Occurrence and Fate of Pharmaceuticals and Personal Care Products (PPCPs) at a Northern Wastewater Treatment Facility

by

Devon James Yacura

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

Master of Science

in

Conservation Biology

Department of Renewable Resources

University of Alberta

Abstract

The occurrence of pharmaceutical and personal care products (PPCPs) in municipal wastewater treatment plants (WWTP) is an emerging environmental issue. Among other concerns, aquatic invertebrates sampled from WWTP have measurable concentrations of PPCPs, which have been found to cause adverse effects in the growth and development of birds when consumed. The Livingstone Trail Environmental Control Facility (LTECF) is the municipal wastewater treatment facility for the City of Whitehorse, Yukon, Canada (lat. 60º43'N, long. 135º03'W). The LTECF is a constructed wetland that hosts a high diversity and abundance of waterfowl, which may be attracted to the facility due to the abundance of aquatic invertebrates. Risk of PPCP accumulation in waterfowl feeding at the LTECF is a concern because the facility may be acting as an ecological trap. This research was the first of its kind at the LTECF and represents a first step in understanding the potential risk to waterfowl feeding at the LTECF. The main objectives of the study were to 1) quantify the occurrence of PPCPs in water, sludge, aquatic invertebrates, and algae, and 2) quantify the removal efficiency, seasonal variation, and bioaccumulation of PPCPs at the LTECF.

Water, sludge, aquatic invertebrates, and algae were sampled from the primary, secondary and tertiary stages of treatment in the spring, summer and fall in 2013 and 2014. The PPCPs with the highest concentrations in water were: acetaminophen (150 µg/L), caffeine (100 µg/L) and ibuprofen (10 µg/L), consistent with other studies of WWTP. The PPCPs with the highest concentrations in sludge, aquatic invertebrates and algae were two antimicrobials, triclosan (93,000 ng/g, 36 ng/g, and 210 ng/g, respectively) and triclocarban (31,000 ng/g; 29 µg/g; 47 ng/g, respectively), also consistent with other WWTP studies. Estrogens and synthetic musks

were among the PPCPs with the lowest concentrations in all media. Generally, PPCP removal efficiencies at the LTECF were equal to, if not exceeding those reported from other WWTP studies, including both conventional wastewater treatment plants and constructed wetlands. The high removal rates at the LTECF may be attributed to the exceptionally long hydraulic retention time and large surface area of the treatment cells, subjecting the chemicals to prolonged periods of photo- and biodegradation. Concentrations of PPCPs were significantly lower in spring than in summer and fall, likely from dilution of the wastewater entering the LTECF during the winter and spring. There was no significant difference between summer and fall PPCP concentrations in any stage of treatment. Triclocarban was the only PPCP at the LTECF to be classified as bioaccumulative, according to the Persistence and Bioaccumulation Regulations in the *Canadian Environmental Protection Act, 1999*. An ecological risk assessment for triclocarban has been recommended for future research at the LTECF.

Acknowledgements

A project such as this would not be possible without the generous help and support from many different people and organizations. First off, I'd like to thank my supervisory committee (i.e., co-supervisors Katie Aitken and Fiona Schmiegelow, and committee members Jim Hawkings and Cameron Eckert) for always being there when I needed them and providing guidance when I wasn't sure where things were going. Thanks in particular to Jim Hawkings and Cameron Eckert for providing their vast knowledge and expertise during the early stages of the research, and helping to define the research objectives. I would like to acknowledge all my financial supporters for their generous contributions and confidence in the project: City of Whitehorse, Northern Research Institute, Yukon College, Environment Yukon, Fish and Wildlife Enhancement Trust Fund, and Ecofor Consulting Ltd. Also, thanks to the Yukon Bird Club for being a continuous supporter and partner of research at the Whitehorse sewage lagoon. Thank you to Yukon College and Alexco Environmental Group for loaning me field equipment to collect my samples. I would also like to thank the City of Whitehorse, specifically Dave Albisser, for being so encouraging to see this research happen. I want to acknowledge Naomi Stock and the staff at the Water Quality Centre at Trent University for performing all the lab analyses, and providing me an affordable rate; this project would not have been possible if it wasn't for your lab. Thank you, Janelle Langlais, for volunteering your precious evenings and weekends to brave the aromas of the lagoon to help with data collection. Your patience over the last five years is greatly appreciated and did not go unnoticed. Finally, I'd like to acknowledge Helmut Grünberg as a great mentor and inspiration to keep pursuing research at the Whitehorse sewage lagoon. His hours of volunteer time and contribution of data has been a big part of the success of this project. I would like to dedicate this thesis to him as I know he would be proud of the work and would like to see this research continue.

Table of Contents

List of Tables

List of Figures

List of Appendices

Abbreviations

BAF – bioaccumulation factor

CEPA – Canadian Environmental Protection Act

CW – constructed wetland

E1 – estrone

E2 – 17 α-estradiol

EE2 – 17 α-ethinylestradiol

HRT – hydraulic retention time

LOQ – limit of quantification

LTECF – Livingstone Trail Environmental Control Facility

LTSP – long-term storage pond

n.d – not detected

PPCP – pharmaceutical and personal care product

R – removal efficiency

WWTP – wastewater treatment plant

Chapter 1: General Overview

1.0 Introduction

Pharma-ecology is an emerging field of research aimed at studying and minimizing the impact of pharmaceutical and personal care products (PPCPs) on the environment (Jjemba, 2008). PPCPs constitute hundreds of chemicals found in prescription and over-the-counter medications, antibiotics, antibacterial agents, estrogens, synthetic fragrances, industrial chemicals, and many others (Miege et al., 2009; Li et al., 2014). PPCPs are organic chemicals designed to be biologically active, meaning they have the ability to affect biochemical and physiological functions of biological systems (Jjemba, 2008). The widespread use of PPCPs in the 21st century has been compared to the early days of the Green Revolution when unlimited quantities of agrochemicals (i.e., pesticides, herbicides, fungicides and fertilizers) were indiscriminately used to increase plant yields (Jjemba, 2008). The widespread use of agrochemicals was later found to be harmful to the ecosystem, as well as human health. Current PPCP consumption levels are similar to those of agrochemical use in the 1960s, and in a number of instances are used in quantities equal to, if not exceeding those of agrochemicals (Hirsch et al., 1999). Although awareness of, and the ability to detect, the occurrence of PPCPs in the environment have increased over the last decades, understanding the ecological risk they pose remains limited. The need to develop a clear understanding of how organisms in the environment interact with, and are affected by, these compounds is critical.

Municipal wastewater treatment plants (WWTP) have been identified as the primary source of PPCPs entering the environment (Kümmerer, 2008; Camacho-Muñoz et al., 2012; Li et al., 2014). Consequently, the majority of PPCP research has focused on studying their occurrence and fate within various types of wastewater treatment systems. In general, municipal WWTP consist of either mechanical or passive treatment systems. Mechanical systems, also referred to as conventional systems, employ advanced wastewater treatment technologies such as advanced oxidation processes, activated carbon adsorption, membrane separation, and membrane bioreactor, among others (Miege et al., 2009; Li et al., 2014). Passive systems, also referred to as constructed wetlands (CW), can be classified into surface flow, horizontal subsurface flow,

vertical subsurface flow, and hybrid systems comprising one or more of these treatments (Li et al., 2014; Verlicchi and Zambello, 2014). Most research to date has focused on conventional WWTP; however, CWs are now attracting increasing attention for their application in the removal of PPCPs from wastewater. Constructed wetlands hold great potential to contribute to the removal of PPCPs due to the presence of many micro-environments that favour the different removal mechanisms, mainly biodegradation, photodegradation, sorption, and tissue accumulation (Verlicchi and Zambello, 2014).

Constructed wetlands have been documented to support significant ecological values (Piest and Sowls, 1985; Hamilton et al., 2005; Zimmerling, 2006). Many sewage lagoon systems (a form of CW) across the world have been identified as hotspots for bird habitat and diversity (Piest and Sowls, 1985; Hamilton et al., 2005; Zimmerling, 2006). It is suspected that sewage lagoons attract birds due to the capacity of lagoons to host an abundance of aquatic invertebrates (Swanson, 1977; Piest and Sowls, 1985), which also makes them highly preferred nesting habitat by waterfowl (Staicer et al., 1994; Hamilton et al., 2005). Recently however, the health of wildlife using sewage lagoons for feeding and breeding has been scrutinized due to the increasing input of PPCPs into wastewater systems. PPCPs have been shown to bioaccumulate in aquatic and terrestrial organisms and reach toxic levels (Aurelien et al., 2013). Lagoons, and other forms of constructed wetlands, may pose an increased risk to wildlife as birds are attracted to these sites to forage on the abundance of aquatic and aerial invertebrates.

Recent studies have found that aquatic invertebrates sampled from WWTP have measurable concentrations of PPCPs (Park et al., 2009; Markman et al., 2011). Many PPCPs have been identified as endocrine disrupting chemicals, disrupting natural hormonal patterns causing effects on development, reproduction and behaviour of fish and wildlife (Crisp et al., 1998; Markman et al., 2011). Passerines feeding on aquatic invertebrates contaminated with PPCPs from a WWTP showed marked changes in brain development, growth rates, behaviour, reproductive success, and immunocompetence (Dods et al., 2005; Markman et al., 2008, 2011). These findings are important from an ecological health perspective as these organisms create the link in the food chain between aquatic and terrestrial ecosystems.

The City of Whitehorse in Yukon, Canada (pop. 29,005) treats its wastewater using a hybrid constructed wetland, consisting of seven, large, gravity-fed lagoon cells connected in series. The complex, known as the Livingstone Trail Environmental Control Facility (LTECF) became operational in 1996 to replace the old lagoon facility that the city had outgrown. Over the years, the large treatment cells and slow moving hydraulic system have combined to create a large and dynamic artificial wetland that hosts many species of birds during the spring, summer and fall. Whitehorse biologists have found that the LTECF has become among the most heavily-used summer moulting and fall staging areas for water birds in the Yukon Southern Lakes region (Jim Hawkings, pers. comm.), and hosts a high diversity and abundance of waterfowl, as well as a wide variety of other bird species (Cameron Eckert, pers. comm.). Waterfowl are particularly abundant at the LTECF because of the abundance of aquatic invertebrates and algae. The potential risk of PPCP accumulation in birds feeding at the LTECF has become a concern because the facility may be acting as an ecological trap.

This study represents a first step in understanding potential risks to birds feeding at LTECF; in particular, waterfowl. It was necessary to complete a baseline assessment of the occurrence and fate of PPCPs at the facility to provide a foundation for further work. The objectives of the baseline assessment were to 1) quantify the occurrence of PPCPs in water, sludge, aquatic invertebrates, and algae, and 2) quantify the removal efficiency, seasonal variation, and bioaccumulation of PPCPs at the LTECF. Results of these preliminary stages were used to identify the PPCPs that may pose the greatest risk to waterfowl using the LTECF, the seasonal periods in which waterfowl are most exposed to PPCPs, and the food sources that provide the greatest exposure of PPCPs to waterfowl.

This is the first study to sample water, sludge, aquatic invertebrates, and algae concurrently from a WWTP. It is also the first study to document the occurrence and fate of PPCPs at a WWTP in northern Canada. The cold, northern climate of Whitehorse may hinder the capability of the LTECF to provide effective treatment of PPCPs, as it is well documented that PPCP concentrations are higher, and removal rates lower, in winter, due to decreased bio- and photodegradation during cold weather months (Li et al., 2013). Therefore, waterfowl, and other

organisms, using the LTECF for feeding and breeding may be exposed to elevated levels of PPCPs.

In chapter 2, I summarize research conducted at the LTECF in 2010, which documented the bird use of the LTECF, with an emphasis on waterfowl. The data collected in 2010 summarized potential waterfowl production at the LTECF, and investigated the spatial and temporal patterns of waterfowl use of the LTECF. The biological and physical features of the LTECF were also evaluated to explore what makes the LTECF attractive to birds. In chapter 3, I summarize two years of water, sludge, aquatic invertebrate, and algae sampling from the primary, secondary and tertiary stages of treatment to quantify the occurrence, removal efficiency, seasonal variation, and bioaccumulation of PPCPs. I compare my data to the literature to determine whether the trends of PPCP occurrence and fate at the LTECF are consistent with those observed elsewhere. Finally, in chapter 4, I discuss the implications of the results of chapter 3 within the broader contexts of wastewater treatment and the relationship of those results with waterfowl ecology at the LTECF. I recommend which PPCPs should be a priority for future research at the LTECF, including the PPCPs that may pose the greatest risk to waterfowl feeding at the LTECF and should be the focus of a future risk assessment. Lastly, I provide recommendations for future research studies to help fill current knowledge gaps.

2.0 Study Area

The LTECF is located approximately 10 km north of Whitehorse, Yukon, Canada (lat. 60°43'N, long. 135°03'W) (Figure 1). Whitehorse is situated in the Yukon Southern Lakes ecoregion, part of the larger Boreal Cordillera ecozone covering half of Yukon (Smith et al., 2004). The Yukon Southern Lakes ecoregion is characterized by broad valleys and large lakes. Lakes and wetlands make up 5% of the land cover in the ecoregion; many of them identified as significant by the Yukon Waterfowl Technical Committee (Smith et al., 2004). Whitehorse is bordered on the east by the Big Salmon Range, on the west by the Miners Range, and is set within the rain shadow of the St. Elias Mountains, making the climate dry and cool (Smith et al., 2004). The climate of the area can be described as continental subarctic having a mean annual temperature of – 1.2°C. The July mean high and low temperatures are 19.9°C and 8.0°C, respectively. The January mean high and low temperatures are – 16.5°C and – 25.2°C, respectively (Whitley and Thirumurthi, 1992). The average annual precipitation at Whitehorse is 15 cm of rainfall and 146 cm of snowfall (Whitley and Thirumurthi, 1992). Willow (*Salix* spp.), white spruce (*Picea glauca*), lodgepole pine (*Pinus contorta*), and trembling aspen (*Populus tremuloides*) are the dominant lowland tree species in the Whitehorse area, as well as on the LTECF property.

Consistent with the relevant classification system, the LTECF can be considered a hybrid constructed wetland, as it consists of seven, gravity-fed lagoon cells connected in series (Li et al., 2014). The seven treatment cells are comprised of two 115,00 m^3 primary lagoons with a combined retention time of 20 days, four 293,000 m^3 secondary lagoons with a combined retention time of 100 days, and a 5,813,000 m^3 tertiary polishing pond, known as the long-term storage pond (LTSP), with approximately a one year retention time (City of Whitehorse, 2014). The facility is located approximately one kilometer from the Yukon River, at an elevation of 675 masl, and treats the wastewater of approximately 25,000 Whitehorse residents (City of Whitehorse, 2014). The property is owned and operated by the City of Whitehorse and is fenced, with restricted access.

In 2014, the average daily flow rate entering the LTECF was 11,236 m^3. Discharge from the LTECF happens once per year, during the fall (September to October). The effluent is discharged

directly to the Yukon River, downstream from Whitehorse. The total discharge volume in 2014 was 4.2 million m^3, discharged over 57 days, beginning September 2. Discharge occurs in September because water treatment occurs during the summer months. The volume remaining in the LTSP after discharge was 0.2 million m^3. The average rate of discharge during that time was 0.85 m^3/s (City of Whitehorse, 2014). Sludge accumulation predominantly occurs in the two primary cells and requires dredging periodically. As of 2014, the estimated volume of sludge in the two primary cells was 23,858 m^3 (City of Whitehorse, 2014), representing accumulation since the facility establishment in 1996. Dredging is expected to occur for the first time during the summer of 2017.

