanich Peninsula Wastewater Treatment Plant with US EPA Estimation Program Interface (EPI) Suite predicted percent loadings to *other removed* in secondary wastewater treatment.

These results provide strong evidence that mean percent loadings of PPCPs partitioned in the SPWWTP are not the same as the US EPA EPI Suite predicted percent loadings partitioned in conventional secondary treatment.

Discussion

Statistically there is a correlation between mean and predicted percent loadings for sludge and statistically the data sets for mean and predicted percent loadings for effluent, sludge, and other removed data sets are distributed equally. *Figure 2*, *Figure 3* and *Figure 4* show that while there is a trend in partitioning behaviour related to the statistical correlation and statistical distributions, there is variation between the measured and predicted values. The variations range from .07% under predicted *other removed* levels for chlortetracycline to 120.54% over predicted sludge levels for triclosan (see *Appendix E*). The variations for individual PPCPs are significant enough to determine that the STPWIN model (sewage treatment plant model) utilizing conventional wastewater treatment plant parameters does not adequately predict the partitioned loadings of pharmaceuticals and personal care products (PPCPs) for the SPWWTP.

The STPWIN model can be set to utilize site-specific biodegradation rates. However, the SPWWTP biodegradation rates are seen to vary from day to day, even hour to hour, as the processes within the SPWWTP are continually adjusted to maximize efficiency (D. Perreault, personal communication, July 28, 2014), thus making it difficult to establish site-specific biodegradation rate constants. The STPWIN model is still constrained to the default treatment system properties such as biomass concentrations and tank parameters for a conventional wastewater treatment plant using activated sludge secondary treatment (Jones, Voulvoulis, & Lester, 2002). Utilizing the STPWIN model with more site-specific biodegradation rates may provide more significant estimations of partitioning behaviours, but the model still operates with limitations that produce uncertainty in any results.

Studies comparing removal efficiencies predicted in models indicate there are significant differences between the models (Kim, Lee, Lee, & Kwon, 2009; Seriki et al., 2008). Removal efficiencies have been found to either under-predicted or over-predicted when compared to experimental data (Besse & Garric, 2010; Kim et al., 2009; Ortiz de Garcia, Pinto Pinto, Encina, & Mata, 2013; Seriki et al., 2008). The US EPA issues a disclaimer that the program is a screening tool (US Environmental Protection Agency, 2014c). However, the STPWIN model, or other models like it, can often be the only recourse for determining partitioning behaviours when there is little available scientific research. It is a safe assumption that due to the variations between treatment plants and the processes, PPCP loadings for other treatment plants with varying biodegradation rates and different system parameters would be equally as difficult to predict using the STPWIN model.

With the exception of eight PPCPs with results of effluent percent loadings exceeding 100%, PPCPs are seen to partition to effluent within the SPWWTP from 0% to 99.87%; estimated effluent percent loadings range from 2.84% to 98.15%. With the exception of triclosan with results of sludge percent loadings exceeding 100%, PPCPs are seen to partition to sludge within the SPWWTP from 0% to 46.85%; estimated sludge percent loadings range from .62% to 39.81%. While these rates appear to follow a trend, individually, most PPCPs exhibit significantly different partitioning behaviours than those predicted. Chlortetracycline, metformin, ibuprofen, and 1,7-dimethylxanthine exhibited similar mean percent loadings to estimated percent loadings within 15% for effluent, sludge, and *other removed*. The remaining

26 PPCPs exhibited variations exceeding 20% between one or all mean to estimated percent loadings for effluent, sludge, and *other removed*.

PPCPs are seen to be removed through processes such as biodegradation within the SPWWTP at .02% to 100%; estimated *other removal* percent loadings range from .09% to 80.36% (see *Appendix E*). Predicted partitioned loadings to *other removed* were calculated from the STPWIN predicted total biodegradation and the model-estimates total to air (US Environmental Protection Agency, 2014c). The SPWWTP partitioned loadings to *other removed* were not measured but calculated from measured sludge and effluent loadings subtracted from the measured influent loadings. The resulting negative values are not indicative of actual partitioned loadings since PPCP concentrations were not actually analyzed for this portion of the wastewater stream. Additional sampling and further analysis of the loadings during secondary treatment are needed to determine where and by what processes within the secondary treatment removal occurs.

PPCPs partitioned to sludge during secondary treatment at rates as low as 0% for tylosin and as high as 176.05% for triclosan (see *Appendix E*). PPCPs partitioned to effluent during secondary treatment at rates as low as 0% for tylosin and as high as 133.46% for carbamazepine (see *Appendix E*). It was anticipated that specific trends would appear in partitioning behaviour amongst groups of PPCPs based on similar pharmacological, therapeutic, or chemical characteristics, but the PPCP removal rates do not follow a predictable partitioning trend even when grouped by American Hospital Formulary Standard first tier classification (AHFS 1) (see *Appendix E*).

The most notable variations in partitioned loadings were the PPCPs with loadings to effluent or sludge that exceeded the influent loadings; loadings that varied significantly from the model-estimates loadings (see *Appendix E*). Clarithromycin (111.90%), lincomycin (103.49%), oxytetracycline (102.89%), trimethoprim (122.26%), albuterol (101.95%), warfarin (104.19%), carbamazepine (133.46%), and fluoxetine (125.52%) effluent mean percent loadings exceeded influent mean percent loadings (100%). Triclosan (176.05%) sludge mean percent loadings exceeded influent mean percent loadings (100%). This potentially indicates processes of reactivation of unaccounted metabolites or conjugates of PPCPs during the treatment process (Leusch et al., 2006; Sun et al., 2008). Transformative processes within the wastewater treatment process increase the difficulty in determining loadings of PPCPs into the environment, and raises further questions regarding the fates and toxicities of the PPCPs and the metabolites in the environment. Further studies would be required to categorize the metabolized compounds of PPCPs for a more comprehensive suite of PPCPs to be included in future fates and effects research.

The effluent and sludge exceedances, along with other variations found in the results, could be attributed to methodology variability in sampling and analysis. Discrete daily grab samples do not provide a representative sample of the intermittently discharged, individual wastewater surges that occur within the wastewater systems (Ort, Lawrence, Reungoat, & Mueller, 2010). The resulting heterogeneity and potential variations in PPCP concentrations within the wastewater systems could produce results of PPCP concentrations in effluent or sludge exceeding PPCP concentration in influent and lead to erroneous conclusions (Ort, Lawrence, Rieckermann, & Joss, 2010). Holding time within the primary and secondary treatment processes and the subsequent transport time through the treatment plant were not accounted for with the daily comparative samples, thus the influent PPCP concentrations do not directly correspond with the effluent and sludge PPCP concentrations. Additionally, the extrapolation of daily PPCP concentrations with annually averaged flow data can introduce systematic errors resulting in data and results uncertainty.

Assessment of Partitioned PPCP Concentrations

This assessment compares the concentrations of pharmaceuticals and personal care products (PPCPs) in effluent and sludge at the Saanich Peninsula Wastewater Treatment Plant (SPWWTP) to the predicted toxicity of PPCPs for the receiving environments.