Figure 1. Location of the Livingstone Trail Environmental Control Facility, Yukon, Canada, as well as configuration and naming of the seven treatment cells.

Chapter 2: Waterfowl Use of the Livingstone Trail Environmental Control Facility

1.0 Introduction

Since the LTECF opened in 1996, Whitehorse ornithologists have monitored the diversity and abundance of birds that use the facility, with close to half the species found in Yukon observed at the LTECF. Over the last two decades, it has become renowned as one of the most heavily-used summer moulting and fall staging areas for waterfowl in the Yukon Southern Lakes region (Jim Hawkings, pers. comm.). Although identified as an important migratory stop for birds of many species, the extent of the diversity and abundance of species nesting at the LTECF was unknown. Therefore, in 2010, I collected data at the LTECF with the purpose of: i) documenting the diversity and abundance of nesting birds, with a particular focus on waterfowl, ii) identifying the spatial and temporal patterns of waterfowl use, and iii) evaluating the biological and physical features of the LTECF that make it attractive to birds. Waterfowl were the focus of the data collection because the combination of treatment cells at the LTECF creates a large and dynamic artificial wetland complex that is particularly attractive to waterfowl. Data collection included waterfowl surveys (nesting and migration) and aquatic invertebrate surveys. Furthermore, an analysis was performed of historic monitoring data collected by local ornithologists, Helmut Grünberg and Cameron Eckert. The waterfowl data presented in this chapter provide critical background information on bird use of the LTECF, which adds important context to the overall implications of this study in regards to the risk to waterfowl.

2.0 Methods

Waterfowl were confirmed nesting by locating a female with her brood of flightless young. All seven treatment cells were surveyed for nesting waterfowl. A complete survey consisted of one or two surveyors walking the perimeter of a cell, flushing broods from shore, while also scanning the water's surface and opposite shoreline for flightless broods. A partial survey consisted of one surveyor walking only part of the perimeter while scanning the entire cell's water surface. All surveys of the secondary cells were complete surveys, beginning on June 29, 2010, and

conducted once a week until August 23, 2010. Complete surveys were conducted on the LTSP on July 8 and August 18, 2010; partial surveys took place on July 13, July 28, and August 1, 2010. Surveys were conducted using a 20-60x spotting scope.

Waterfowl production measures such as broods per hectare, average brood size, and estimated number of fledged young were calculated using an aging classification system developed by Gollop and Marshall (1954). The number of fledged young was calculated based on the number of broods, and the average brood size at fledging age. Average brood size at fledging age was calculated based on the average number of ducklings to survive to a minimum age of 19 days. This age was used because there is apparently little mortality from 19 days old to fledging (Gollop and Marshall, 1954).

Historic monitoring data spanning 1998 – 2010 were analyzed to determine the temporal use of the LTECF by waterfowl. Monitoring surveys from 1998 – 2010 were mostly conducted on the LTSP and did not include a thorough survey of the entire pond. Surveys included surveyors scanning the main body of the LTSP from a raised berm on the west side of the LTECF. Scans were completed with a combination of spotting scope and binoculars. Species included in this report were limited to dabbling and diving ducks; swans and geese were not included in the analyses because the data did not differentiate swans and geese flying over from those actually using the treatment cells. The data summaries presented include the earliest observation in spring, the latest observation in fall, peak spring and fall migration periods, and highest spring and fall counts. The peak migration periods have been deduced from the maximum number of birds observed during each two week time period on any visit in any year. Finally, the peak spring and fall counts from 1998 – 2010, and the specific dates of these counts, were identified.

Three days of aquatic invertebrate sampling occurred at the LTECF during the summer of 2010. The focus of the surveys was on collection and quantification of *Daphnia* and chironomid species. These groups were selected for collection because anecdotal observations suggested they were the most numerous invertebrates consumed by nesting waterfowl. *Daphnia* concentrations were collected from, and quantified, for the LTSP and four secondary cells using Luer-Lok syringes (10 ml, 20 ml and 60 ml). Chironomid samples were obtained using a dip net

with a surface area of 413 cm^2. Three horizontal sweeps of the dip net through the water column were completed along the entire length of the shoreline of each secondary cell, and at the effluent location of the LTSP. Following the sweeps, the contents of the dip net were placed in plastic containers and the number of chironomid was counted. Samples were not collected from the primary cells because visual inspection of the water suggested that no *Daphnia* or chironomid were present. Eckman dredge sediment samples found no benthic invertebrates were present in the sludge.

3.0 Results

3.1 Nesting Waterfowl

A total of 56 broods of seven species of nesting waterfowl were documented at the LTECF during the summer of 2010 (Table 1). Green-winged Teal (*Anas crecca*) was the most abundant nesting species, with a total of 15 broods, while Mallard (*Anas platyrhynchos*) (13 broods), and American Wigeon (*Anas americana*) (11 broods) were the next most abundant. Lesser Scaup (*Aythya affinis*) (8 broods) was the only diving duck nesting at the LTECF, although nesting Ruddy Duck (*Oxyura jamaicensis*) have been found in the past. Other nesting species were Gadwall (*Anas strepera*) (6 broods), Northern Pintail (*Anas acuta*) (2 broods), and Northern Shoveler (*Anas clypeata*) (1 brood).

Among the 56 broods documented, 54 were observed on the secondary cells and only 2 were observed on the LTSP. Among all brood observations on the secondary cells, 38% were on secondary cell 3, 36% were on secondary cell 2, 18% were on secondary cell 4, and 8% were on secondary cell 1. The earliest brood observed at the LTECF was Mallard, which had a suspected nest initiation date of May 9. Broods of Northern Shoveler, Green-winged Teal, American Wigeon and Gadwall were observed approximately two to three weeks later. Lesser Scaup and Northern Pintail nested even later, approximately one month after Mallard. The earliest nest initiation date was calculated based on the age of the first brood observed, and the average incubation period for each species based on Birds of North America data (Mini et al., 2014). Overall, sample sizes were low for all species and especially low for some species (e.g.,

Northern Shoveler, n = 1; Northern Pintail, n = 2), suggesting caution is warranted when extrapolating results.

The number of broods per hectare on the secondary cells and LTSP were 1.05 and 0.012, respectively. There were an estimated 293 fledglings raised on the secondary cells, and 8 fledglings raised on the LTSP. Most of the broods remained on the secondary cells until fledging. This was confirmed by continuous monitoring of the treatment cells. It is unknown where the waterfowl nests were located. No nests were located during the nesting waterfowl surveys.

Table 1. Nesting species production data for the secondary cells and long-term storage pond including number of broods, broods per hectare, estimated fledged young, fledglings per hectare, and earliest estimated nest initiation date.

Species	# of broods		broods/hectare		# of fledglings		fledglings/hectare		Nest Initiation Date
	Secondary Cells	*LTSP*	*Secondary Cells*	*LTSP*	*Secondary Cells*	*LTSP*	*Secondary Cells*	*LTSP*	
Gadwall	6	-	0.12	0	27	-	0.53	-	June 2
American Wigeon	10	1	0.2	0.006	61	3	1.20	0.02	June 1
Mallard	13	-	0.25	0	89	-	1.75	-	May 9
Northern Shoveler	1	-	0.02	0	2	-	0.04	-	May 25
Northern Pintail	2	-	0.04	0	8	-	0.16	-	June 12
Green-winged Teal	14	1	0.27	0.006	68	5	1.33	0.03	May 29
Lesser Scaup	8	-	0.16	0	38	-	0.75	-	June 14
TOTAL	**54**	**2**	**1.05**	**0.012**	**293**	**8**	**5.76**	**0.05**	

3.2 Spatial and Temporal Waterfowl Use

Based on monitoring data from 1998-2010, waterfowl were the first birds to arrive at the LTECF during spring migration. They arrived in mid-April and occupied the ice-free areas of the LTSP, feeding on available vegetation and aquatic invertebrates. As spring migration progressed, an increasing diversity and abundance of species accumulated at the LTECF, with peak numbers occurring in May (Table 2). The peak numbers for most duck species during spring migration at the LTECF was the first two weeks in May. By June, most waterfowl have left the LTECF to continue their northern migration to selected nesting grounds across the Yukon; however, some remained to breed, feed, and molt.

Throughout the entire open water season at the LTECF, the primary cells were not used by waterfowl. They likely did not use the primary cells because of the absence of a food source. Secondary cell 1 was the least used secondary cell by waterfowl, likely because of an absence of a food source as well. Secondary cells 2, 3, and 4 were used by many species of waterfowl for raising broods as well as feeding during migration. The secondary cells were used more than the LTSP for brood rearing likely because of the presence of vegetative cover around the perimeter of the cells. The LTSP is the largest treatment cell and was used by the highest diversity and abundance of species. The LTSP was also used by Northern Shoveler and Lesser Scaup for molting. These two species were often found at the LTECF in highest numbers during the summer, during the molting period.

The peak fall migration period for dabbling ducks at the LTECF was August 16 – September 15, with a slightly later peak period for diving ducks (Table 2). During fall migration, the number of ducks present at the LTECF exceeded those found in spring. This is a trend commonly observed amongst waterfowl species in natural lakes and wetlands in Yukon (Sinclair et al., 2003). In fall, the dabbling ducks were found in greatest numbers, with American Wigeon reported in the highest numbers. High numbers of American Wigeon were likely associated with the high quantities of filamentous algae present in the LTECF during the fall migration period. By October, ice developed on the treatment cells and most ducks have departed the LTECF. From November until April, the treatment cells are ice-covered, and therefore have no waterfowl activity.

Table 2. Summary of migration data for the most commonly observed duck species at the Livingstone Trail Environmental Control Facility from 1998 – 2010.

Species	Spring Migration		Fall Migration	
	Earliest	**Peak Period (#)**	**Latest**	**Peak Period (#)**
Gadwall (*Anas strepera*)	25 Apr 05	May 16 – 30 (150)	2 Nov 07	Sep 1 – 15 (400)
American Wigeon (*Anas americana*)	21 Apr 04	May 1 – 15 (300)	1 Nov 09	Aug 16 – 30 (3500)
Mallard (*Anas platyrhynchos*)	21 Apr 04	May 1 – 15 (100)	29 Oct 03	Aug 16 – 30 (800)
Blue-winged Teal (*Anas discors*)	13 May 06	May 16 – 30 (11)	28 Aug 04	Aug 1 – 15 (30)
Northern Shoveler (*Anas clypeata*)	21 Apr 04	May 16 – 30 (300)	27 Oct 07	Aug 16 – 30 (2500)
Northern Pintail (*Anas acuta*)	21 Apr 04	May 1 – 15 (700)	2 Nov 07	Sep 1 – 15 (500)
Green-winged Teal (*Anas crecca*)	21 Apr 04	May 1 – 15 (200)	27 Oct 06	Aug 16 – 30 (300)
Canvasback (*Aythya valisineria*)	24 Apr 06	May 1 – 15 (400)	29 Oct 03	Sep 16 – 30 (66)
Redhead (*Aythya americana*)	27 Apr 05	May 1 – 15 (15)	20 Oct 04	Sep 1 – 15 (20)
Ring-necked Duck (*Aythya collaris*)	21 Apr 04	May 1 – 15 (200)	27 Oct 06	Sep 16 – 30 (200)
Greater Scaup (*Aythya marila*)	6 May 04	May 1 – 15 (100)	26 Oct 05	Aug 16 – 30 (150)
Lesser Scaup (*Aythya affinis*)	24 Apr 06	May 1 – 15 (799)	2 Nov 07	Aug 16 – 30 (300)
Surf Scoter (*Melanitta perspicillata*)	8 May 04	May 16 – 30 (200)	27 Oct 06	Jul 16 – 30 (150)
White-winged Scoter (*Melanitta fusca*)	3 May 03	May 16 – 30 (40)	22 Oct 06	Aug 1 – 15 (80)
Long-tailed Duck (*Clangula hyemalis*)	6 May 04	May 16 – 30 (150)	27 Oct 06	Oct 16 – 30 (40)
Bufflehead (*Bucephala albeola*)	21 Apr 04	May 1 – 15 (350)	5 Nov 10	Aug 16 – 30 (1000)
Common Goldeneye (*Bucephala clangula*)	21 Apr 04	May 1 – 15 (300)	2 Nov 07	Oct 16 – 30 (150)
Barrow's Goldeneye (*Bucephala islandica*)	21 Apr 04	May 1 – 15 (315)	27 Oct 06	Sep 16 – 30 (100)
Ruddy Duck (Oxyura jamaicensis)	25 Apr 05	May 16 – 30 (60)	25 Oct 03	Sep 1 – 15 (154)

3.3 Aquatic Invertebrates

Among the aquatic invertebrates sampled, *Daphnia* were more abundant than chironomid in secondary cell 3 and 4 and the LTSP (Table 3). However, chironomid were more abundant than *Daphnia* in secondary cell 1 during both sampling events, and were more abundant than *Daphnia* in secondary cell 2 on August 23. Overall, the *Daphnia* concentration increased from secondary cell 1 to secondary cell 4, and the concentration in the LTSP was similar to the average *Daphnia* concentration in the four secondary cells. Absence of *Daphnia* in secondary cell 1 may be due to ammonia concentrations (15 mg/L) that were toxic for their survival, as they have been reported to withstand a maximum concentration of 8 mg/L (Swanson, 1977; Horne and Goldman, 1994). Anecdotal observations found *Daphnia* concentrations at the LTECF peak in July and August, which corresponds to the peak of the waterfowl nesting season, including brood rearing.

Other invertebrates collected, but not quantified, included: caddisflies (Trichoptera,) leeches (Hirudinea), riffle beetles (Coleoptera), water boatmen (Hemiptera), mosquitos and crane flies (Diptera). These invertebrates, in addition to chironomid and *Daphnia*, are generally tolerant to some degree of environmental stress (e.g., low oxygen, pollution, acidity, etc.), which explains their presence in the sewage lagoon ponds (Horne and Goldman, 1994). These invertebrates, along with chironomids and *Daphnia*, were likely the main food sources for most water birds. No molluscs were collected, which may be due to inadequate survey methodology. The chironomids collected in September are likely those that are over-wintering as they are unlikely to emerge that late.

Table 3. Abundance of *Daphnia* and chironomid in the four secondary cells and long-term storage pond of the Livingstone Trail Environmental Control Facility, Yukon, Canada, collected on August 10, August 23, and September 17, 2010.

	***Daphnia* per litre (August 10)**	**chironomid per litre (August 23)**	**chironomid per litre (September 17)**
Secondary Cell 1	0	2	12
Secondary Cell 2	11	16	4
Secondary Cell 3	69	12	5
Secondary Cell 4	434	12	19
LTS Pond	143	9	2

4.0 Discussion

In the Southern Lakes region of the Yukon, the LTECF was found to be a regionally significant wetland for many species of waterfowl during the spring, summer and fall for feeding, breeding and molting. It is suggested that a combination of biological and physical features likely contributes to the diversity and abundance of waterfowl at the LTECF. One of the most important factors may be the abundance of aquatic invertebrates, serving as a critical food source for nesting and migrating waterfowl. The importance of chironomids as a protein source for females during egg production, and for early development of ducklings, is widely recognized (e.g., Swanson, 1977; Mowbray, 1999). Furthermore, it is well documented that sewage lagoons host an abundance of aquatic invertebrates (Swanson, 1977; Piest and Sowls, 1985), making lagoons highly preferred nesting habitat by waterfowl (Staicer et al., 1994; Hamilton et al., 2005). A U.S. Fish and Wildlife Service study determined that invertebrates comprised 98% of the diet of adult and immature Mallard and Gadwall on sewage lagoon ponds, and that chironomid and *Daphnia* each made up 44% of the diet (Swanson, 1977). Empirical evidence as well as anecdotal observations found *Daphnia* and chironomid were the most numerous invertebrates at the LTECF and were consumed the most by brooding waterfowl. *Daphnia* and chironomid concentrations peaked in summer, which corresponded to the hatch of ducklings. Adult and juvenile ducks were observed feeding on emerging chironomids on the secondary cells and LTSP.