Methodology

The methods used to prepare and assess the data for the comparison of partitioned concentrations from the SPWWTP to the predicted toxicities for the receiving environments are outlined in the following section.

Data preparation.

The sludge concentration data was converted from µg/kg to mg/kg for comparison to predicted acute terrestrial toxicity levels reported in mg/kg. The effluent concentration data was converted from µg/L to mg/L for comparison to predicted acute and chronic aquatic toxicity levels reported in mg/L. The effluent concentration data was also adjusted by the SPWWTP 153:1 *outfall marine dilution factor* used by the Capital Regional District (CRD) Environmental Services Marine Programs (Hay & Company Consultants, 2005). This is the minimum initial dilution of effluent confined to approximately 50 m from the marine outfall measured during a dye study. The average initial dilution factor is actually much higher. The maximum initial dilution factor is estimated to be 1578:1within the 50 m initial dilution region; becoming orders of magnitude larger beyond the 50 m region as a result of rapid dilution due to horizontal deflection from the ambient currents. The 153:1 *outfall marine dilution factor* provides a conservative predictor of environmental concentrations. The effluent concentration data was converted from µg/L to mg/L for comparison to predicted acute marine toxicity levels.

Predicted acute and chronic toxicity values for freshwater daphnid species, predicted acute toxicity values for marine or marine-equivalent species, and predicted acute toxicity values for mice were acquired from the US EPA ECOSAR (US Environmental Protection Agency, 2014b), National Centers for Coastal Ocean Science (NCCOS) Pharmaceuticals in the Environment database (National Centers for Coastal Ocean Science, 2014), US National Library of Medicine Hazardous Substances Data Bank (HSDB) (US National Library of Medicine, 2013), and US National Library of Medicine ChemID*plus* database (US National Library of Medicine, 2012) and are presented in *Appendix I.*

For the purposes of qualitative assessment, groupings were applied to the PPCPs based on the American Hospital Formulary Standard first tier classification (AHFS 1) of the AHFS Pharmacologic-Therapeutic Classification system developed by the American Society of Health-System Pharmacists and provided to the CRD by the Ministry of Health (2014). The AHFS classification provides groupings of drugs with similar pharmacological, therapeutic, or chemical characteristics. Magnitudes of differences were calculated between the mean PPCP concentrations for effluent, effluent with SPWWTP 153:1 *outfall marine dilution factor,* and sludge and the predicted toxicity levels for freshwater acute and chronic, marine acute, and terrestrial acute.

Statistical assessments.

Two statistical methods were used to assess the differences between the concentrations in effluent and sludge from the SPWWTP and the predicted toxicity values for receiving environments. All statistical tests were conducted using a statistical significance level of α=.05.

The total (liquid and solid) PPCP concentrations in effluent and sludge were assessed separately for normality using non-parametric Anderson-Darling test for distribution.

The mean PPCP concentrations for effluent, effluent with SPWWTP 153:1 *outfall marine dilution factor*, and sludge and the predicted toxicity levels for freshwater acute and chronic, marine acute, and terrestrial acute were assessed separately for normality using the non-parametric Anderson-Darling test for distribution.

The total PPCP concentrations for effluent, effluent with SPWWTP 153:1 *outfall marine dilution factor*, and sludge were assessed for median ranked differences against the predicted toxicity levels for freshwater acute and chronic, marine acute, and terrestrial acute using the non-parametric Wilcoxon 1-tailed t-test for distribution equality.

The mean PPCP concentrations for effluent, effluent with SPWWTP 153:1 *outfall marine dilution factor*, and sludge and the predicted toxicity levels for freshwater acute and chronic, marine acute, and terrestrial acute levels were assessed for mean ranked differences using the non-parametric Wilcoxon paired signed-rank test for distribution equality.

Results

The mean PPCP concentrations in effluent, effluent with SPWWTP 153:1 *outfall marine dilution factor*, and sludge in the SPWWTP and the predicted toxicity levels for freshwater acute and chronic, marine acute, and terrestrial acute, grouped by AHFS 1 classification, are presented in *Appendix I*. The magnitude of the difference between mean PPCP concentrations in the SPWWTP and the predicted toxicity levels for receiving environments are presented in *Appendix N*. The mean concentrations in effluent are between 64 and $19x10^5$ times less than the predicted freshwater daphnid chronic toxicity levels, representing the least comparative difference (see *Appendix N*). The mean concentrations in effluent with SPWWTP 153:1 *outfall marine dilution factor* are between $15x10^3$ and $48x10^9$ times less than the predicted marine or marine equivalent species acute toxicity levels, representing the greatest comparative difference (see *Appendix N*). The mean concentrations in sludge are between 525 and $97x10^7$ times less than the predicted terrestrial toxicity levels. Triclosan represents the PPCP with the least comparative difference between treatment plant concentrations and predicted toxicity levels: mean concentrations in effluent (.00130 mg/L) are 68 times less than predicted freshwater Daphnid chronic toxicity levels (.089 mg/L); mean concentrations in effluent (.00130 mg/L) are 100 times less than predicted marine or marine equivalent species acute toxicity levels (.131 mg/L); mean concentrations in effluent (.00130 mg/L) are 359 times less than predicted freshwater daphnid acute toxicity levels (.469 mg/L); mean concentrations in sludge (8.63 mg/kg) are 525 times less than the predicted terrestrial toxicity levels (4530 mg/kg); and mean concentrations in effluent with SPWWTP 153:1 *outfall marine dilution factor* (.00000853 mg/L) are $15x10^3$ times less than the predicted marine or marine equivalent species acute toxicity levels (.131 mg/L).

There are no apparent patterns associated with pharmaceutical AHFS 1 groups for concentrations in SPWWTP and toxicity levels for the receiving environments (see *Appendix I*) or patterns associated with groups for differences between concentrations and toxicity levels (see *Appendix N*).

Diltiazem and triclosan concentrations in effluent (2 of 30 PPCPs) and 1,7-dimethylxanthine, albuterol, cimetidine, codeine, cotinine, gemfibrozil, metformin, and trimethoprim total concentrations in sludge (8 of 30 PPCPs) meet the assumption of normal distribution (n = 25, p > .05) (see *Appendix J*). The mean concentrations for effluent, effluent with SPWWTP 153:1 *outfall marine dilution factor*, and sludge and the predicted toxicity levels for freshwater acute and chronic, marine acute, and terrestrial acute did not meet the assumption of normal distribution (n = 30, p < .05) (see *Appendix K*).

The total concentrations in effluent with freshwater acute toxicity levels, effluent with freshwater chronic, effluent diluted by SPWWTP 153:1 *outfall marine dilution factor* with marine acute, and sludge with terrestrial acute toxicity levels meet the alternative assumption that concentrations are less than predicted toxicity levels (n = 30, p < .05) (see *Appendix L*).

The mean concentrations in effluent with freshwater acute toxicity levels, effluent with freshwater chronic, effluent diluted by SPWWTP 153:1 *outfall marine dilution factor* with marine acute, and sludge with terrestrial acute toxicity levels did not meet the assumption for distribution equality (n = 30, p < .05) (see *Appendix M*).

These results provide strong evidence that concentrations of PPCPs partitioned in the SPWWTP are less than the predicted toxicity levels in the receiving environments.

Discussion

The PPCPs concentrations in effluent, effluent diluted by SPWWTP 153:1 *outfall marine dilution factor*, and sludge are less than the predicted toxicity levels for freshwater acute and chronic toxicity levels, marine acute toxicity levels, and terrestrial acute toxicity levels. The statistically significant differences between concentrations and predicted toxicity levels are shown in *Appendix I*; *Appendix N* shows the magnitude of the differences. It was anticipated that specific trends would appear in concentrations and toxicity levels amongst groups of PPCPs based on similar pharmacological, therapeutic, or chemical characteristics, but the PPCP concentration and toxicity levels do not follow a predictable trend even when grouped by American Hospital Formulary Standard first tier classification (AHFS 1) (see *Appendix I*).

The US EPA ECOSAR integrates QSAR) models, predicted octanol-water partition coefficient (K_{ow}), and measured toxicity values for a set of 111 known chemical classes and applies it to estimate the general toxicity of the class constituents (US Environmental Protection Agency, 2014b). Predicted acute (LC50, 96hr) and chronic (ChV) toxicity values for freshwater daphnid species were acquired from the ECOSAR model, providing a consistent series of toxicity endpoints for comparison. The most conservative effect level was used for assessment when the ECOSAR model provided results for multiple classes for a PPCP. There are studies providing widely varying toxicity endpoints such as acetaminophen for *Daphnia magna* at 9.2 mg/L EC50 48hr and 293 mg/L EC50 24hr (National Centers for Coastal Ocean Science, 2014). The ECOSAR predicted value is lower at 1.652 mg/L. Predicted acute (LC50, 96hr) toxicity values for marine mysid species were acquired from the US EPA ECOSAR model providing a consistent series of endpoints for comparison, and when unavailable, substituted with predicted acute (LC50, 96hr) toxicity values for marine fish, freshwater mysid, or freshwater fish, respectively (US Environmental Protection Agency, 2014b). There are studies providing widely varying marine toxicity endpoints such as erythromycin for saltwater whiteleg shrimp (*Penaeus vannamei*) at 22.7 mg/L EC50 48hr post larvae and >500 mg/L EC50 1st stage post larvae (US National Library of Medicine, 2013). The ECOSAR predicted value for freshwater mysid (used as saltwater mysid substitute) is lower at .587 mg/L. The substitution for marine mysid endpoints reduces the continuity and accuracy of the assessment of concentrations compared to predicted toxicity levels. Another study provided a triclosan toxicity endpoint of EC50 96hr of .0007 mg/L for freshwater algae (*Scenedesmus subspicatus*); below the ECOSAR predicted toxicity value for daphnid of .469 mg/L and exceeded by the SPWWTP effluent concentration of .00130 mg/kg. This endpoint may be for a common freshwater representative species, but is not for the locally representative marine receiving environment consisting of different species with different tolerances to stressors.

The use of acute toxicity values for mice provided a consistent series of endpoints for comparison, and were acquired from the National Centers for Coastal Ocean Science (NCCOS) Pharmaceuticals in the Environment database (National Centers for Coastal Ocean Science, 2014), US National Library of Medicine Hazardous Substances Data Bank (HSDB) (US National Library of Medicine, 2013), and US National Library of Medicine ChemID*plus* database (US National Library of Medicine, 2012). Mice are mainly used as human analogues to determine toxicity levels for humans on a weight per weight basis. Most PPCP toxicity endpoints are not available for more representative terrestrial species with the potential to be exposed to sludge or biosolids through landfill disposal or land application. The purpose of this study was not to verify the predicted toxicity endpoints, but toxicity endpoints determined through studies were compared for context. There are studies providing terrestrial species endpoints such as the Northern bobwhite bird >2000 mg/kg oxytetracycline LD50 for 18 week old, Northern bobwhite bird 825 mg/kg triclosan LD50 for 21 week old, and Northern bobwhite bird 625 mg/kg warfarin LC50 15 day for 14 day old (National Centers for Coastal Ocean

Science, 2014). The NCCOS mice value for oxytetracycline is greater at 7200 mg/kg; for triclosan is greater at 4530 mg/kg, and for warfarin is greater at 374 mg/kg. It is worth noting that the above Northern bobwhite toxicity endpoints determined by studies are between 10^2 and 10^7 magnitude of difference above SPWWTP sludge concentrations (US National Library of Medicine, 2013).

Even with quantified values, variations in endpoints, exposures, target species, and receiving environments, it is difficult to determine an environmental toxicity level for comparison to chemical concentrations entering the environment. The US EPA issues a disclaimer that the ECOSAR program is a screening tool (US Environmental Protection Agency, 2014b). ECOSAR provides conservative toxicity endpoint estimates; useful in the absence of measured or the presence of conflicting data. Further studies are required to provide a robust database of environmental toxicity endpoints for new and emerging contaminants for all receiving environments.

The results of this study indicate erythromycin, codeine, and triclosan concentrations in effluent are within an order of magnitude of 100 to the freshwater aquatic chronic toxicity values, while triclosan concentrations in effluent are within an order of magnitude of 100 to the marine acute toxicity levels. It was found that PPCP concentrations in sludge from the SPWWTP are significantly below the toxicity levels for terrestrial toxicity levels of mice, and that PPCP concentrations in effluent and in effluent diluted by SPWWTP 153:1 *outfall marine dilution factor* are significantly below the marine species acute toxicity levels. Effluent concentrations versus marine species acute toxicity levels were intended to assess the effects of undiluted effluent in the immediate vicinity of the marine outfall. It is worth noting that the

SPWWTP 153:1 *outfall marine dilution factor* is the minimum observed dilution of wastewater effluent confined to approximately 50 m from the marine outfall as a result of rapid dilution due to horizontal deflection from the ambient currents. The maximum dilution is predicted to be 1578:1 within the 50 m region, and beyond 50 m from the marine outfall the dilution factor becomes orders of magnitude larger (Hay & Company Consultants, 2005). The results also indicate triclosan represents the PPCP with the least comparative difference between SPWWTP concentrations and predicted toxicity levels.

It was found that the least comparative difference between SPWWTP concentrations and predicted toxicity values is between effluent and chronic toxicity values. The PPCPs assessed in this study do not exceed predicted toxicity levels for the local receiving environments of the SPWWTP, though triclosan, erythromycin, codeine, fluoxetine, and caffeine warrant further study based on SPWWTP concentrations being relatively close to the predicted and assessed toxicity endpoints. There is little available data worldwide on the chronic toxicity of PPCPs, on the toxicity of PPCPs in the marine and terrestrial receiving environments, or on the toxicity of PPCPS on locally representative organisms in locally represented marine and terrestrial environments of the SPWWTP.