The large algae blooms that form in summer are likely another important factor for attracting large numbers of waterfowl in the late summer. Filamentous algae are a primary food source for many species of waterfowl that have a preference for a herbivorous diet, especially American Wigeon (Mini et al., 2014). The large algae blooms supply a virtually endless source of food for dabbling ducks. All species of dabbling ducks were observed feeding on the algae; however, American Wigeon particularly exploited this abundant food source. American Wigeon are mostly herbivorous during fall migration, eating plants that are most abundant (Mini et al., 2014). Record high numbers of American Wigeon in Yukon have been recorded at the LTECF during fall migration (Sinclair et al., 2003), likely because of the abundance of algae. An

estimated 3500 American Wigeon were observed feeding on the algae in the LTSP at the LTECF in September 2010.

Other biological factors that may contribute to the diversity and abundance of birds at the LTECF include the presence of thick willows and emergent vegetation around the perimeter of the treatment cells, providing cover for nesting adults and juvenile birds. Studies have shown that nesting females of most species of dabbling ducks prefer the presence of dense vegetative cover to provide security from predators (Piest and Sowls, 1985). The dense thickets around the secondary cells were regularly used by broods for protection and cover. Broods often scattered from the willows into the water when surveyors walked past.

Physical features of the LTECF that may attract nesting waterfowl include the predator-reduced environment created by the perimeter fencing. Duebbert and Lokemoen (1980) showed exceptionally high nesting duck densities and hatching rates when predators were controlled, and found that the number of nesting pairs increased over time in a predator-reduced environment. Lastly, the physical location of the LTECF likely contributes to attracting high numbers of birds of many species. The surrounding geography has an abundance of rich wetlands, ponds, and large lakes that provide some of the most important waterfowl staging areas in the Yukon (Sinclair et al., 2003).

Compared to other natural and artificial wetlands in North America, waterfowl productivity is intermediary at the LTECF, with an average of 1.05 broods per hectare. Mossop (1991) reports 0.125 broods per hectare at the Needlerock wetland in south central Yukon from 1985-87. Notably, that study also found American Wigeon, Green-winged Teal, and Mallard were the most abundant nesting dabblers, and Lesser Scaup was the most abundant nesting diving duck. Piest and Sowls (1985) found average densities less than 4 pair/ha in wetlands in the prairie pothole region of southern Canada and northern United States, but an annual average in Arizona of 0.4 pair/ha. Breeding pair densities of dabbling ducks as high as 30.3 pairs/ha have been reported in South Dakota, in a rigidly controlled predator-reduced wetland complex (Duebbert and Lokemoen, 1980). It should be noted that there are likely more nesting pairs of waterfowl using the LTECF than what this study found. Studies show it is very difficult to account for all

broods present on ponds and sloughs. One study found that after as many as three successive "beat-outs" by two surveyors and a dog plus a search of the surrounding upland, not all of the young present were seen (Gollop and Marshall, 1954).

The results presented in this chapter are consistent with other studies: sewage lagoons are hotspots of bird diversity (Piest and Sowls, 1985; Hamilton et al., 2005; Zimmerling, 2006). The regional significance of the LTECF as a feeding, breeding and molting site for waterfowl in the Yukon Southern Lakes region, suggests there is potential for this area to act as an ecological trap, given recent research concerning the occurrence of pharmaceuticals and personal care products (PPCPs) in municipal wastewater treatment plants. The bioaccumulation of PPCPs in aquatic invertebrates and algae has been documented in a number of studies (Dods et al., 2005; Coogan et al., 2007; Kümmerer, 2008; Park et al., 2009). The consumption of contaminated aquatic invertebrates at wastewater treatment plants has been identified as a significant exposure route of PPCPs to terrestrial ecosystems (Dods et al. 2005; Park et al., 2009; Markman et al., 2011). Recent studies have reported PPCP concentrations found in the environment have caused adverse effects on fish and wildlife, including birds; effects on reproduction, growth rate, and behaviour, among other things, have all been documented (Dods et al., 2005; Park et al., 2009; Markman et al., 2011). These recent studies, in conjunction with the results presented in this chapter, establish the rationale for conducting the research presented in chapter 3, in order to establish a foundation for assessing the risk to waterfowl using the LTECF.

Chapter 3: Occurrence and Fate of Pharmaceuticals and Personal Care Products at the Livingstone Trail Environmental Control Facility

1.0 Introduction

Pharmaceuticals and personal care products (PPCPs) are biologically active compounds, classified according to their chemical structure, mode of action, or therapeutic purpose (Jjemba, 2008). They consist of prescription and over-the-counter drugs, natural and synthetic estrogens, antibiotics, antibacterials, synthetic musks, industrial chemicals, and many others. They are found in many household products such as cosmetics, soaps, shampoos, deodorants, lotions, cleaning products, furniture, kitchen ware, toys, etc (Jjemba, 2008; Kümmerer, 2008). Recently, the presence of PPCPs in wastewater treatment plants (WWTPs) has been identified as an emerging environmental issue (Miege et al., 2009; Zhang et al., 2014; Wang and Wang, 2016), which represent global threats to aquatic animals and ecosystems (Melvin and Leusch, 2016). Although awareness of this issue has been documented since the 1980s, only recently has international research focused on quantifying the occurrence and fate of PPCPs in WWTPs. This surge of research has been aided by the advancement of new analytical techniques, which allows trace levels of these micro-pollutants to be detected (Rivera-Utrilla et al., 2013).

Recent research has mainly focused on quantifying the occurrence of PPCPs in wastewater and sludge. However, the ecological risk these PPCPs pose to the environment is still largely unknown. Some PPCPs have been identified as endocrine disrupting chemicals, causing effects on development, reproduction and behaviour of fish and wildlife (Crisp et al., 1998; Markman et al., 2011). Recent studies have found that aquatic invertebrates sampled from WWTP have measurable concentrations of PPCPs (Park et al., 2009; Markman et al., 2011). Studies by Dods et al. (2005) and Markman et al. (2008 and 2011) found that European Starlings and Tree Swallows feeding on aquatic invertebrates contaminated with PPCPs from a WWTP showed marked changes in brain development, growth rates, behaviour, reproductive success and immunocompetence. Given the regional importance of the LTECF as a stopover site for feeding and breeding waterfowl in the Yukon Southern Lakes region, the potential risk of PPCPs causing

acute or chronic toxicity to waterfowl is of concern. Quantifying the occurrence and fate of PPCPs at the LTECF was the first step towards understanding this risk.

Data collection for this research was conducted in two phases. The first phase was exploratory and was performed to determine which PPCPs were present and at what concentrations. Guided by the results from the exploratory sampling, I refined my research objectives and implemented a more robust and targeted sampling program for the second phase. I based my research objectives on those common in this emerging field, but specific to my study system. My objectives were: 1) to quantify the occurrence of PPCPs in water, sludge, *Daphnia* and algae at the LTECF, 2) to evaluate the removal efficiency and seasonal variation of PPCPs in water at the LTECF, and 3) to calculate bioaccumulation factors for PPCPs in *Daphnia* and algae at the LTECF.

This research is unique as it is the first project of its kind to sample water, sludge, aquatic invertebrates, and algae concurrently from a WWTP. Furthermore, this project is unique because it is the first research of its kind in northern Canada. In fact, very limited pharma-ecology research is occurring in Canada, with the majority of research occurring on non-passive WWTPs in temperate climates. The cold climate in Whitehorse may pose distinct challenges to the capacity of the LTECF for effective treatment of PPCPs, given that PPCP concentrations are higher and removal efficiencies are lower during winter months (Li et al., 2013). Cold climates reduce bio- and photodegradation rates, two of the main processes for PPCP removal (Verlicchi and Zambello, 2014). Therefore, environments receiving effluent discharges from northern WWTP may be more vulnerable to PPCP contamination. This is of concern because many northern communities rely on wild food sources, and expanding populations in the north requiring increased water treatment capacity, could increase risk. Understanding how these chemicals react in northern environments is critical to understanding the risk they pose.

2.0 Methods

2.1 Field Sampling

Samples were collected from the primary, secondary, and tertiary stages of treatment in 2013 and 2014, in three seasons (spring, summer and fall) (Table 4). The seasons were defined relative to

the three different seasonal periods of peak waterfowl use of the LTECF. Spring samples were collected in May corresponding to the peak of waterfowl use during spring migration. Summer samples were collected in July corresponding with the peak of the waterfowl nesting season, including brood rearing. Fall samples were collected in September corresponding to the peak of waterfowl use during fall migration, as well as the period of effluent discharge to the Yukon River. Furthermore, the samples were collected during spring, summer and fall in order to quantify any seasonal variation in sample concentrations.

Water samples were collected from the primary, secondary and tertiary cells during the spring, summer and fall in 2013 and 2014. Sludge samples were collected from the primary, secondary and tertiary cells during the spring, summer and fall in 2013, but only from secondary cell 2 in summer in 2014. Aquatic invertebrates were only collected from secondary cell 2, in the summer of both 2013 and 2014. Aquatic invertebrates were collected from secondary cell 2 because this was the earliest stage of treatment on which waterfowl were rearing their broods. Furthermore, aquatic invertebrate sampling in 2010 found no *Daphnia* were present in secondary cell 1. Ten aquatic invertebrate samples were collected in 2013, including seven *Daphnia*, two chironomid, and one Tipulidae. *Daphnia* and chironomid species were targeted for sampling during the summer because of their abundance and importance as a food source for waterfowl, especially during the breeding season. One Tipulidae sample was collected to determine if there was a difference in concentration between various invertebrate groups. In 2014, only three aquatic invertebrate samples were collected, which were all *Daphnia*. *Daphnia* was selected as the target organism during 2014 because results from samples collected in 2013 indicated little difference in PPCP concentrations between *Daphnia*, chironomid, and Tipulidae samples. Only three *Daphnia* samples were collected in 2014 due to limited financial resources allocated for aquatic invertebrate sampling. Algae samples were collected from the LTSP in the fall, when large algal mats develop, providing an attractive food source for many species of dabbling ducks during migration.

Samples from the primary stage of treatment were collected in primary cell B, which is the first treatment cell of the LTECF and represents the influent concentration of the system. Samples from the secondary stage of treatment were collected in secondary cell 2, which represents the

middle of the overall treatment process and is the second stage of the four-stage secondary treatment. The long-term storage pond was considered the tertiary stage of treatment, which is the final stage of treatment and represents the effluent concentration of the system. Samples collected from the LTSP in the fall represent the discharge concentrations to the Yukon River.

Table 4. Summary of complete sampling programs conducted in 2013 and 2014 at the Livingstone Trail Environmental Control Facility, Yukon, Canada.

		Spring	Summer	Fall
Sampling Date		*Apr 8*	*Jul 11/Aug 14*	*Sep 8*
2013	***Primary***	water (n=1) sludge (n=1)		
	Secondary		water (n=3) sludge (n=2) invertebrate (n=10)	water (n=1) sludge (n=1)
	Tertiary			water (n=1) sludge (n=1)
Sampling Date		*May 7*	*Jul 30/31*	*Sep 21*
2014	***Primary***	water (n=3)	water (n=3)	water (n=3)
	Secondary	water (n=3)	water (n=3) sludge (n=3) invertebrate (n=3)	water (n=3)
	Tertiary	water (n=3)	water (n=3)	water (n=3) algae (n=3)

Water samples were collected from a canoe as grab samples at the surface of the water (Brun et al., 2006; Hijosa-Valsero et al., 2010; Li et al., 2013). Grab sample results can be limiting because they only capture a snapshot concentration at a specific time and place. In contrast, 24-hr composite sampling collects many small samples over a 24-hour period to make one complete sample; therefore, reducing the variability in the results. Grab samples were used for collecting water samples in this study due to budget constraints. However, to address limitations of this method, three water samples were collected from each treatment cell (i.e., influent, middle, effluent) to support calculation of an average concentration for each cell (n=3) (Lin et al., 2010).

An Ekman dredge was used to collect sludge samples from the bottom of each treatment cell. Aquatic invertebrate samples were collected using a 30-cm diameter Wildco plankton net, dragged behind a canoe at a depth of 30 cm. This was repeated until a sample meeting the dry weight requirement for analysis of 0.5 g was collected. Algae samples were collected from the water column using a 30 cm diameter dip net.

One field blank was collected during each day of sampling in spring, summer and fall. Distilled water used for the field blank was provided by Maxxam Analytics. All samples were collected in sterile, HDPE plastic 500 mL bottles provided by Fisher Scientific. Water and sludge samples to be analyzed for synthetic musks required that no head space be left in the bottle. After collection, all samples were immediately put on ice in an insulated 16 qt cooler. Samples were frozen overnight and shipped the following day in insulated coolers to the Water Quality Centre, Trent University, Peterborough, Ontario, for analysis. The samples were mailed with dry ice and arrived to the laboratory still frozen.

2.2 Analyses

2.2.1 Target PPCPs

In 2013, a total of 33 PPCPs were tested for in the water, sludge, and aquatic invertebrate samples collected during the exploratory sampling (Table 5). These 33 PPCPs comprise the entire list of PPCPs the laboratory was capable of analysing. Based on the results of the exploratory sampling, 15 of the 33 PPCPs were targeted for subsequent sampling and testing, based on their known occurrence at the LTECF and their propensity to bioaccumulate. These 15 PPCPs were: ibuprofen and naproxen (non-prescription anti-inflammatory drugs), triclosan and triclocarban (antimicrobials), gemfibrozil (lipid regulator), estrone and 17 α-estradiol (natural estrogens), 17 α-ethinylestradiol (synthetic estrogen), and galaxolide, tonalide, traseolide, cashmeran, celestolide, phantolide, and musk ketone (synthetic musks, also known as fragrance chemicals).

Table 5. Sources and classification of pharmaceutical and personal care products sampled for in this study.

	Therapeutic Classes	Representative chemicals	Sources
Pharmaceuticals	Antibiotics	Sulfamethoxazole Sulfapyridine Trimethoprim	Prescription drugs
	Estrogens/Hormones	Estrone (E1) 17 α-estradiol (E2) 17 α-ethinylestradiol (EE2)	Natural and synthetic hormones; hormone therapy
	Industrial Estrogens	Bisphenol A (BPA) Octylphenol Nonylphenol	Industrial processes
	Beta Blockers	Atenolol Metoprolol Propranolol Sotalol	Prescription drugs
	Anti-inflammatory drugs	Ibuprofen Acetaminophen Naproxen	Non-prescription drugs (eg. Tylenol, Advil, Motrin)
	Anti-depressant drugs	Citalopram Cotinine Venlafaxine	Prescription drugs
	Antiepileptic drugs	Carbamazepine	Prescription drug
	Blood lipid regulators	Gemfibrozil	Prescription drug
	Stimulants	Caffeine	Coffee, tea, pop, etc.
	Perfluorinated Compounds	Perfluorooctane Sulfonate (PFOS) Perfluorooctanoate (PFOA)	Industrial processes
Personal Care Products	Antibacterial agents/Disinfectants	Triclosan Triclocarban	Cosmetics, toothpaste, deodorants, kitchenware, furniture, plastics, etc.
	Synthetic Musks/Fragrances	Galaxolide Tonalide Traseolide Cashmeran Celestolide Phantolide Musk Ketone	Cosmetics, soaps, shampoos, deodorants, detergents, perfume, air fresheners, cleaning products

2.2.2 Analytical Methods

All PPCPs samples were analyzed by the Water Quality Centre, Trent University, Peterborough, Ontario, using the following procedures.