Conclusions

A comparative assessment was conducted for the Saanich Peninsula Wastewater Treatment Plant (SPWWTP) effluent, sludge, and transformative loss loadings of pharmaceutical and personal care product (PPCP) against the United States Environmental Protection Agency (US EPA) Estimation Programs Interface (EPI) Suite STPWIN model (sewage treatment plant model) predicted loadings of PPCPs to influent, effluent, sludge, and transformative loss during conventional activate sludge secondary wastewater treatment. It was found that the data sets of PPCP partitioning rates from the STPWIN estimation model and from the SPWWTP had statistically similar distribution patterns. However, the variations between individual PPCP predicted and measured loadings were enough to determine that the US EPA EPI Suite STPWIN estimation model results is not representative of the PPCP partitioning occuring during secondary treatment at the SPWWTP. The STPWIN model can be set for site specific biodegradation rates, but this was found to be problematic in determining a treatment plant specific biodegradation rate as the SPWWTP processes are constantly being adjusted to maximize performance. The STPWIN model also operates on a set of default parameters for treatment plant processes and designs that cannot be adjusted to match specific treatment plant specifications.

The partitioned PPCP loadings found within the SPWWTP show that PPCPs are being removed and being partitioned to sludge during secondary treatment. The partitioning rates for effluent, sludge, and removal vary from 0% to 176% with no discernable trend, and do not follow a predictable partitioning trend when grouped by American Hospital Formulary Standard first tier classification (AHFS 1). Negative values for removed loadings were a result of calculations of effluent and sludge loadings subtracted from influent loadings, and are not representative of actual loadings. Additional analysis of liquid and solid streams within the wastewater treatment process is required to quantify the amount of PPCPs removed during treatment.

Analysis of liquid stream samples taken from the primary clarifiers, along with the analysis of the primary sludge samples taken from Site 3 (primary treatment) (see *Figure 1*), would provide further understanding of the removal rates of PPCPs during primary treatment processes. Analysis of liquid stream samples from the secondary clarifiers, along with the analysis of the return activated sludge samples taken from Site 4 (secondary treatment) (see *Figure 1*), would provide further understanding of the removal rates of PPCPs during secondary treatment processes.

The effluent and sludge PPCP loadings for some parameters exceed the influent PPCP loadings leading to the assumption that metabolized PPCP chemicals were not captured in the initial influent analysis and are reactivated during secondary treatment. This also leads to the conclusion that the presence of PPCPs is underreported in this study due to metabolites and transformative products present and produced in the wastewater streams and discharged to the receiving environments.

A comparative assessment was conducted for the PPCP concentrations discharged from the SPWWTP to the marine and terrestrial receiving environments against a limited set of predicted and known toxicity endpoint levels for aquatic and terrestrial species to determine priority PPCPs of concern being discharged to the Capital Regional District receiving environments. It was found that concentrations of PPCPs discharged from the SPWWTP to the marine and terrestrial receiving environments were below the predicted and known toxicity endpoints for the respective aquatic and terrestrial receiving environments. Effluent with SPWWTP 153:1 *outfall marine dilution factor* had the greatest comparative difference between the predicted marine or marine equivalent species acute toxicity levels; this is the most representative of the receiving environments for the SPWWTP effluent. However, the lack of known marine and terrestrial toxicity endpoints for local species provides no direct effects correlation to the SPWWTP effluent and sludge PPCP concentrations discharged to the marine and terrestrial receiving environments.

The purpose of this study was not to verify predicted or experimental toxicity endpoints. However, additional literature review into toxicity endpoints for chronic and representative marine and terrestrial receiving environments provided evidence that the predicted toxicity endpoints derived from the United States Environmental Protection Agency (US EPA) Ecological Structure Activity Relationship Class Program (ECOSAR) model were for the most part conservative estimates compared to experimental toxicity endpoints found in literature. Erythromycin, codeine, and triclosan concentrations were within a magnitude of difference of 100 or less of the predicted or known toxicity endpoint. SPWWTP effluent concentrations had the least comparative difference between chronic toxicity values, though there is a little available known chronic toxicity endpoint data for direct effects correlation. This study found SPWWTP concentrations of PPCPs did not exceed toxicity endpoint levels.

There is little available research on the cumulative effects of PPCPS in the environment and on the effects of PPCP mixtures in the environment to determine effects from the continual loadings introduced into the environment from wastewater treatment systems. Erythromycin, fluoxetine, codeine, and triclosan are considered contaminants of concern due to concentrations found in the SPWWTP wastewater. These concentrations, though less than the toxicity endpoints for the receiving environments, are close enough to warrant further investigation. With the variations and limitations in predicted and reported toxicity endpoints, these PPCPs are of concern more as a precautionary measure until more representative toxicity data is available in the literature.

Recommendations

Studies have shown secondary wastewater treatment has reduced the loadings of pharmaceuticals and personal care products (PPCPs) entering the marine receiving environment. The removal rate and partitioning rate to sludge during secondary treatment are unique to each PPCP. The PPCP removal rate in the Saanich Peninsula Wastewater Treatment Plant (SPWWTP) is as much as 100% and as little as 0% for individual PPCPs. Partitioning to sludge, considered removal from the effluent stream and marine receiving environment, is as much as 176% and as little as 0%. However, partitioning to effluent is as much as 133% and as little as 0%. It is expected that, with the addition of secondary treatment for the Capital Regional District (CRD) core municipalities, the loadings of PPCPs into the marine environment would be reduced for most PPCPs.

Effluent and sludge concentrations exceeding influent concentrations of 100% were likely a result of the reactivation of metabolized PPCPs otherwise not captured in the influent analysis. Further studies are therefore required to categorize metabolized and transformative compounds for a more comprehensive suite of PPCPs.

There is little available data worldwide on chronic or cumulative effects of PPCPS in the environment and on the effects of PPCP mixtures in the environment. The effects from the continual low-dose loadings of PPCPs into the environment from wastewater treatment systems are also not known. Data is also lacking on marine and terrestrial species toxicity endpoints. . Further studies are required to determine representative toxicity endpoints for chronic and cumulative exposures to PPCPs, exposures to mixtures of PPCPs, and effects on both aquatic and terrestrial species.

Source control initiatives would be effective in addressing the identified PPCPs of concern in the CRD. Triclosan has been identified by Environment Canada as having potential bioaccumulative effects in the environment and is being targeted for voluntary removal as a non-essential ingredient from some personal care products. The CRD commissioned a study into source control strategies for triclosan. The study determined that including triclosan into the ongoing public outreach initiatives of the CRD would be an effective addition to the national efforts of Canada and the United States. Including erythromycin and other antibiotic drugs in the ongoing public outreach initiatives of the CRD would be an effective addition to the *Do Bugs Need Drugs?* campaign provincially promoted throughout British Columbia and Alberta.