2.2.2.1 Pharmaceuticals and Antimicrobials

Samples were extracted and analyzed using liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS). Water samples were extracted using a modified version of EPA 1694 solid phase extraction (SPE) method. Samples, 200 to 600 mL depending on the analyte, were decanted, adjusted to pH 2 and Na_2EDTA (1 g/L) was added. When the Na_2EDTA was dissolved, samples were spiked with isotopically labelled standards and extracted using Water's Oasis HLB cartridges (6cc, 150 mg). Cartridges were conditioned with 6 mL of methanol, 6 mL of milli-Q water, and 6 mL of milli-Q water at pH 2. Samples were passed through the cartridges at a rate of approximately 5 mL/min. Analytes were eluted using two aliquots of methanol and one aliquot of methanol/acetonitrile (50/50 by volume). Sludge/biosolid and biota samples were centrifuged to remove water and freeze dried (usually 24 hours). Freeze-dried samples were homogenized and spiked with isotopically labelled standards. Samples were extracted using 5 mL methanol and sonication for 20 minutes, followed by centrifugation and removal of the solvent layer. This was repeated two additional times and solvent layers (15 mL total) were combined.

All sample extract volumes were reduced using a nitrogen evaporator to either 200 or 400 µL. Sample extracts were analyzed on either an AB Sciex API 3000 triple quadruple mass spectrometer coupled with a Shimadzu 10A liquid chromatography (LC) instrument and Perkin Elmer Series 200 Autosampler or an AB Sciex Qtrap 5500 mass spectrometer coupled with an Agilent 1100 series LC and autosampler. Analytes were separated using an Agilent Zorbax Eclipse XDB-C8 column (4.6 x 150 mm) with either methanol or acetonitrile and 20 µM ammonium acetate in milli-Q water as mobile phases. Injection volume was 20 µL. PPCPs in group I, as well as antibiotics and β-blockers were analyzed in positive ion mode, while PPCPs in group II, as well as estrogens, industrial estrogens and perfluorinated compounds were

analyzed in negative ion mode. Samples were analyzed in multiple reaction monitoring mode and quantitated using internal calibration of the isotopically labelled standards.

2.2.2.2 Synthetic Musks/Fragrances

Samples were extracted and analyzed using gas chromatography- mass spectrometry (GC-MS). Water samples, 200 mL, were decanted and extracted into 25 mL of hexanes in a separatory funnel. This extraction was repeated two additional times and the solvent layers (75 mL total) were combined. The hexane extract was dried using sodium sulphate. Sludge/biosolid and biota samples were centrifuged to remove water. Samples were extracted with 5 mL hexanes for 20 minutes, followed by centrifugation and removal of the solvent layer. This was repeated two additional times and solvent layers (15 mL total) were combined. The hexane extract was dried using sodium sulphate.

The volumes of the sample extracts were reduced using a nitrogen evaporator to 400 µL. Sample extracts were analyzed using a Varian CP-3800 gas chromatogram (GC) instrument with a CP-8410 autosampler and a Saturn 2200 ion trap mass spectrometer. Injector was at 275ºC and operated in splitless mode; the injection volume was 1 µL. Analytes were separated on a Restek Rtk-5MS 30 m column (0.25 mm ID and 0.25 ⌈m film thickness). Initial conditions of the GC oven were 50ºC and held for 1.5 minutes. Temperature was ramped 10ºC/min to 150ºC and 20ºC/min to 190ºC where it was held for 1 minute. The oven temperature was then increased by 0.5ºC/min to 191ºC, held for 1 minute, increased by 0.5ºC/min to 193ºC and held for 1 minute, and finally increased by 50ºC/min to 290ºC while it was held for 2.5 minutes. The total run time was 26 minutes. Samples were analyzed in single ion storage mode and quantitated using external calibration.

2.2.3 Data Analysis

When a laboratory result was reported as less than the limit of quantification (LOQ), the value used for analysis was half the concentration of the LOQ. For example, when a value of <4.0 ng/L was reported, a value of 2.0 ng/L was used for the appropriate calculations. A reported value of n.d (not detected) was treated as zero for all appropriate calculations. The 2013 data collection

was exploratory, and is considered complementary to the 2014 data. However, due to limited sampling and lack of replication in 2013, results should be interpreted with caution. Results for two estrogens, E2 and EE2, were not available from 2014 spring water samples due to laboratory error.

2.2.3.1 Removal Efficiency, R

Removal efficiency (R) for each PPCP was calculated by the following equation:

$R = (C_i - C_f)/C_{primary}*100\%$, where R is expressed in %

C_i is the mean concentration of the PPCP detected in the initial stage of treatment (either primary or secondary), C_f is the mean concentration of the PPCP detected in the subsequent stage of treatment (either secondary or tertiary), and $C_{primary}$ is the mean concentration of the PPCP detected in the primary stage of treatment. The maximum R obtainable is 100%. A negative R results if the concentration of a PPCP is higher in a later stage of treatment than in earlier stages.

Removal efficiencies were calculated for each PPCP across the primary stage of treatment, secondary stage of treatment, and the overall treatment process. The primary stage of treatment is defined as the difference in mean concentrations between primary cell B and secondary cell 2. The secondary stage of treatment is defined as the difference in mean concentrations between secondary cell 2 and the LTSP. The overall treatment process is defined as the difference in mean concentrations between primary cell B and the LTSP.

In 2013, removal rates for each PPCP were calculated based on one sample from each of the primary and tertiary cells and the mean of four samples from secondary cell 2. In 2014, removal rates for each PPCP were calculated based on the mean concentration within each stage of treatment (n=9), calculated by averaging the three concentrations within each treatment cell across the three seasons. Results for the estrogens, E2 and EE2, were based on six samples because spring water samples were not available.

Overall removal efficiencies for each PPCP in 2013 and 2014 were compared to mean removal rates documented by Miege et al. (2009), who compiled results from 117 research papers

covering a period from 1997 – 2006. These data were selected for comparison as it was the most comprehensive in terms of number of papers and number of PPCPs included. However, the review was focussed on conventional activated sludge systems, not lagoon systems.

The Kruskal-Wallis test for non-parametric data was used to test for differences in PPCP concentrations across the three stages of treatment (primary, secondary and tertiary). Kruskal-Wallis test results were followed by a multiple pair-wise comparison using Dunn's test to identify where differences existed between treatment stages. A significance level of 95% was used.

2.2.3.2 Seasonal Variation

Water samples were collected in spring, summer and fall, from the three stages of treatment, in order to examine potential seasonal variation in concentrations during periods relevant to waterfowl species using the LTCEF. Concentrations for each PPCP were normalized to a relative percentage between 0 and 1.0, by dividing each sample concentration (n=3), collected during each sampling event, by the maximum concentration in that set of 3 samples. For example, the normalized values for the following concentrations, 2.0 ng/L, 4.0 ng/L, and 8.0 ng/L, are 0.25, 0.5, and 1.0, respectively. Normalizing the concentrations allowed the concentrations of each PPCP to be combined together, within each season, to examine composite patterns across all PPCPs, while maintaining the integrity of the relative concentrations with each PPCP.

For each PPCP, concentrations across the three seasons, in each treatment cell, were normalized (n=9). Then, the normalized concentrations for each PPCP in each treatment cell, within each season, were grouped (n=39; 3 samples x 13 PPCPs) and a mean normalized value was obtained. This equates to a total of nine mean values; one for each stage of treatment within each season. The mean normalized values were then compared to determine whether a significant difference in seasonal concentrations existed. A seasonal variation analysis was performed for each PPCP individually, as well as all PPCPs grouped together.

The Kruskal-Wallis test for non-parametric data was used to test for seasonal differences in individual and grouped PPCP concentrations across treatment levels at the LTECF. Where

results were significant, a multiple pair-wise comparison using Dunn's test was used to identify where the differences existed between seasons. Note that seasonal variation could only be analyzed in 2014 due to the limited data set in 2013. Seasonal variation could not be analyzed for the estrogens, E2 and EE2, because laboratory results were not available for water samples collected in spring.

2.2.3.3 Bioaccumulation

To assess the bioaccumulation of PPCPs at the LTECF, aquatic invertebrates and algae were sampled because they are the dominant food sources for nesting and migrating waterfowl at the LTECF and therefore, act as a proxy for what PPCPs may be accumulated in the waterfowl themselves. Two main approaches are used to assess bioaccumulation in aquatic organisms: i) an empirical approach that uses laboratory or field data to calculate bioaccumulation factors (BAF), and; ii) a deterministic modeling approach that uses kinetic or equilibrium models to predict aspects of bioaccumulation (U.S. EPA, 2000). This study used the empirical approach, which is simpler and more reliable as it uses actual concentrations rather than predicted ones (U.S. EPA, 2000). The empirical approach is preferred because the field based measurements provide the most direct evidence of the occurrence of bioaccumulation (Gobas, 2001).

A BAF is defined as the ratio of the concentration of a substance in the tissue of an aquatic organism to the concentration of that substance in the ambient water (U.S. EPA, 2000). BAFs for each PPCP were calculated for *Daphnia* and algae using the following equation:

$$BAF = C_t/C_w$$

where BAF is expressed in L/kg of tissue. C_t is the concentration of the contaminant in the tissue of the organism (ng/g), and C_w is the concentration of the contaminant in the water (ng/L).

The BAF values calculated through empirical field data at the LTECF were compared to the BAF threshold identified in the Persistence and Bioaccumulation Regulations as part of the *Canadian Environmental Protection Act, 1999* (CEPA), which defines a substance to be

bioaccumulative when the BAF is greater than or equal to 5000 (Canadian Environmental Protection Act, 1999).

3.0 Results

3.1 Occurrence

All 33 PPCPs were detected in at least one of the media sampled (i.e., water, sludge, *Daphnia* and algae). Nonylphenol was the only PPCP not detected in any water samples. Musk ketone was the only PPCP not detected in any sludge samples. Celestolide was the only PPCP not detected in any *Daphnia* samples. Naproxen, celestolide, musk ketone and cashmeran were not detected in any algae samples. Concentrations of many PPCPs were detected at levels below the LOQ. This was especially true for concentrations in *Daphnia* and algae. The synthetic musks and estrogens were the PPCPs most often reported below the LOQ. Field blanks showed no detections above the LOQ for any of the PPCPs except estrone in the July field blank; the concentration was 4.6 ng/g, while the detection limit was 3 ng/g. It should be noted that the 2013 data collection was exploratory, and is considered complementary to the 2014 data. However, due to limited sampling and lack of replication in 2013, results should be interpreted with caution.

3.1.1 Water

Among all PPCPs tested for within primary cell B in 2013, acetaminophen (150,000 ng/L), caffeine (100,000 ng/L) and ibuprofen (10,000 ng/L) had the highest concentrations (Figure 2). In 2014, ibuprofen (9,300 ng/L), triclosan (1,000 ng/L) and galaxolide (340 ng/L) had the highest concentrations in primary cell B; acetaminophen and caffeine were not tested for in 2014. The estrogens and synthetic musks had lower concentrations than any of the other PPCPs throughout all stages of treatment, especially musk ketone, which was not detected above the LOQ in any stage of treatment. Among the three estrogens, 17 α-estradiol (E2) was found at the highest concentration (90 ng/L). Concentrations of all estrogens remained low and stable throughout the treatment stages. Among the three antibiotics analyzed in primary cell B in 2013, all were reported above the detection limit: sulfapyridine (170 ng/L), sulfamethoxazole (150 ng/L), and

trimethoprim (90 ng/L). Among the four beta blockers, atenolol (850 ng/L) and metaprolol (440 ng/L) had the highest concentrations. Bisphenol A had a concentration of 220 ng/L in primary cell B. Carbamazepine concentrations ranged between 200 – 306 ng/L in primary cell B, and had little variation throughout the treatment stages.

There was large annual variability in spring concentrations of ibuprofen, naproxen and triclosan in primary cell B. Concentration of ibuprofen, naproxen and triclosan were 33, 87 and 87 times greater, respectively, in the spring of 2013 compared to spring of 2014; however, concentrations of gemfibrozil, triclocarban, and galaxolide were similar between years. Raw data of PPCP concentrations in water are reported in Appendix 1, Appendix 2, Appendix 3 and Appendix 4.

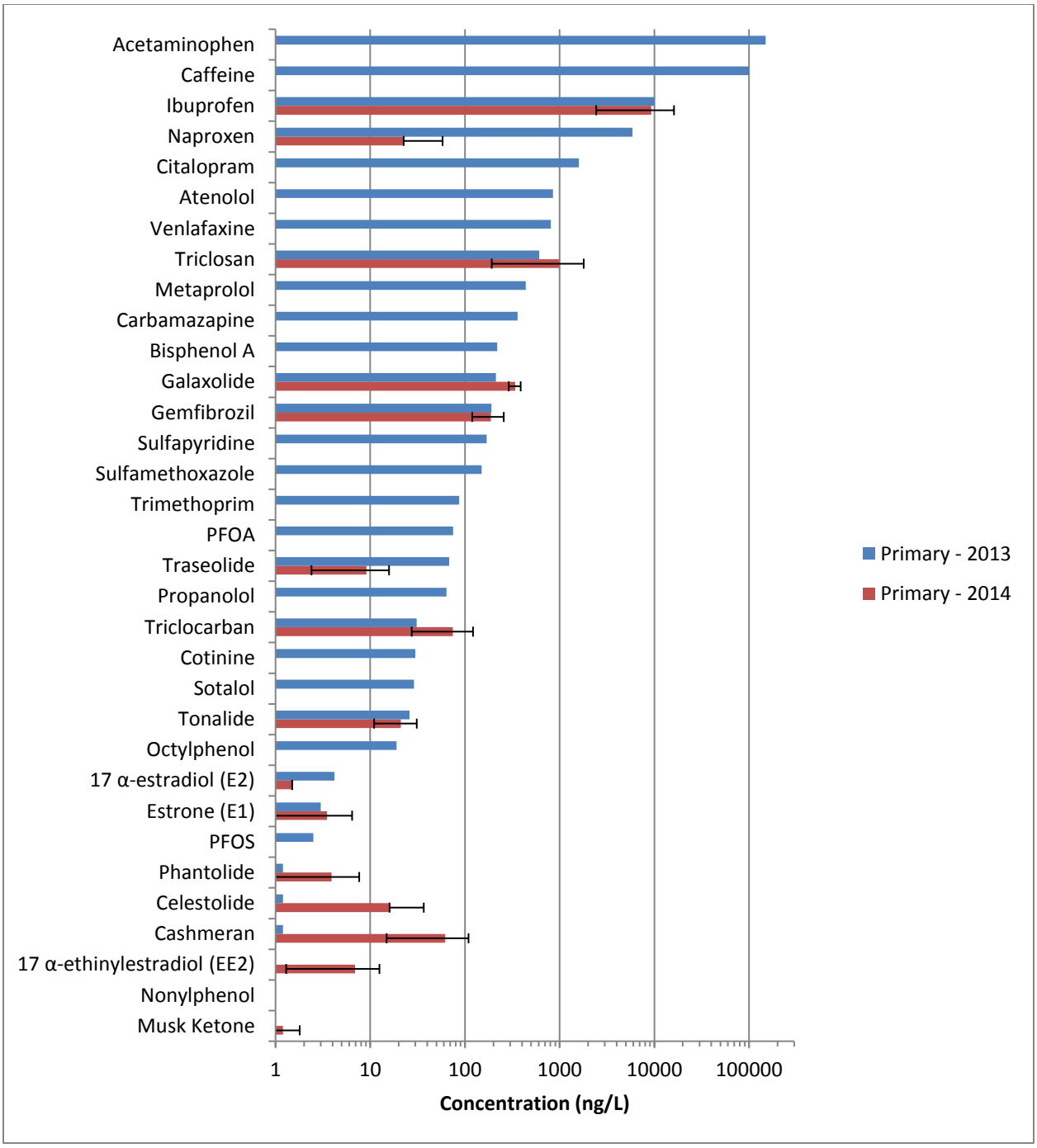

Figure 2. Mean concentration and standard deviation of PPCPs tested in water samples collected from primary cell B of the Livingstone Trail Environmental Control Facility, Yukon, Canada, in 2013 (n=1) and 2014 (n=9). (PFOA: perfluorooctanoate; PFOS: perfluorooctane sulfonate)

3.1.2 Sludge

Among all PPCPs tested in the sludge of primary cell B in 2013, triclosan (93,000 ng/g), triclocarban (31,000 ng/g) and citalopram (6,200 ng/g) had the highest concentrations (Figure 3). Triclosan (9,000 ng/g), triclocarban (1,200 ng/g), and citalopram (2,100 ng/g) also had the highest concentrations in sludge from secondary cell 2. Naproxen (170 ng/g), triclosan (100 ng/g), and propanolol (80 ng/g) had the highest concentrations in sludge from the LTSP. Among the synthetic musks, galaxolide and tonalide had the highest concentrations, while musk ketone was not detected in any sludge samples. Nonylphenol was not detected in the sludge from primary cell B, but was detected at 51 ng/g in the LTSP. Concentrations of all antibiotics were slightly above the LOQ in primary cell B, and were below the LOQ in the secondary and tertiary stages of treatment. Among the estrogens, 17 α-ethinylestradiol (EE2) had the highest concentration, 18 ng/g, found in primary cell B in spring of 2013. The highest concentration of carbamazepine in sludge was 90 ng/g, and was detected in secondary cell 2. Bisphenol A had a sludge concentration of 110 ng/g in primary cell B. Concentrations of acetaminophen, caffeine and ibuprofen in sludge from primary cell B were 130 ng/g, 830 ng/g and 720 ng/g, respectively.

There was large variability in PPCP concentrations among replicate sludge samples collected in summer, secondary cell 2, 2013. Concentrations of triclosan, triclocarban and citalopram ranged from 200 – 9,000 ng/g, 100 – 1,200 ng/g, and <4.0 – 2,100 ng/g, respectively. Furthermore, summer concentrations of triclosan in sludge in secondary cell 2 varied between years; the maximum concentration in 2013 was 9,000 ng/g compared to a maximum concentration of 1,900 ng/g in 2014. Raw data of PPCP concentrations in sludge are reported in Appendix 5 and Appendix 6.

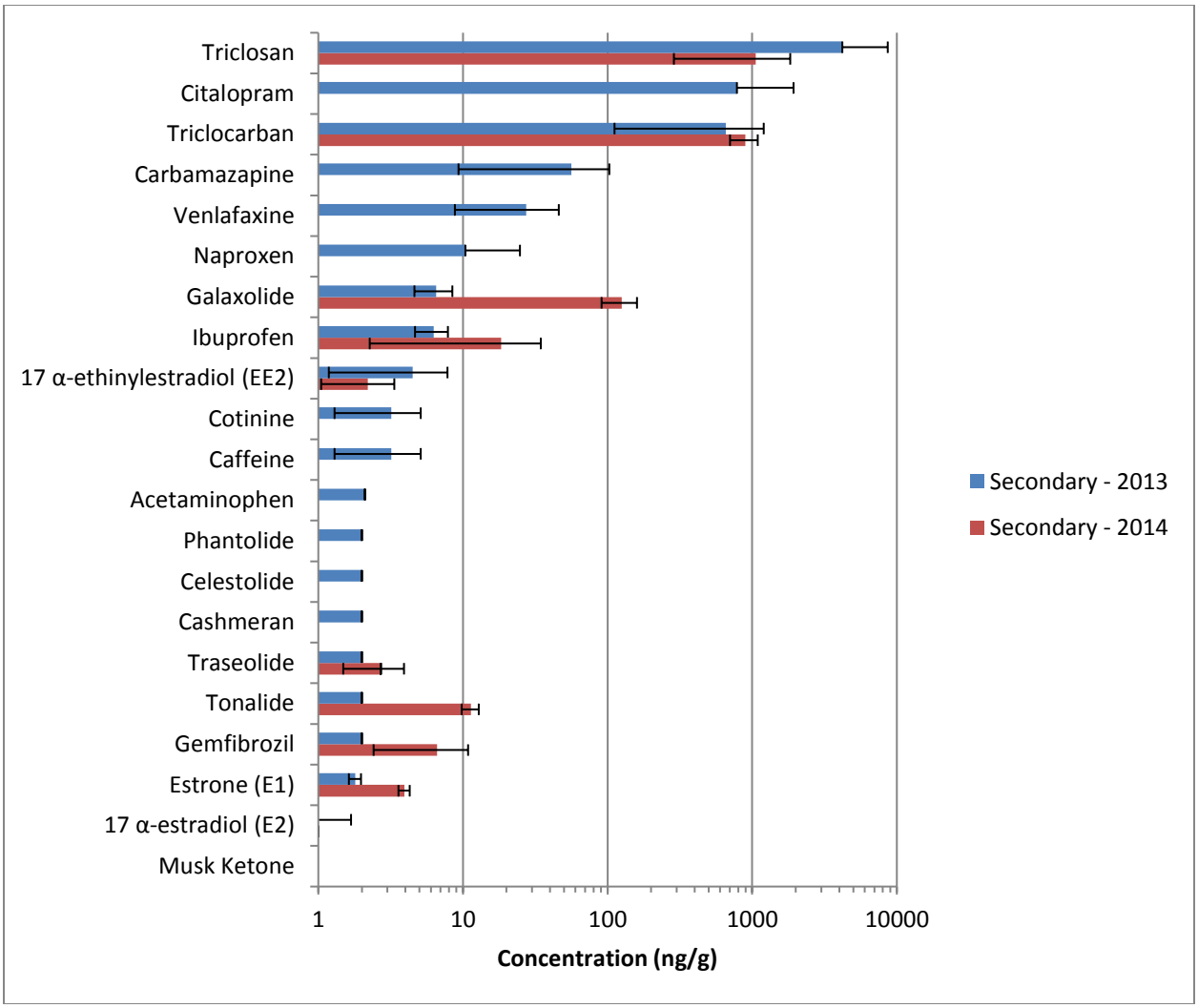

Figure 3. Mean concentration and standard deviation of PPCPs tested in sludge samples, collected from secondary cell 2 of the Livingstone Trail Environmental Control Facility, Yukon, Canada, in 2013 (n=3) and 2014 (n=3).

3.1.3 Aquatic Invertebrates

All PPCPs, except celestolide, were detected in aquatic invertebrate samples from 2013 and 2014. However, only triclosan, triclocarban and musk ketone had concentrations above the LOQ in 2013 and 2014. Concentrations of triclosan, triclocarban and musk ketone ranged from 4.0 – 36 ng/g, 3.0 – 25 ng/g, and <4.0 – 8.1 ng/g, respectively (Figure 4). Notably, while musk ketone was not quantified in any water or sludge samples, it was the PPCP with the third highest concentration in *Daphnia* in 2013 and 2014. There was little difference between PPCP concentrations in *Daphnia*, chironomid, and Tipulidae samples collected in 2013; triclosan and triclocarban exceeded the LOQ in all three invertebrate groups. Raw data of PPCP concentrations in *Daphnia*, chironomid, and Tipulidae are reported in Appendix 7 and Appendix 8.

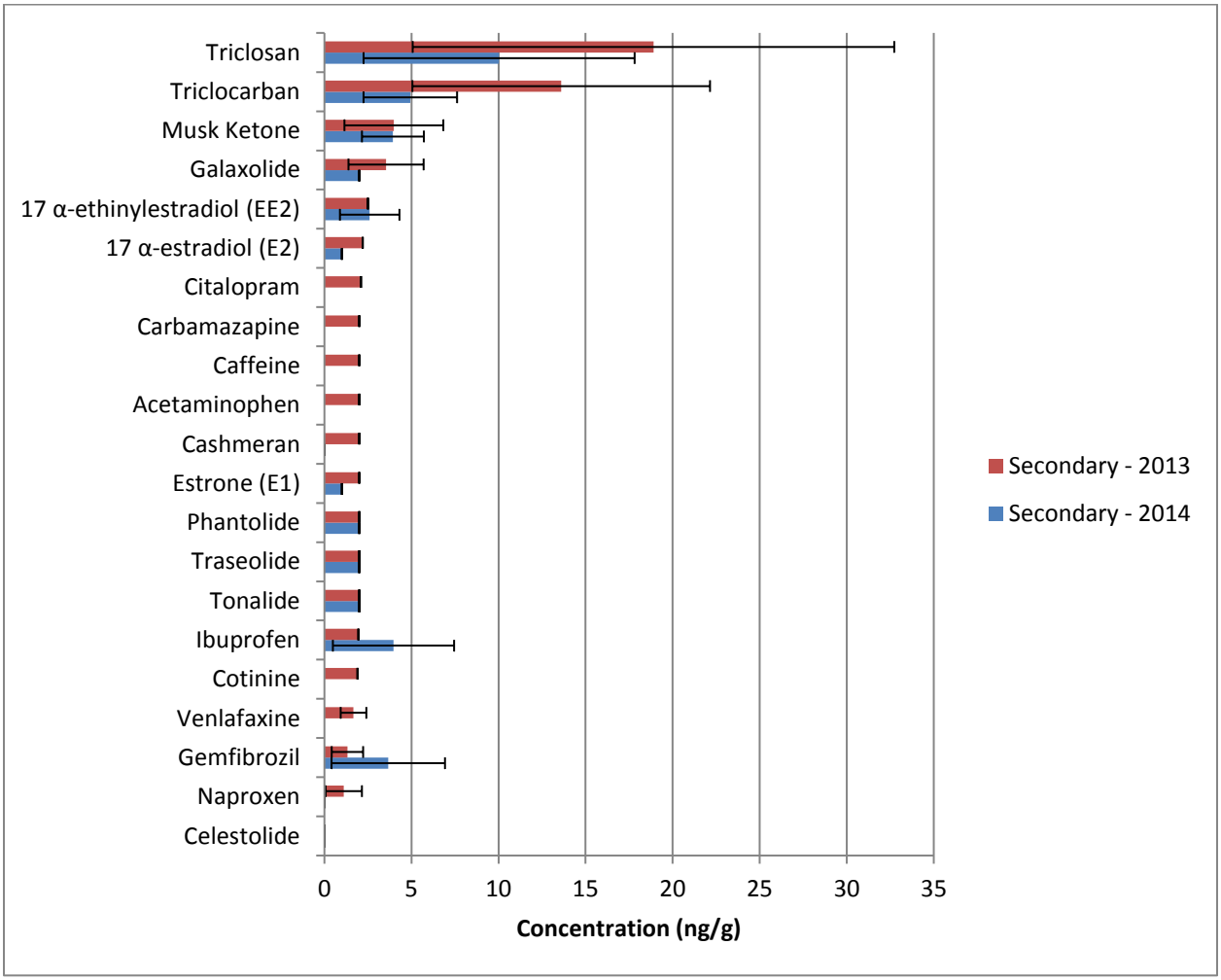

Figure 4. Mean concentration and standard deviation of PPCPs tested in *Daphnia* samples, collected from secondary cell 2 of the Livingstone Trail Environmental Control Facility, Yukon, Canada, in 2013 (n=7) and 2014 (n=3).

3.1.4 Algae

Triclosan (61 – 210 ng/g), triclocarban (4.7 – 47 ng/g), gemfibrozil (9.7 – 46 ng/g), and ibuprofen (15 – 46 ng/g) had the highest concentrations in algae samples collected from the tertiary pond (Figure 5). Naproxen, cashmeran, celestolide, and musk ketone were not detected in any algae samples. Among the estrogens, E1 and EE2 were not detected above the LOQ, while E2 was detected only slightly above the LOQ. Phantolide was the only synthetic musk with a concentration above the LOQ (i.e., 11 ng/g). Raw data of PPCP concentrations in algae are reported in Appendix 9.

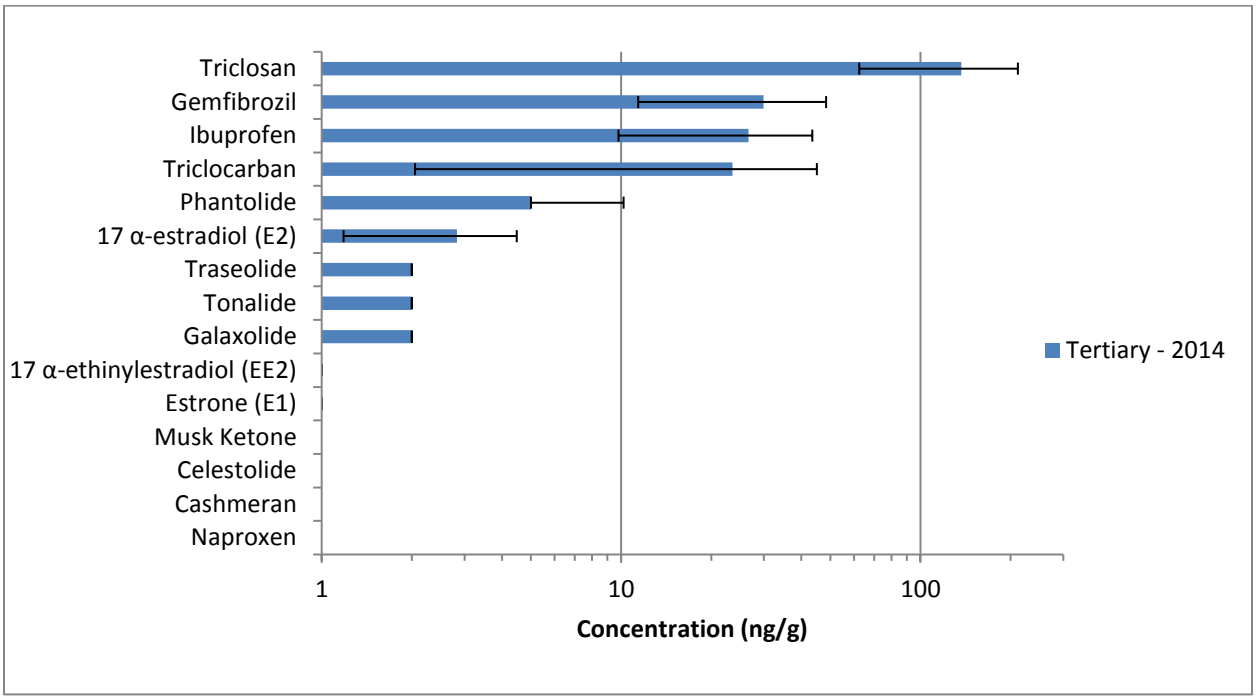

Figure 5. Mean concentration and standard deviation of PPCPs tested in algae samples, collected from the long-term storage pond of the Livingstone Trail Environmental Control Facility, Yukon, Canada, in 2014 (n=3).