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Appendices

Appendix A. Limit of detection (LOD) and descriptive statistics for total concentrations of sample parameters in wastewater influent

Sample Parameter	Descriptive Statistics N	Min (µg/L)	Max (µg/L)	Median (µg/L)	Mean (µg/L)	SD	RSD (%)	LOD (µg/L)
1,7-Dimethylxanthine	25	10.9	33.9	11.0	13.92	6.35	45.6	7.29
Acetaminophen	25	6.40	163	57.2	64.1	42.8	66.8	4.27
Albuterol	25	0	.0307	.0292	.0260	.0096	37.1	.0584
Caffeine	25	24.1	81.0	46.1	48.6	13.0	26.8	2.29
Carbamazepine	25	.145	1.14	.147	.293	.225	76.7	.0967
Chlortetracycline	25	0	.0334	.03	.0191	.0159	83.4	.0215
Cimetidine	25	.287	1.36	.597	.633	.225	35.5	.0942
Clarithromycin	25	.157	1.46	.346	.430	.328	76.3	.105
Codeine	25	.699	3.76	1.73	1.93	.881	45.7	.466
Cotinine	25	.454	1.38	.458	.820	.454	55.3	.907
Diltiazem	25	.233	2.17	.632	.731	.400	54.8	.0122
Doxycycline	25	.301	1.44	.484	.655	.356	54.4	.0823
Erythromycin	25	.00115	41.6	.228	3.28	8.74	266	.454
Fluoxetine	25	.000384	.115	.0928	.0588	.0470	80.0	.184
Gemfibrozil	25	.0514	1.48	.336	.349	.262	75.1	.00875
Ibuprofen	25	6.65	24.8	14.2	14.1	5.04	35.7	.432
Lincomycin	25	0	.0550	.0178	.0222	.0233	105	.0355
Metformin	25	28.4	67.6	43.0	43.7	7.29	16.7	1.76
Oxytetracycline	25	0	.0710	.0588	.0347	.0315	90.8	.00413
Ranitidine	25	.0507	4.72	.873	1.61	1.57	97.3	.0338
Roxithromycin	25	0	.0271	0	.00206	.00715	347	.00762
Sulfamethazine	25	0	.0463	.0154	.0130	.0165	127	.0309
Sulfamethizole	25	0	.0452	.0151	.0157	.0180	115	.0299
Sulfamethoxazole	25	.381	2.97	.758	1.04	.655	63.1	.0687
Sulfathiazole	25	0	.119	.0398	.0351	.0239	68.1	.0796
Tetracycline	25	.190	1.57	.948	.900	.413	45.9	.0469
Triclosan	25	1.74	7.18	4.87	4.84	1.38	28.6	2.61
Trimethoprim	25	.0763	.582	.229	.213	.121	56.8	.151
Tylosin	25	0	.00278	0	.000111	.00056	500	.0841
Warfarin	25	.000657	.0764	.0261	.0257	.0212	82.6	.00112

Note. SD = standard deviation. RSD% = relative standard deviation percent.

Appendix B. Limit of detection (LOD) and descriptive statistics for total concentrations of sample parameters in wastewater effluent

	Descriptive Statistics							
Sample Parameter	N	Min (µg/L)	Max (µg/L)	Median (µg/L)	Mean (µg/L)	SD	RSD (%)	LOD (µg/L)
1,7-Dimethylxanthine	25	0	3.65	0	1.31	1.79	136	7.29
Acetaminophen	25	0	2.13	2.13	1.11	1.09	98.1	4.27
Albuterol	25	0	.0292	.0292	.0245	.0109	44.5	.0584
Caffeine	25	0	3.44	1.15	1.51	1.03	68.2	2.29
Carbamazepine	25	.145	1.06	.358	.362	.230	63.5	.0967
Chlortetracycline	25	0	.0323	.0323	.0177	.0158	89.5	.0215
Cimetidine	25	.0471	.925	.141	.297	.247	83.0	.0942
Clarithromycin	25	.157	1.17	.452	.446	.292	65.3	.105
Codeine	25	.233	2.96	.698	.851	.864	101	.466
Cotinine	25	0	.453	.453	.399	.150	37.7	.907
Diltiazem	25	.182	.906	.454	.453	.181	40.1	.0122
Doxycycline	25	.0412	.298	.123	.152	.0682	45.0	.0823
Erythromycin	25	0	22.0	.227	1.57	4.56	290	.454
Fluoxetine	25	0	.699	0	.0684	.139	203	.184
Gemfibrozil	25	.00438	.319	.0847	.127	.107	83.7	.00875
Ibuprofen	25	0	4.81	0	.443	1.29	290	.432
Lincomycin	25	0	.0533	.0178	.0213	.0235	110	.0355
Metformin	25	2.64	31.2	7.51	10.3	7.68	74.4	1.76
Oxytetracycline	25	0	.0698	.0578	.0331	.0316	95.6	.00413
Ranitidine	25	.114	3.10	.327	.641	.731	114	.0338
Roxithromycin	25	0	.00381	0	.000305	.00106	346	.00762
Sulfamethazine	25	0	.0463	.0154	.0117	.0128	109	.0309
Sulfamethizole	25	0	.0150	.0150	.00838	.00758	90.5	.0299
Sulfamethoxazole	25	.103	1.46	.342	.429	.351	82.0	.0687
Sulfathiazole	25	0	.0398	.0398	.0303	.0174	57.4	.0796
Tetracycline	25	.192	.599	.341	.361	.123	34.1	.0469
Triclosan	25	1.30	1.30	1.30	1.30	2.27×10^{-16}	1.74×10^{-14}	2.61
Trimethoprim	25	.0757	.739	.227	.242	.152	63.1	.151
Tylosin	25	0	0	0	0	0		.0841
Warfarin	25	0	.156	.0193	.0248	.0321	129	.00112

Note. SD = standard deviation. RSD% = relative standard deviation percent. NA = not available.

Appendix C. Limit of detection (LOD) and descriptive statistics for total concentrations of sample parameters in wastewater sludge

	Descriptive Statistics							
Sample Parameter	N	Min (µg/L)	Max (µg/L)	Median (µg/L)	Mean (µg/L)	SD	RSD (%)	LOD (µg/L)
1,7-Dimethylxanthine	25	0	74.2	35.4	32.8	18.2	55.7	7.29
Acetaminophen	25	0	185	0	24.9	49.4	199	4.27
Albuterol	25	0	1.73	.917	.788	.564	71.5	.0584
Caffeine	25	0	588	155	171	125	73.2	2.29
Carbamazepine	25	21.1	760	43.4	77.0	144	187	.0967
Chlortetracycline	25	0	.272	0	.0217	.0751	346	.0215
Cimetidine	25	20.3	92.4	52.4	51.5	16.0	31.0	.0942
Clarithromycin	25	4.83	98.0	28.8	29.9	21.3	71.4	.105
Codeine	25	0	73.9	25.7	27.0	18.2	67.2	.466
Cotinine	25	0	4.66	2.39	2.22	1.54	69.5	.907
Diltiazem	25	36.0	697	165	207	165	79.4	.0122
Doxycycline	25	3.18	94.5	56.0	56.1	23.9	42.5	.0823
Erythromycin	25	.613	23.5	3.78	6.45	5.31	82.3	.454
Fluoxetine	25	10.2	52.5	20.2	21.6	9.22	42.7	.184
Gemfibrozil	25	1.11	13.2	7.21	7.01	3.26	46.6	.00875
Ibuprofen	25	0	67.2	0	7.47	18.1	242	.432
Lincomycin	25	0	1.35	0	.0542	.271	500	.0355
Metformin	25	9.49	334	138	143	67.9	47.7	1.76
Oxytetracycline	25	0	9.14	2.92	3.27	2.28	69.6	.00413
Ranitidine	25	4.83	70.4	12.9	14.9	12.9	86.7	.0338
Roxithromycin	25	0	.284	0	.100	.116	116	.00762
Sulfamethazine	25	0	.735	0	.0510	.158	310	.0309
Sulfamethizole	25	0	.501	.0854	.100	.113	113	.0299
Sulfamethoxazole	25	0	8.33	.833	1.34	1.79	134	.0687
Sulfathiazole	25	0	2.90	0	.202	.704	349	.0796
Tetracycline	25	0	6.20	.623	1.50	1.92	128	.0469
Triclosan	25	1253	12880	9520	8630	2953	34.2	2.61
Trimethoprim	25	3.85	35.4	19.4	20.5	10.2	50.0	.151
Tylosin	25	0	0	0	0	0		.0841
Warfarin	25	0	41.3	11.2	12.2	8.53	70.0	.00112

Note. SD = standard deviation. RSD% = relative standard deviation percent. NA = not available.