3.2 Removal Efficiency, R

Overall removal efficiencies for 11 PPCPs in 2013 and 2014 were categorized according to a classification system developed by Li et al. (2014), as: “readily removed” (>70%), “moderately removed” (50-70%), “low removed” (20-50%), and “hardly removed” (<20%). All PPCPs, except gemfibrozil and the three estrogens, were readily removed in both years, often greater than 90% (Figure 6). The PPCPs that were readily removed were readily removed during the primary and early stages of secondary treatment (i.e., primary cell A, primary cell B, secondary cell 1, and secondary cell 2). Significant overall removal rates were observed for all PPCPs that were readily removed, as well as gemfibrozil, which was moderately removed in 2013, and low removed in 2014.

Removal efficiency results for the three estrogens did not follow the same patterns that were observed for the other PPCPs. The removal efficiency of estrone in 2013 was the only circumstance when an estrogen had a removal efficiency greater than zero. All three estrogens exhibited negative overall removal rates, at least one of the two years. The estrogen concentrations were often higher in secondary cell 2 and the LTSP, compared to primary cell B. Note that some of the rates are calculated based on half the value of the LOQ, and therefore, may not represent the actual R. However, there was consistency in the pattern of results between 2013 and 2014, despite the fact that R was calculated with one sample in 2013 and nine samples in 2014.

Table 6. Removal efficiency (R) of pharmaceuticals and personal care products across the primary, secondary and overall treatment stages in 2013 (n=1) and 2014 (n=9) at the Livingstone Trail Environmental Control Facility, Yukon, Canada. Statistically significant ($p<0.05$) concentration differences between treatment stages are indicated by an asterisk (*).

PPCPs (ng/L)	Year	Primary Removal (Primary→Secondary=R)	Secondary Removal (Secondary→Tertiary=R)	Overall Removal (Primary→Tertiary=R)
Gemfibrozil	2013	190 → 117 = 38%	117 → 87 = 16%	190 → 87 = 54%
	2014	188 → 160 = 15%	160 → 108 = 28%	**188 → 108 = 43% ***
	2014	p = 0.6269	p=0.2281	**p=0.0103**
Ibuprofen	2013	10,000 → 91 = 99%	91 → 180 = -1%	10,000 → 180 = 98%
	2014	**9,289 → 394 = 96% ***	394 → 244 = 1%	**9,289 → 244 = 97% ***
	2014	**p = 0.0315**	p = 0.3096	**p = 0.0027**
Naproxen	2013	5,900 → 196 = 97%	196 → 5.5 = 3%	5,900 → 5.5 = 100%
	2014	23 → 0 = 100%	0 → 5 = -22%	23 → 5 = 78%
	2014	p = 0.0665	p = 0.1456	p = 0.4020
Triclosan	2013	610 → 66 = 89%	66 → 22 = 7%	610 → 22 = 96%
	2014	995 → 123 = 88%	123 → 118 = 0.5%	**995 → 118 = 88% ***
	2014	p = 0.0605	p = 0.7189	**p = 0.0429**
Triclocarban	2013	31 → 10 = 68%	10 → 5.5 = 14%	31 → 5.5 = 82%
	2014	**75 → 0.5 = 99% ***	0.5 → 10 = -12%	**75 → 10 = 87% ***
	2014	**p = 0.0001**	p = 0.0662	**p = 0.0019**
Estrone (E1)	2013	3.0 → 1.5 = 50%	1.5 → 0.8 = 23%	3.0 → 0.8 = 73%
	2014	3.5 → 7.5 = -117%	7.5 → 4.6 = 85%	3.5 → 4.6 = -32%
	2014	p = 0.1191	p = 0.5890	p = 0.4792
17 α-estradiol (E2)	2013	4.2 → 9.1 = -117%	9.1 → 8.0 = 26%	4.2 → 8.0 = -90%
	2014	**1.5 → 47 = -3022% ***	47 → 15 = 2155%	1.5 → 15 = -866%
	2014	**p = 0.0222**	p = 0.1715	p = 0.1396
17 α-ethinylestradiol (EE2)	2013	1.0 → 1.7 = -70%	1.7 → 4.0 = -230%	1.0 → 4.0 = -300%
	2014	6.9 → 13 = -88%	13 → 6.9 = 88%	6.9 → 6.9 = 0%
	2014	p = 0.1262	p = 0.1062	p = 0.9349
Galaxolide	2013	213 → 18 = 92%	18 → 1.2 = 8%	213 → 1.2 = 99%
	2014	**339 → 88 = 74% ***	**88 → 17 = 21% ***	**339 → 17 = 95% ***
	2014	**p = 0.0006**	**p = 0.0429**	**p = 0.0003**
Tonalide	2013	26 → 1.2 = 95%	1.2 → 1.2 = 0%	26 → 1.2 = 95%
	2014	**21 → 11 = 47% ***	**11 → 3.7 = 35% ***	**21 → 3.7 = 82% ***
	2014	**p = 0.0214**	**p = 0.0029**	**p = 0.0006**
Traseolide	2013	68 → 6.4 = 91%	6.4 → 3.4 = 4%	68 → 3.4 = 95%
	2014	9.0 → 4.9 = 45%	**4.9 → 0.8 = 46% ***	**9.0 → 0.8 = 91% ***
	2014	p = 0.0532	**p = 0.0176**	**p = 0.0003**

*Underlined values represent one half of the limit of quantification for that PPCP

Removal rates in 2013 and 2014 for ibuprofen, galaxolide, traseolide, triclosan, tonalide, and naproxen were higher at the LTECF than the mean removal rates reported in the review by Miege et al. (2009) (Figure 6). The R of synthetic musks (galaxolide, traseolide, tonalide) were particularly high at the LTECF, compared to the mean values in Miege et al. (2009). No R for triclocarban was available in Miege et al. (2009). Removal rates of 54% and 43% for gemfibrozil at the LTECF were similar to the mean removal rate of 52% for gemfibrozil in Miege et al. (2009). Mean removal rates for the three estrogens, E1, E2, and EE2, in Miege et al. (2009) were 74%, 80%, and 67%, respectively, while R at the LTECF for E1, E2, and EE2 were often negative. Note the number of papers compiled in the review by Miege et al. (2009) to obtain the mean removal rates for each PPCP.

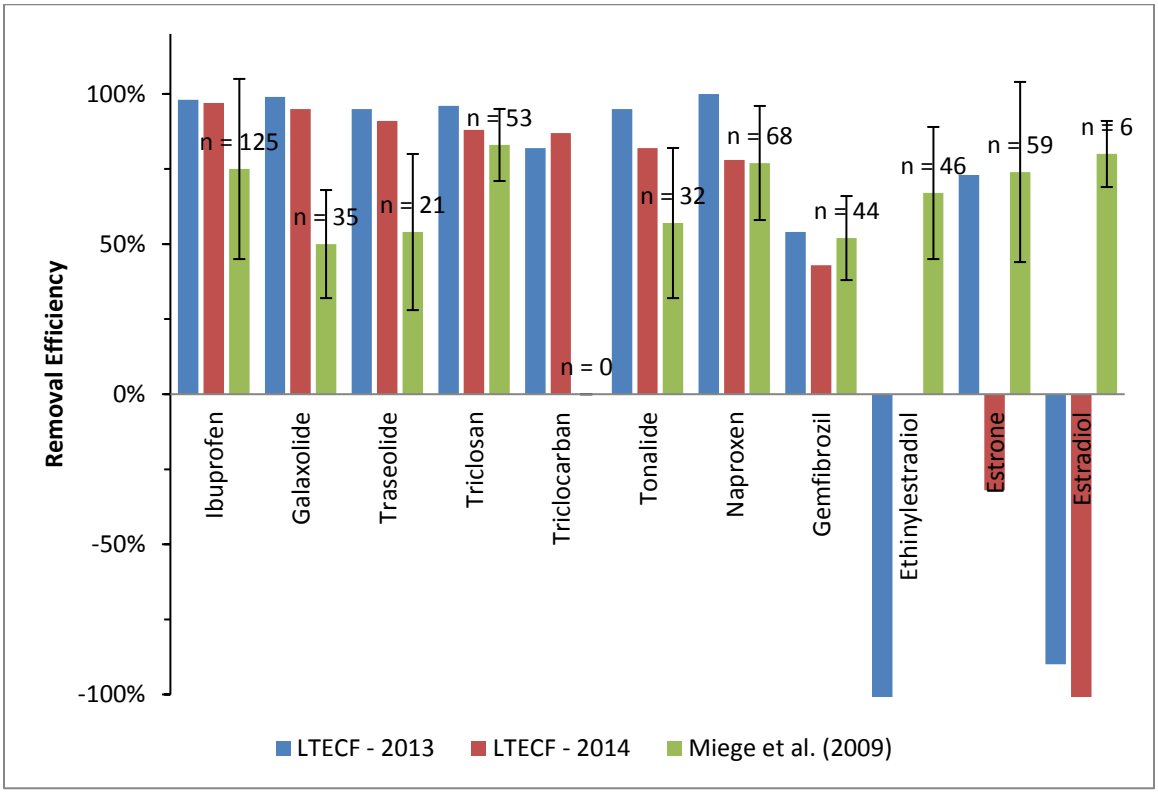

Figure 6. Comparison of overall removal efficiencies at the Livingstone Trail Environmental Control Facility in 2013 (n=1) and 2014 (n=9), to mean removal efficiencies and standard deviations reported in Miege et al. (2009). Note number of papers (n) compiled in Miege et al. (2009) to obtain mean and standard deviation removal rates for each chemical.

3.3 Seasonal Variation

When all PPCPs were grouped, there were significant differences in seasonal concentrations in each stage of treatment, with concentrations lower in spring than in summer and fall, across all stages of treatment (Figure 7; Table 7). No significant difference existed between summer and fall concentrations within any of the three treatment stages.

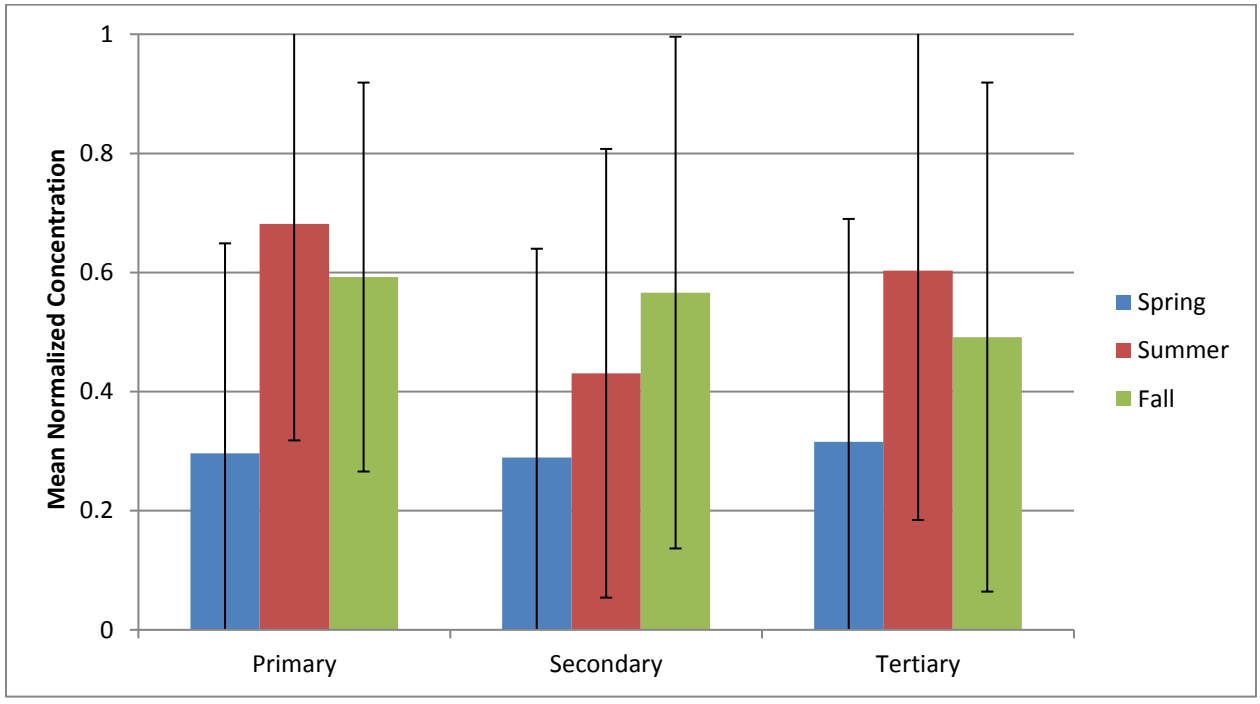

Figure 7. Comparison of the seasonal variability of mean normalized concentrations and standard deviation for all PPCPs combined (n=13), between spring, summer and fall in the primary, secondary and tertiary stages of treatment at the Livingstone Trail Environmental Control Facility, Yukon, Canada.

Table 7. Results of Dunn's nonparametric comparison following a Kruskal-Wallis test for seasonal differences in concentrations of 13 PPCPs combined in the primary, secondary and tertiary cells (n=39), as well as all cells combined (n=117).

Seasonal Variation	Primary	Secondary	Tertiary	Overall
Spring – Summer	****	---	**	****
Spring – Fall	**	**	*	****
Summer – Fall	---	---	---	---

--- no statistical difference; * p = <0.05 – ≥0.01; ** p = <0.01 – ≥0.001; *** p = <0.001 – ≥0.0001; **** p = <0.0001

3.4 Bioaccumulation

Triclocarban had the highest BAF values amongst all *Daphnia* and algae samples (Table 8), with BAF values in algae ranging from >5,000 to >50,000 L/kg, and BAF values for *Daphnia* ranging from 350 to >8,000 L/kg. Triclocarban was the only PPCP to have a BAF ≥5,000, meaning triclocarban was the only PPCP to be defined as bioaccumulative according to the Persistence and Bioaccumulation Regulations in CEPA. The synthetic musks, phantolide and musk ketone, had the second and third highest BAF, with values of >4,500 L/kg and >2,400 L/kg, respectively. Many BAF values were quantified as ">" or "<" because either the concentration in the organism, or the concentration in the water was <LOQ. When the concentrations in the organism and in the water were both <LOQ, the BAF was not quantifiable (i.e., n/q). Bioaccumulation factors of zero were observed for naproxen, cashmeran, celestolide, and musk ketone.

Table 8. Bioaccumulation factors (BAF) for pharmaceuticals and personal care products in *Daphnia* samples collected from secondary cell 2 in 2013 (n=7) and 2014 (n=3), and algae samples collected from the long-term storage pond in 2014 (n=3) at the Livingstone Trail Environmental Control Facility, Yukon, Canada.

	$BAF_{daphnia}$ (L/kg)		BAF_{algae} (L/kg)
	2013	2014	2014
Gemfibrozil	<31	<11 – 40	75 – 425
Ibuprofen	<33	<20 – 80	75 – 225
Naproxen	<15	0	0
Triclosan	50 – 450	50 – 200	750 – 2,500
Triclocarban	350 – 2,300	>3000 – >8,000	>5000 – >50,000
Estrone (E1)	<2,350	<300	<400
17 α-estradiol (E2)	<600	<400	660 – >1,400
17 α-ethinylestradiol (EE2)	<1,700	<250 – 550	<200
Galaxolide	325-400	<100	n/q
Tonalide	n/q	<350	<800
Traseolide	<500	n/q	n/q
Cashmeran	n/q	0	0
Celestolide	0	0	0
Phantolide	<800	<1,200	>4500
Musk Ketone	>2,400	n/q	0

n/q: not quantifiable

4.0 Discussion

4.1 Occurrence

This is the first study to quantify the occurrence of PPCPs in the water, sludge, aquatic invertebrates, and algae concurrently at a WWTP. Most pharma-ecology research involves sampling only the water or sludge, sometimes both, but never also the aquatic invertebrates and algae. Quantifying the occurrence of PPCPs at the LTECF was a necessary first step to establish a baseline data set, which could be used for comparison with other WWTP, as well as for future research and monitoring of PPCPs at the LTECF and other WWTP throughout Yukon.