Appendix D. Daily and annual mean loadings of sample parameters in wastewater treatment

	Daily Average Loadings (mg/day)			Annual Average Loadings (g/year)		
Sample Parameter	Influent	Effluent	Sludge	Influent	Effluent	Sludge
1,7-Dimethylxanthine	126044	12837	293	46070	4685	107
Acetaminophen	580047	10852	222	212010	3961	81.1
Albuterol	235	240	7.05	85.9	87.5	2.57
Caffeine	439802	14788	1527	160750	5398	557
Carbamazepine	2649	3540	689	968	1292	251
Chlortetracycline	173	173	.194	63.1	63.0	.0709
Cimetidine	5726	2904	460	2093	1060	168
Clarithromycin	3894	4364	267	1423	1593	97.5
Codeine	17463	8324	242	6383	3038	88.3
Cotinine	7427	3902	19.8	2715	1424	7.23
Diltiazem	6617	4426	1855	2419	1616	677
Doxycycline	5933	1483	502	2169	541	183
Erythromycin	29696	15386	57.7	10854	5616	21.0
Fluoxetine	532	669	193	195	244	70.5
Gemfibrozil	3161	1244	62.6	1155	454	22.9
Ibuprofen	127738	4329	66.8	46689	1580	24.4
Lincomycin	201	208	.484	73.5	76.1	.177
Metformin	396018	100969	1275	144747	36853	465
Oxytetracycline	314	324	29.3	115	118	10.7
Ranitidine	14619	6269	133	5343	2288	48.6
Roxithromycin	18.7	2.98	.894	6.83	1.09	.326
Sulfamethazine	117	115	.456	42.9	41.9	.167
Sulfamethizole	142	82	.891	52.0	29.9	.325
Sulfamethoxazole	9388	4191	12.0	3431	1530	4.36
Sulfathiazole	318	296	1.80	116	108	.658
Tetracycline	8149	3532	13.5	2979	1289	4.91
Triclosan	43773	12758	77169	15999	4657	28167
Trimethoprim	1930	2363	183	706	863	66.8
Tylosin	1.01	0	0	.368	0	0
Warfarin	232	243	109	85.0	88.5	39.8

Appendix E. Mean and predicted percent loadings in secondary wastewater treatment, grouped by AHFS 1 classification

	Mean Percent Loadings			Predicted Percent Loadings		
Substance by AHFS 1 Group	Effluent	Sludge	Other Removed	Effluent	Sludge	Other Removed
Anti-infective Agents						
Clarithromycin	111.90	6.85	-18.75	92.70	7.17	.14
Doxycycline	24.95	8.45	66.60	91.22	1.65	7.13
Erythromycin	51.74	.19	48.07	93.77	6.11	.13
Lincomycin	103.49	.24	-3.73	78.01	1.45	20.54
Oxytetracycline	102.89	9.31	-12.20	91.22	1.65	7.13
Roxithromycin	15.93	4.78	79.29	95.95	3.94	.11
Sulfamethazine	97.57	.39	2.04	78.01	1.45	20.54
Sulfamethiazole	57.44	.63	41.93	77.99	1.46	20.55
Sulfamethoxazole	44.57	.13	55.30	77.95	1.47	20.57
Sulfathiazole	92.98	.57	6.46	78.02	1.45	20.54
Tetracycline	43.28	.16	56.55	91.22	1.65	7.13
Trimethoprim	122.26	9.46	-31.73	91.17	1.68	7.15
Tylosin	0	0	100.00	97.98	1.92	.09
Autonomic Drugs						
Albuterol	101.95	2.99	-4.95	24.90	.63	74.47
Cotinine	52.47	.27	47.27	78.02	1.45	20.54
Blood Formation, Coagulation, and Thrombosis						
Warfarin	104.19	46.85	-51.04	49.32	2.56	48.12
Cardiovascular Drugs						
Diltiazem	66.80	28.00	5.21	88.22	3.51	8.26
Gemfibrozil	39.30	1.98	58.72	2.84	39.81	57.35
Central Nervous System Agents						
1,7-Dimethylxanthine	10.17	.23	89.60	24.95	.62	74.45
Acetaminophen	1.87	.04	98.09	24.91	.63	74.46
Caffeine	3.36	.35	96.30	24.93	.62	74.45
Carbamazepine	133.46	25.97	-59.43	75.49	2.42	22.09
Codeine	47.60	1.38	51.01	91.13	1.71	7.16
Fluoxetine	125.52	36.22	-61.75	62.21	20.37	17.41
Ibuprofen	3.38	.05	96.56	5.07	14.57	80.36
Gastrointestinal Drugs						
Cimetidine	50.64	8.03	41.33	78.00	1.45	20.54
Ranitidine	42.82	.91	56.27	78.01	1.45	20.54
Hormones and Synthetic Substitutes						
Metformin	25.46	.32	74.22	24.94	.62	74.44
Skin and Mucous Membrane Agents						
Chlortetracycline	99.87	.11	.02	98.15	1.76	.09
Triclosan	29.10	176.05	-105.16	16.90	55.51	27.59

Note: Shaded results indicate values greater than 100% and less than 0%. AHFS 1= American Hospital Formulary Standard first tier classification.

Appendix F. Anderson-Darling test results for mean and predicted percent loadings in wastewater treatment

	Mean Percent Loadings			Predicted Percent Loadings		
Anderson-Darling	Effluent	Sludge	Other Removed	Effluent	Sludge	Other Removed
A^2 *test statistic*	.79	6.36	.59	2.42	6.54	2.46
$p<.05$	.0360	<.0001	.1146	<.0001	<.0001	<.0001

Note: Shaded results indicate no rejection of the null hypothesis that the distribution is normal.

Appendix G. Spearman's Rank Correlation Coefficient test results for mean and predicted percent loadings in wastewater treatment

	Percent Loadings		
Spearman Rank Correlation	Effluent	Sludge	Other Removed
rs Correlation	.186	.368	.138
t-approximation	1.00	2.09	.74
df	28	28	28
p<.05	.3258	.0454	.4674

Note: Shaded results indicate no rejection of the null hypothesis that the variables are independent.

Appendix H. Wilcoxon Paired Signed-Rank test results for mean and predicted percent loadings in wastewater treatment

	Percent Loadings		
Wilcoxon Paired Signed-Rank	Effluent	Sludge	Other Removed
t-statistic	274.00	215.00	208.00
Z-approximation	.85	-.36	-.50
p<.05	.3933	.7189	.6143

Note: Shaded results indicate no rejection of the null hypothesis that the shift in location between distributions is equal to 0.

Appendix I. Mean concentrations in wastewater treatment and predicted toxicity levels for receiving environments, grouped by AHFS 1 classification

	Concentrations			Predicted Toxicity			
Substance by AHFS 1 Groups	Effluent (mg/L)	Effluent (153:1) (mg/L)	Sludge (mg/kg)	Daphnid (acute, mg/L)[a]	Daphnid (chronic, mg/L)[b]	Marine species (acute, mg/L)	Mouse (acute, oral, mg/kg)
Anti-infective Agents							
Clarithromycin	.000446	.00000292	.0299	3.307	.31	15.993[d]	1230[h]
Doxycycline	.000152	.000000991	.0561	2.893	.6	1105.971[c]	1007.45[h]
Erythromycin	.00157	.0000103	.00645	1.078	.101	.587[e]	2580[i]
Lincomycin	.0000213	.000000139	.0000542	101.623	6.879	65.08[c]	13900[h]
Oxytetracycline	.0000331	.000000216	.00327	4.442	1.85	10501.22[c]	7200[h]
Roxithromycin	.000000305	.00000000199	.000100	6.717	.601	40.249[e]	13900[h]
Sulfamethazine	.0000117	.0000000766	.0000510	2.045	.078	23.995[c]	50000[i]
Sulfamethiazole	.00000838	.0000000547	.000100	2.001	.096	36.746[c]	>10000[i]
Sulfamethoxazole	.000429	.00000280	.00134	1.872	.086	31.313[c]	2650[g]
Sulfathiazole	.0000303	.000000198	.000202	1.878	.074	23.26[c]	4500[h]
Tetracycline	.000361	.00000236	.00150	2.866	.585	1048.946[c]	678[h]
Trimethoprim	.000242	.00000158	.0205	2.134	.083	317.91[f]	3960[h]
Tylosin	0	0	0	79.099	5.836	1113.663[e]	10000[h]
Autonomic Drugs							
Albuterol	.0000245	.000000160	.000788	2.209	1.303	261.829[d]	>2000[g]
Cotinine	.000399	.00000261	.00222	1917.981	35.948	26.368[c]	1604[i]
Blood Formation, Coagulation, and Thrombosis							
Warfarin	.0000248	.000000162	.0122	.783	.031	39.665[e]	374[i]
Cardiovascular Drugs							
Diltiazem	.000453	.00000296	.207	3.129	.281	2.422[c]	740[i]
Gemfibrozil	.000127	.000000832	.00701	4.933	.0979	.0982[e]	316[i]
Central Nervous System Agents							
1,7-Dimethylxanthine	.00131	.00000858	.0328	17.796	.505	39.31[c]	127[g]
Acetaminophen	.00111	.00000725	.0249	1.652	1.239	24.844[c]	338[g]
Caffeine	.00151	.00000988	.171	11.925	.333	18.687[c]	127[g]
Carbamazepine	.000362	.00000237	.0770	14.902	1.171	6.716[c]	529[g]
Codeine	.000851	.00000556	.0270	.976	.06	7.438[f]	250[h]
Fluoxetine	.0000684	.000000447	.0216	.175	.019	1.063[f]	248[h]
Ibuprofen	.000443	.00000289	.00747	27.848	4.305	11.666[e]	740[h]
Gastrointestinal Drugs							
Cimetidine	.000297	.00000194	.0515	10.781	.297	13.002[c]	2550[g]
Ranitidine	.000641	.00000419	.0149	78.001	5.284	797.927[f]	884[i]
Hormones and Synthetic Substitutes							
Metformin	.0103	.0000675	.143	577000	17071.402	27737.172[f]	1450[i]
Skin and Mucous Membrane Agents							
Chlortetracycline	.0000177	.000000115	.0000217	2.609	.395	436.951[c]	48[g]
Triclosan	.00130	.00000853	8.63	.469	.089	.131[f]	4530[h]

Note. AHFS 1= American Hospital Formulary Standard first tier classification. LC50 = lethal concentration. FW = freshwater. SW = saltwater. [a](Daphnid, FW, LC50, 96hr). [b](Daphnid, FW, ChV). [c](Mysid, SW, LC50, 96hr). [d](Fish, SW, LC50, 96hr). [e](Mysid, FW, LC50, 96hr). [f](Fish, FW, LC50, 96hr). (US Environmental Protection Agency, 2014b).
[g](National Centers for Coastal Ocean Science, 2014). [h](US National Library of Medicine, 2013). [i](US National Library of Medicine, 2012).

Appendix J. Anderson-Darling test results for concentrations in wastewater treatment

	Effluent Concentrations		Sludge Concentrations	
Anderson-Darling	*A^2 test statistic*	*$p<.05$*	*A^2 test statistic*	*$p<.05$*
1,7-Dimethylxanthine	4.80	<.0001	.55	.1443
Acetaminophen	4.34	<.0001	4.45	<.0001
Albuterol	7.14	<.0001	.62	.0970
Caffeine	4.63	<.0001	1.08	.0063
Carbamazepine	1.69	.0002	6.54	<.0001
Chlortetracycline	3.66	<.0001	8.49	<.0001
Cimetidine	1.54	.0004	.45	.2486
Clarithromycin	1.15	.0043	.87	.0214
Codeine	2.93	<.0001	.29	.5961
Cotinine	7.81	<.0001	.29	.5961
Diltiazem	.65	.0809	1.77	.0001
Doxycycline	5.21	<.0001	.36	.4210
Erythromycin	6.83	<.0001	1.31	.0017
Fluoxetine	4.60	<.0001	1.55	.0004
Gemfibrozil	1.14	.0044	.38	.3701
Ibuprofen	7.01	<.0001	6.15	<.0001
Lincomycin	2.75	<.0001	9.14	<.0001
Metformin	1.62	.0003	.22	.8064
Oxytetracycline	3.24	<.0001	.76	.0420
Ranitidine	2.97	<.0001	2.52	<.0001
Roxithromycin	8.49	<.0001	3.52	<.0001
Sulfamethazine	2.74	<.0001	6.69	<.0001
Sulfamethiazole	4.42	<.0001	1.25	.0023
Sulfamethoxazole	1.90	<.0001	2.72	<.0001
Sulfathiazole	5.98	<.0001	8.28	<.0001
Tetracycline	.44	.2728	3.74	<.0001
Triclosan		NA	.81	.0313
Trimethoprim	3.71	<.0001	.43	.2766
Tylosin		NA		NA
Warfarin	2.04	<.0001	.96	.0126

Note. Shaded results indicate no rejection of the null hypothesis that the distribution is normal. NA = not available.