Samples collected from primary cell B provide information about the consumption patterns of PPCPs among Whitehorse residents. Samples collected from the discharge location of the LTSP are important from an environmental point of view, as those results indicate what concentrations are being released into the environment. Sludge samples are also important from an environmental point of view as the disposal of sludge is a primary mechanism for PPCPs to find their way into the environment (Jelic et al., 2011). Quantifying the occurrence of PPCPs in aquatic invertebrates and algae is important from an ecological health perspective, as these organisms are dominant food sources for waterfowl using the LTECF, and create the link in the food chain between aquatic and terrestrial ecosystems. Therefore, observations of PPCP concentrations in aquatic invertebrates and algae can provide insight into potential PPCP contamination of waterfowl using the LTECF.

4.1.1 Water

High concentrations of acetaminophen (150,000 ng/L), caffeine (100,000 ng/L) and ibuprofen (10,000 ng/L) at the LTECF are in agreement with the literature, which consistently reports these chemicals to have the highest concentrations among all PPCPs tested. High concentrations of these chemicals are not unexpected as these drugs are easily obtained and widely consumed by the public. A review published by Verlicchi et al. (2012) found ibuprofen was the compound with the highest concentration (373,000 ng/L) in raw urban wastewater samples collected in conventional WWTPs (i.e., activated sludge and membrane bioreactors), followed by

acetaminophen (246,000 ng/L); acetaminophen and ibuprofen also had the highest average raw influent concentrations of 38,000 ng/L and 37,000 ng/L, respectively. Lin et al. (2010) determined that among 61 PPCPs tested in six different conventional WWTPs, acetaminophen, caffeine and ibuprofen were detected at the highest concentrations: 31,000 ng/L and 23,000 ng/L, and 18,000 ng/L, respectively, whereas a review by Liu and Wong (2013) found caffeine and ibuprofen were PPCPs with the highest detection frequencies and concentrations in various forms of WWTPs. In a review of 117 studies of activated sludge WWTPs, Miege et al. (2009) reported a mean influent ibuprofen concentration of 14,600 ng/L.

Other PPCP concentrations at the LTECF are similarly in general agreement with published studies. The influent and effluent concentrations from the LTECF were compared to results in the comprehensive database compiled by Miege et al. (2009), who reported the influent and effluent minimum, maximum and median concentrations for 184 compounds, 16 of which were studied at the LTECF. Concentrations for all PPCPs at the LTECF, except ibuprofen, E2 and EE2, were below the median concentrations of the influent and effluent in the database; acetaminophen and caffeine were not included. Effluent concentrations of E2 and EE2 at the LTECF were higher than the maximum effluent concentrations reported in the database, based on 9 papers and 33 papers, respectively.

4.1.2 Sludge

High concentrations of triclosan and triclocarban in the sludge at the LTECF are also consistent with other studies (Hydromantis Inc., 2010), and not unexpected given the intense usage of these chemicals in household products (McClellan and Halden, 2010). In 2001, the U.S. EPA performed a national sewage sludge survey, which determined the mean concentrations of 72 PPCPs in 110 biosolid samples from 94 WWTP. Results of that survey found triclosan and triclocarban were the most abundant PPCPs, with mean concentrations of 13,000±400 and 36,000±800 ng/g respectively, accounting for 65% of the total PPCP mass (McClellan and Halden, 2010). In 2009, the U.S. EPA performed another national sewage sludge survey, which found triclosan and triclocarban at concentrations up to 133,000 and 441,000 ng/g, respectively, with mean concentrations of 12,000±18,000 and 39,000±60,000 ng/g (n=74). Furthermore,

triclosan and triclocarban have been observed in a number of U.S. studies of sewage sludge with concentrations ranging from 530 to 30,000 ng/g, and 6,000 to 51,000 ng/g, respectively (McClellan and Halden, 2010).

The 2001 U.S. EPA national sewage sludge survey also reported mean and maximum concentrations of ibuprofen, naproxen, gemfibrozil, caffeine and carbamazepine (McClellan and Halden, 2010). Concentrations of ibuprofen, caffeine and triclosan in sludge of primary cell B at the LTECF were higher than the maximums reported in the national sewage sludge survey; however, concentrations of naproxen, gemfibrozil, carbamazepine and triclocarban at the LTECF were below the mean concentrations in the database. High concentrations of the synthetic musk, galaxolide, at the LTECF are consistent with results from a review performed by Hydromantis Inc. (2010), that summarized sludge sample results collected across various Canadian WWTPs and found galaxolide had the third highest median concentration among all PPCPs. The concentrations of estrogens in sludge at the LTECF were lower than those found by Martin et al. (2012).

4.1.3 Aquatic Invertebrates

This is the first study to analyze PPCP concentrations in *Daphnia* and chironomid samples collected from a WWTP. Similar to sludge and algae, triclosan and triclocarban had the highest concentrations in aquatic invertebrate samples, consistent with the suggestion by Brausch and Rand (2011) that triclosan and triclocarban are the PPCPs most likely to accumulate to the highest concentrations in aquatic organisms. Brausch and Rand (2011) also suggested triclosan and triclocarban could affect benthic invertebrates at concentrations reported in the environment.

Although no studies have reported specifically on the effects of triclosan and triclocarban on birds, recent studies have found that European Starlings and Tree Swallows feeding on aquatic invertebrates contaminated with PPCPs from a WWTP showed marked changes in brain development, growth rates, behaviour, reproductive success and immunocompetence (Dods et al., 2005; Markman et al., 2008; Markman et al., 2011). This is of concern because my study confirms that PPCPs are being accumulated in *Daphnia* and chironomid, two important food sources for waterfowl at the LTECF, especially breeding females and developing ducklings. A

U.S. Fish and Wildlife Service study determined that aquatic invertebrates comprised 98% of the diet of adult and immature Mallard and Gadwall on sewage lagoon ponds, and that chironomid and *Daphnia* each made up 44% of the diet (Swanson, 1977). Furthermore, aquatic invertebrates are known to serve as a critical protein source for female ducks during egg production, and for early development of ducklings (Swanson, 1977; Mini et al., 2014).

Among synthetic musks, musk ketone had the highest concentrations in *Daphnia* at the LTECF, in both 2013 and 2014, consistent with results reported by Hu et al. (2011), who studied synthetic musk concentrations in fish from the Haihe River in China. In that study, galaxolide and tonalide were the most prevalent musks in fish samples; however, musk ketone concentrations were higher than galaxolide and tonalide when musk ketone was detected. The concentrations of galaxolide, tonalide, and musk ketone ranged between 2.9 and 7.9 ng/g, which are similar to the concentrations found in *Daphnia* at the LTECF. Interestingly, musk ketone was not detected in the water or sludge of the LTECF. However, Hydromantis Inc. (2010) also reported no detection of musk ketone in treated sludge or biosolids samples.

Kannan et al. (2005) investigated galaxolide and tonalide concentrations in various vertebrates, including waterfowl. Both musks were reported in liver tissues of Common Merganser, Lesser Scaup, Greater Scaup, and Mallard. Concentrations of galaxolide in Mallard, scaup and merganser ranged from 1.9 to 4.2 ng/g, which are similar to, or slightly lower than concentrations of galaxolide and tonalide in *Daphnia* at the LTECF. The limit of quantification for analysis of galaxolide and tonalide in *Daphnia* for this study was 4.0 ng/g, which is higher than the concentrations measured in Kannan et al. (2005).

4.1.4 Algae

This is also the first study to report PPCP concentrations in algae samples collected from a WWTP. Similar to sludge and *Daphnia*, triclosan and triclocarban had the highest concentrations in algae samples, although concentrations were higher in algae compared to *Daphnia*. The maximum concentration of triclosan in algae was 210 ng/g, compared to 36 ng/g in *Daphnia*. Results at the LTECF are consistent with those reported by Coogan et al. (2007), who sampled triclosan and triclocarban in algae samples collected in a creek downstream from a WWTP, and

found concentrations ranging from 50-400 ng/g. These results are concerning for waterfowl species such as American Wigeon that select for algae as a preferred food source (Mini et al., 2014). During late summer, algae density peaks in the tertiary pond of the LTECF, which attracts thousands of American Wigeon, thereby exposing them to levels of triclosan and triclocarban that may cause deleterious effects. As a result, the LTECF may be acting as an ecological trap for birds such as American Wigeon that select the facility as preferred habitat due to an abundant food source.

Long-term exposure tests found algae was the most sensitive trophic group to triclosan concentrations, among fish, invertebrates and vascular plants (Brausch and Rand, 2011). Similarly, Wilson et al. (2003) observed a consistent reduction of algal genus diversity at triclosan concentrations equal to those that have been reported in the environment. Toxicity tests by Liu and Wong (2013) reported that exposure to triclosan and triclocarban at concentrations of 0.4 – 10 µg/L for 3 days resulted in growth inhibition of the algae *Pseudokirchneriella subcapitata*. High triclosan sensitivity in algae is likely due to the antibacterial characteristics of the compound (Brausch and Rand, 2011). Only minimal aquatic toxicity data exist for triclocarban, but recent studies indicate that triclocarban is slightly more toxic to aquatic invertebrates and fish for both short- and long-term exposures than triclosan (Brausch and Rand, 2011). No studies report on the toxicity of triclosan or triclocarban specifically to birds.

4.2 Removal Efficiency, R

Removal efficiency (R) is an important criterion to evaluate the performance of a WWTP, and is influenced by many factors (Li et al., 2014; Verlicchi and Zambello, 2014). Most literature suggests that the most significant factors affecting R are photodegradation and biodegradation (Imfeld et al., 2009; Li et al., 2014; Verlicchi and Zambello, 2014). Extending the length of time that wastewater is treated (i.e., hydraulic retention time, HRT) can increase bio- and photodegradation rates, and therefore, have a significant impact on the R of a WWTP. Verlicchi and Zambello (2014) suggest that the longer the HRT, the greater the R. Likewise, Zhang et al. (2011) found that the R of all target PPCPs in a pilot-scale system were linearly proportional to the HRT. Conkle et al. (2008) and Llorens et al. (2009) demonstrated that a series of lagoons

exhibiting a high HRT (up to 30 days) guaranteed better removal of common PPCPs compared to conventional WWTP, which have low HRT (10-60 hr depending on the system).

The HRT at the LTECF is up to one year, which is unique among those presented in the literature; the HRT in many CWs is less than a week, and often only a matter of hours for conventional systems (Conkle et al., 2008; Li et al., 2014;). It is suspected that the long HRT of wastewater at the LTECF is the major contributing factor to consistently higher removal rates at the LTECF than rates reported in the literature, for both CWs and conventional systems. The HRT of the LTECF is allowed to be so long due to the large size, and therefore storage capacity, of the treatment cells, as well as the number of treatment cells. The large surface area and volume of each treatment cells likely facilitates high rates of bio- and photodegradation, as well as volatilization, the vaporization of dissolved substances, a recognized removal pathway, especially for synthetic musks (Osemwengie and Gerstenberger, 2004; Imfeld et al., 2009).

Results of this study support recent suggestions that constructed wetlands hold great potential for their application in effective removal of PPCPs from wastewater (Li et al., 2014). CWs generally have longer HRT than conventional WWTP, which is the suspected cause for their effectiveness of PPCP removal (Miege et al., 2009; Li et al., 2014; Verlicchi and Zambello, 2014). A review by Li et al. (2014) compared R in constructed wetlands to those in conventional WWTPs and found most removal efficiencies in CW were as good as or even higher than those in conventional WWTPs. Likewise, Hijosa-Valsero et al. (2010) found that CWs were at least as efficient in PPCP removal as the conventional WWTP. However, a study by Ying et al. (2008) found that sewage lagoons in series were the least effective of four methods (i.e., Plant A: conventional activated sludge; Plant B: two oxidation ditches; Plant C: three bioreactors; Plant D: 10 lagoons in series) evaluated for removal of pharmaceuticals and hormones from wastewater. Qiang et al. (2013) investigated the R of four different WWTP (i.e., Plant A: activated sludge; Plant B: micro-power biofilm reactor; Plant C: constructed wetland; Plant D: stabilization pond) and found that mechanical activated sludge was the most effective, while CW, stabilization ponds, and biofilm reactors are less effective. MacLeod and Wong (2010) found no difference in R between conventional systems and CW.

Verlicchi and Zambello (2014) compiled occurrence and removal data from 47 peer reviewed journal articles from various types of constructed wetlands treating municipal wastewater. The paper reviewed investigations carried out on 136 different constructed wetlands, including surface flow, horizontal, and vertical subsurface flow acting as primary, secondary or tertiary treatments. Of those 136 constructed wetlands, 11 were classified as hybrid types, like the LTCEF, although none of the 11 facilities compared to the size and HRT of the LTECF. Results from this study were the most comparable to the LTECF in terms of treatment system. The mean removal rates for ibuprofen, naproxen, triclosan, triclocarban, and gemfibrozil were equal to, if not higher at the LTECF compared to the mean removal rates in Verlicchi and Zambello (2014). These results emphasize the effectiveness of the LTECF for removal of PPCPs. The only PPCPs that did not have higher removal efficiencies at the LTECF were the estrogens.

Removal rates for 11 PPCPs at the LTECF were also compared to mean removal rates in a review by Miege et al. (2009). This study was the most comprehensive compilation of R found in the literature, in terms of number of papers and PPCPs documented, although it only included data from conventional activated sludge systems. Regardless, eight of the eleven PPCP removal rates at the LTECF were higher than the mean removal rates in Miege et al. (2009); only estrogens did not have higher removal rates at the LTECF.

It is unclear why the removal efficiencies of estrogens at the LTECF were negative. Most studies report relatively good removal of estrogens, regardless of the type of treatment system (Ying et al., 2008; Miege et al., 2009). One explanation is that the estrogen concentrations are so low in the LTCEF that the slightest error in instrumentation, resulting in an inaccurate reading of 1-2 ng/L, could cause the removal efficiency to vary drastically (Li et al., 2014). Another explanation for an apparent increase in PPCP concentrations is the inter-conversion of estrogens in the later stages of treatment between the various forms (i.e., E2 and EE2), caused from microbial metabolization (Shi et al., 2010; Qiang et al., 2013). Similarly, the reformation of broken bonds by enzymatic processes during the treatment process to re-form the PPCP has also been reported (Li et al., 2014). Finally, it is possible the estrogen results for 2014 presented in this study are not representative of the actual concentrations in the LTECF. The Water Quality Centre had to re-run the estrogen samples due to inaccurate results following the first

analysis. The samples were re-anlayzed using a different methodology to account for the error. The samples were frozen and thawed between the two analyses, which may have had an effect on the concentration. The 2014 estrogen results have been displayed and discussed but should be viewed critically, if not discounted altogether.