Appendix K. Anderson-Darling test results for mean concentrations in wastewater treatment and predicted toxicity levels for receiving environments

	Mean Concentrations			Predicted Toxicity Levels			
Anderson-Darling	Effluent	Effluent (153:1)	Sludge	Freshwater Daphnid (LC50, 96hr)	Freshwater Daphnid (ChV)	Marine Mysid(SW/FW) Fish(SW/FW) (LC50, 96hr)	Terrestrial Mouse (oral)
A^2 test statistic	6.23	6.23	10.46	11.06	11.06	8.90	5.03
$p<.05$	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Note. All results indicate rejection of the null hypothesis in favour of the alternative hypothesis that the distribution is not normal. LC50 = lethal concentration. ChV = chronic value. FW = freshwater. SW = saltwater.

Appendix L. Wilcoxon 1-tailed t-Test results for concentrations in wastewater treatment with predicted toxicity levels for receiving environments

	Concentrations vs Toxicity Levels				
	Effluent vs FW Acute	Effluent vs FW Chronic	Effluent vs Marine Acute	Effluent 153:1 vs Marine Acute	Sludge vs Terrestrial Acute
Wilcoxon 1-tailed t-Test	*p<.05*	*p<.05*	*p<.05*	*p<.05*	*p<.05*
1,7-Dimethylxanthine	<.0001	<.0001	<.0001	<.0001	<.0001
Acetaminophen	<.0001	<.0001	<.0001	<.0001	<.0001
Albuterol	<.0001	<.0001	<.0001	<.0001	<.0001
Caffeine	<.0001	<.0001	<.0001	<.0001	<.0001
Carbamazepine	<.0001	<.0001	<.0001	<.0001	<.0001
Chlortetracycline	<.0001	<.0001	<.0001	<.0001	<.0001
Cimetidine	<.0001	<.0001	<.0001	<.0001	<.0001
Clarithromycin	<.0001	<.0001	<.0001	<.0001	<.0001
Codeine	<.0001	<.0001	<.0001	<.0001	<.0001
Cotinine	<.0001	<.0001	<.0001	<.0001	<.0001
Diltiazem	<.0001	<.0001	<.0001	<.0001	<.0001
Doxycycline	<.0001	<.0001	<.0001	<.0001	<.0001
Erythromycin	<.0001	<.0001	<.0001	<.0001	<.0001
Fluoxetine	<.0001	<.0001	<.0001	<.0001	<.0001
Gemfibrozil	<.0001	<.0001	<.0001	<.0001	<.0001
Ibuprofen	<.0001	<.0001	<.0001	<.0001	<.0001
Lincomycin	<.0001	<.0001	<.0001	<.0001	<.0001
Metformin	<.0001	<.0001	<.0001	<.0001	<.0001
Oxytetracycline	<.0001	<.0001	<.0001	<.0001	<.0001
Ranitidine	<.0001	<.0001	<.0001	<.0001	<.0001
Roxithromycin	<.0001	<.0001	<.0001	<.0001	<.0001
Sulfamethazine	<.0001	<.0001	<.0001	<.0001	<.0001
Sulfamethiazole	<.0001	<.0001	<.0001	<.0001	<.0001
Sulfamethoxazole	<.0001	<.0001	<.0001	<.0001	<.0001
Sulfathiazole	<.0001	<.0001	<.0001	<.0001	<.0001
Tetracycline	<.0001	<.0001	<.0001	<.0001	<.0001
Triclosan	<.0001	<.0001	<.0001	<.0001	<.0001
Trimethoprim	<.0001	<.0001	<.0001	<.0001	<.0001
Tylosin	<.0001	<.0001	<.0001	<.0001	<.0001
Warfarin	<.0001	<.0001	<.0001	<.0001	<.0001

Note: All results indicate rejection of the null hypothesis in favour of the alternative hypothesis that the median is less than the predicted toxicity level. All *t-statistic* = 0. FW -= freshwater.

Appendix M. Wilcoxon Paired Signed-Rank test results for mean concentrations in wastewater treatment with predicted toxicity levels for receiving environments

	Mean Concentrations vs Toxicity Levels				
Wilcoxon Paired Signed-Rank	Effluent vs FW Acute	Effluent vs FW Chronic	Effluent vs Marine Acute	Effluent 153:1 vs Marine Acute	Sludge vs Terrestrial Acute
t-statistic	465.00	465.00	465.00	465.00	406.00
Z-approximation	4.78	4.78	4.78	4.78	4.62
p<.05	<.0001	<.0001	<.0001	<.0001	<.0001

Note: All results indicate rejection of the null hypothesis in favour of the alternative hypothesis that the shift in location between distributions is not equal to 0. FW = freshwater.

Appendix N. Magnitude of difference results between mean concentrations in wastewater treatment and predicted toxicity levels for receiving environments, grouped by AHFS 1 classification

	Magnitude Difference				
Substance by AHFS 1 Groups	Effluent vs FW Acute	Effluent vs FW Chronic	Effluent vs Marine Acute	Effluent 153:1 vs Marine Acute	Sludge vs Terrestrial Acute
Anti-infective Agents					
Clarithromycin	7410	695	35837	5483083	41170
Doxycycline	19078	3957	7293405	1115890920	17953
Erythromycin	685	64	373	57076	400092
Lincomycin	4767145	322695	3052910	467095159	256568947
Oxytetracycline	134201	55892	317261196	48540963000	2199276
Roxithromycin	22032019	1971303	132018275	20198795999	139075101
Sulfamethazine	174385	6651	2046144	313060010	979608018
Sulfamethiazole	238887	11461	4386877	671192230	100304982
Sulfamethoxazole	4368	201	73063	11178714	1981760
Sulfathiazole	62076	2446	768838	117632214	22311296
Tetracycline	7934	1619	2903737	444271781	450586
Trimethoprim	8829	343	1315246	201232706	193624
Tylosin	NA	NA	NA	NA	NA
Autonomic Drugs					
Albuterol	90055	53120	10674058	1633130943	2538330
Cotinine	4806140	90080	66074	10109292	723761
Blood Formation, Coagulation, and Thrombosis					
Warfarin	31563	1250	1598917	244634356	30664
Cardiovascular Drugs					
Diltiazem	6912	621	5350	818605	3567
Gemfibrozil	38771	7694	772	118086	45106
Central Nervous System Agents					
1,7-Dimethylxanthine	13555	385	29943	4581260	3875
Acetaminophen	1488	1116	22385	3424868	13595
Caffeine	7885	220	12356	1890477	744
Carbamazepine	41157	3234	18549	2837938	6866
Codeine	1146	70	8737	1336739	9242
Fluoxetine	2558	278	15536	2376982	11486
Ibuprofen	62902	9724	26351	4031690	99013
Gastrointestinal Drugs					
Cimetidine	36304	1000	43783	6698741	49533
Ranitidine	121664	8242	1244583	190421186	59415
Hormones and Synthetic Substitutes					
Metformin	55877459	1653218	2686105	410974096	10173
Skin and Mucous Membrane Agents					
Chlortetracycline	147791	22375	24751824	3787029136	2210716
Triclosan	359	68	100	15362	525

Note. Shaded results indicate magnitude of difference less than or equal to 100. AHFS 1= American Hospital Formulary Standard first tier classification. FW = freshwater.