Lastly, the presence of plants in constructed wetlands has been shown to play an important role in removal of PPCPs in wastewater (Imfeld et al., 2009; Carranza-Diaz et al., 2014; Verlicchi and Zambello, 2014; Zhang et al., 2014). Li et al. (2014) found high temperatures and strong sunlight enhanced the activities of plants and microorganisms in CW, resulting in increased R of ibuprofen, naproxen, triclosan, carbamazepine, and caffeine. The mechanisms responsible for the removal included the degradation of PPCPs via metabolic processes after being taken up in plant tissues. Furthermore, Li et al. (2014) found plants stimulate development and activities of microbial populations, which are responsible for biodegradation of PPCPs. For example, plants in constructed wetlands are able to release oxygen around their root tips, which favors the development of aerobic microorganisms inducing more efficient biodegradation processes. The removal of PPCPs at the LTECF via the occurrence of plants and accumulation in other biota is likely a contributing factor, which is supported by the occurrence of PPCPs within algae and aquatic invertebrate samples. Anecdotal observations suggest increasing incursion of vegetation in the LTSP, and continued vegetation growth within all stages of treatment at the LTECF should be encouraged.

4.3 Seasonal Variation

Seasonal differences in PPCP concentrations have been observed in other studies. Generally, PPCP concentrations are higher in winter than in summer, and removal efficiencies are higher in summer than in winter (Liu and Wong, 2013; Qiang et al., 2013). This would be expected, as summer weather brings warmer temperatures and more daylight, which promotes bio- and photodegradation, two key processes in the degradation of PPCP compounds (Verlicchi and Zambello, 2014). Various studies have reported poor R during the winter months, due either to a lack of microbial activity or lack of photodegradation (Li et al., 2013; Liu and Wong, 2013; Qiang et al., 2013). Matamoros et al. (2008) reported lower R in winter than summer for

naproxen and diclofenac. MacLeod and Wong (2010) detected higher concentrations of a variety of PPCPs in the effluent of a lagoon system in winter compared to summer. Similarly, Reyes-Contreras et al. (2012) found salicylic acid and caffeine are more easily removed in summer than in winter. In a review by Verlicchi et al. (2012), lower R was observed in winter compared to summer for many PPCPs in multiple WWTPs in Italy. Likewise, a Finnish study found the rate of removal decreased an average of 25% in winter (Vieno et al., 2005). At the old Whitehorse sewage lagoon, used prior to the establishment of LTECF, Whitley and Thirumurthi (1992) found little difference in removal rates of biological oxygen demand between spring, summer and fall; however, there was a noticeable decrease in removal during the winter months.

Although winter samples were not collected at the LTECF, concentrations for most PPCPs were significantly lower in spring than in summer and fall; however, naproxen and galaxolide had significantly higher concentrations in spring than summer and fall. The lower concentrations of most PPCPs in spring may be a result of dilution, as the monthly sewage pumpage summary for the LTECF for previous years shows that winter months have higher flow volumes than summer months (City of Whitehorse, 2014). Whitehorse is located in a subarctic climatic zone; therefore, during the winter months, the City runs bleeders through the underground water pipes to supply a continuous flow of water to ensure the pipes do not freeze. These bleeders use clean water and dilute the wastewater entering the LTECF during the winter. The bleeders are not shut off until June, to ensure the pipes do not freeze as a result of a late frost (Dave Albisser, pers. comm.). This dilution over the course of the winter may explain why most PPCP concentrations were lower in spring among all three treatment cells. Furthermore, dilution of wastewater may occur during spring freshet (the melting of winter snow and ice). During spring sample collection from secondary cell 2, surface ice was in the process of melting; the ice on the tertiary cell had only recently melted. This surface ice melt may have diluted the surface layer of wastewater in each pond where grab samples were being collected. Primary cell B was ice free during May sampling but would still be under the influence of the bleeders. Snow melt around the perimeter of the treatment cells would further dilute the cells of the LTECF in spring.

Effects of seasonality on evaporation and evapotranspiration may in turn have an effect on PPCP concentrations at the LTECF. Studies have shown that evaporation and evapotranspiration (i.e.,

uptake of water through vegetation) result in water losses, causing a concentrating effect on PPCP concentrations (Verlicchi and Zambello, 2014). Lake evaporation data and annual precipitation data from the Whitehorse area suggest that this water loss may be occurring at the LTECF. In the Whitehorse area, the total mean lake evaporation for the period May – September is 48.3 cm; however, the average annual precipitation from rainfall is only 15 cm (Whitley and Thirumurthi, 1992). A study that examined evapotranspiration at sewage lagoons found water loss is greatly increased in vegetated treatment cells and tends to be highest in summer (Verlicchi and Zambello, 2014). In unvegetated treatment cells, the rate of evaporation is mainly dependent on air temperature and relative humidity, and is also highest in summer months (Verlicchi and Zambello, 2014). The secondary and tertiary treatment cells at the LTECF have large surface areas of 50 and 150 ha, respectively, and are increasingly vegetated each year. It is possible that evaporation and evapotranspiration are contributing to a concentrating effect of PPCPs, causing an increase in PPCP concentrations during summer and fall. That may also explain why concentrations for some PPCPs (e.g., estrogens) are higher in a later stage of treatment in summer. However, as mentioned previously, bio- and photodegradation rates are also highest in summer, resulting in lower concentrations. The strength of the effect from increased evaporation and evapotranspiration on PPCP concentrations, and the rate of removal caused from increased bio- and photodegradation in summer, is unknown.

Minimal research has analyzed seasonal variation of PPCP concentrations in a cold climate, especially a region with shortened daylight hours in winter. Vieno et al. (2005) performed an analysis of seasonal variation effects on PPCP concentrations at a WWTP in Finland, and found concentrations of pharmaceuticals were three to five times higher in winter compared to other seasons, concentrations in recipient water were higher in winter, and PPCPs were carried further downstream when the river was covered with snow and ice. These results suggest that cold seasons in boreal areas can increase the environmental risk of PPCPs. However, at the LTECF, the treatment cells are frozen during winter months and no discharge into the environment occurs. Therefore, the exposure to PPCPs during winter is low, because access to the chemicals is restricted. The exposure of PPCPs to birds at the LTECF is likely highest during summer and fall, when PPCP concentrations are highest, and exposure of birds to contaminated food is greatest.

4.4 Bioaccumulation

Triclocarban had the highest BAF results for both *Daphnia* and algae at the LTECF, and was the only PPCP to be classified as bioaccumulative according to the criteria in the Persistence and Bioaccumulation Regulations of CEPA. Although minimal bioaccumulation data exist for triclocarban, results at the LTECF seem to be consistent with the literature. A review by Brausch and Rand (2011) found that triclocarban demonstrated a propensity to bioaccumulate more than triclosan in aquatic organisms. Likewise, Coogan et al. (2007) calculated BAF for triclocarban and triclosan in algae collected from a creek that receives effluent from a WWTP. Results from that study found BAF for triclocarban were greater than BAF for triclosan, which was similar to results from the LTECF. Furthermore, a study by Xu et al. (2015) found that triclocarban was more toxic than triclosan to a species of aquatic crustacean, *Artemia salvina*, which is related to *Daphnia*. Results from my study, with support from the literature, suggest that triclocarban should be further investigated for environmental effects at the LTECF.

Although triclosan was found at measurable levels in both algae and *Daphnia* at the LTECF, the BAF values were not above the threshold in the Persistence and Bioaccumulation Regulations of CEPA. These results are consistent with the Assessment Report of Triclosan, which found while triclosan accumulates in organisms to levels that can cause adverse effects, it does not meet the bioaccumulation criteria as set out in the Persistence and Bioaccumulation Regulations of CEPA (Government of Canada, 2016). Coogan et al. (2007) reported a BAF ranging from 900 to 2,100 L/kg ww for triclosan in algae collected in a creek receiving the effluent from a WWTP. These results are very similar to the range of BAF for triclosan in algae at the LTECF (750-2,500 L/kg dw). Likewise, Coogan and La Point (2008) reported a BAF of 500 for triclosan in snails that had been caged in the same creek for 2 weeks, which is similar to the BAF calculated for triclosan in *Daphnia* at the LTECF (50-450 L/kg dw).

Among the synthetic musks at the LTECF, BAF were highest for phantolide (>4,500 L/kg) and musk ketone (>2,500 L/kg), which may be classified as bioaccumulative at the LTECF according to criteria in the Persistence and Bioaccumulation Regulations. Hu et al. (2011) also found higher BAF values for phantolide and musk ketone, than for galaxolide and tonalide, in water and fish

samples collected from the Haihe River in China. A similar trend was also observed in a study by Gatermann et al. (2002), who examined BAF of galaxolide, tonalide, and musk ketone in fish collected from a pond that received WWTP effluent. Results of that study found musk ketone had the highest BAF (60-1,300), followed by tonalide (40-670) and galaxolide (20-580). It must be noted that although trends were similar, the aquatic organisms used in the calculations were not the same. Interestingly, musk ketone was not detected in many water and sludge samples in Hu et al. (2011); however, it was detected in biota samples. This pattern is consistent with results found at the LTECF.

Although BAF values for the estrogens at the LTECF were not ≥5,000 L/kg, accumulation in *Daphnia* and algae did occur. This warrants further study, as adverse effects of estrogens on the development and endocrine function of birds have been reported at concentrations similar to those found at the LTECF (Markman et al., 2008; Markman et al., 2011). Markman et al. (2011) found exposure of European Starling nestlings to a mixture of estrogens on a daily basis, between 1 and 15 days old, resulted in reduced growth and immunocompetence. Furthermore, Markman et al. (2008) found that male European Starlings exposed to low concentrations of synthetic and natural estrogens developed longer and more complicated songs compared to control males, which in turn attracted more females. The key brain area controlling male song complexity was significantly enlarged in the contaminated birds. Lastly, a study by Dods et al. (2005) examined reproductive, immunological, and growth endpoints in Tree Swallows exposed to 4-nonylphenol (i.e., an industrial estrogen found in the sludge of secondary cell 2 and LTSP). They found clutch size and fledgling success were significantly lower, and mean mass of nestling livers was significantly higher, in swallows breeding at the WWTP compared to the reference site. These studies suggest that birds at the LTECF that are feeding on algae and *Daphnia* may be subject to increased risk of adverse health effects.

5.0 Summary

Water, sludge, aquatic invertebrate, and algae samples were collected in 2013 and 2014 in the primary, secondary and tertiary stages of treatment, in the spring, summer and fall, at the Livingstone Trail Environmental Control Facility. The objective of the sampling was to establish

a baseline data set for the occurrence and fate of pharmaceutical and personal care products (PPCPs) in the wastewater treatment facility. The occurrence of 33 PPCPs analyzed was detected in at least one of the water, sludge, aquatic invertebrate, and algae samples. The PPCPs with the highest concentrations in water were acetaminophen, caffeine and ibuprofen. The PPCPs with the highest concentrations in sludge, aquatic invertebrates, and algae were the two antimicrobial chemicals, triclosan and triclocarban. Estrogens and synthetic musks were among the PPCPs with the lowest concentrations in all media. Many PPCPs, especially those in aquatic invertebrates and algae, were measured at levels below their limit of quantification. PPCP concentrations at the LTECF were comparable to those reported in other studies (Miege et al., 2009; McClellan and Halden, 2010; Brausch and Rand, 2011; Yu et al., 2011; Chen et al., 2013, Verlicchi and Zambello, 2014; Government of Canada, 2016).

Removal efficiencies for most PPCPs at the LTECF were equal to, if not exceeding, those in the literature; for both conventional WWTPs and CWs. It is suspected that the high removal rates at the LTECF are due to the large size of the treatment cells and the long HRT. These factors allow the chemicals to be subjected to prolonged periods of bio- and photodegradation, two of the main processes involved in PPCP removal. Despite the fact that the treatment cells at the LTECF are ice-covered for half the year, the removal rates were higher than those from WWTP in temperate climates with short HRT. Based on the results of this study, and supported by findings of other published work (Conkle et al., 2008; Matamoros et al., 2008; Llorens et al., 2009; Miege et al., 2009; Hijosa-Valsero et al., 2010; Li et al., 2014; Verlicchi and Zambello, 2014), constructed wetlands hold great potential as a wastewater treatment method for efficiently removing PPCPs.

Concentrations of PPCPs were significantly lower in spring than in summer and fall, in all three stages of treatment at the LTECF. However, it is well documented that PPCP concentrations are lower in summer than winter, and removal efficiencies are lower in winter than in summer (Li et al., 2013; Liu and Wong, 2013; Qiang et al., 2013). Bleeder use throughout the winter and early spring in the underground water pipes of the City of Whitehorse is likely responsible for diluting the wastewater entering the LTECF over the winter, causing significantly lower concentrations in spring. There was no significant difference between summer and fall concentrations in any stage of treatment. The exposure of birds to PPCPs at the LTECF is highest during summer and

fall, when PPCP concentrations are highest, and adult and young birds are feeding most heavily on contaminated food items (i.e., aquatic invertebrates and algae).

Triclocarban was the only PPCP at the LTECF to be classified as bioaccumulative, as defined by the Persistence and Bioaccumulation Regulations in CEPA. These results are consistent with published studies that suggest that triclocarban and triclosan are the only two personal care products to present significant environmental hazard, among those that were studied at the LTECF. Furthermore, Brausch and Rand (2011) suggested that triclocarban demonstrated a propensity to bioaccumulate more than triclosan in aquatic organisms, which was observed at the LTECF for both *Daphnia* and algae. Based on this information, a risk assessment for triclocarban at the LTECF is warranted.

Chapter 4: Implications and Recommendations

This study was the first of its kind in a northern wastewater treatment plant in Canada. Therefore, it is uniquely positioned to contribute to our understanding of effective and appropriate wastewater management in northern systems. Furthermore, to the best of the author's knowledge, this was the first study of its kind anywhere to sample water, sludge, aquatic invertebrates, and algae concurrently from a WWTP. Most studies in this field sample either the water or sludge, sometimes both, but never also the aquatic invertebrates and algae. Also unique about this study, is the engineering design of the LTECF among constructed wetlands used for municipal wastewater treatment. For example, the sizes of the treatment cells are much larger than any other treatment cells reported in the literature. Secondly, the HRT of wastewater at the LTECF is longer than the HRT reported in other studies. Thirdly, the long-term storage pond is unique simply due to its presence following secondary treatment, and due to its size (Li et al., 2014).

Although unique in its engineering design, the concentrations and trends of PPCP occurrence and fate at the LTECF are consistent with the literature. For example, acetaminophen, caffeine, and ibuprofen had the highest concentrations in influent water samples (Lin et al., 2010; Verlicchi et al., 2012; Liu and Wong, 2013). Triclosan and triclocarban had the highest concentrations in sludge samples (Hydromantis Inc., 2010; McClellan and Halden, 2010). Triclosan and triclocarban were the most bioaccumulative substances, and triclocarban was more bioaccumulative than triclosan (Brausch and Rand, 2011). Among synthetic musks, musk ketone was more bioaccumulative than galaxolide and tonalide (Gatermann et al., 2002; Hu et al., 2011). In general, removal efficiencies at the LTECF were equal to, if not higher than, removal efficiencies in the literature; this was consistent for both conventional WWTPs, as well as CWs (Miege et al., 2009; Li et al., 2014).

Evidence suggests that the unique engineering design of the LTECF contributes to the effectiveness of treatment at the facility. Prior to beginning this research, it was suspected that the cold, northern climate of Whitehorse may hinder the capability of the LTECF to provide effective treatment of PPCPs. It is well documented that PPCP concentrations are higher and

removal rates are lower in winter, due to decreased bio- and photodegradation during cold weather months (Li et al., 2013). Results from this study found that despite the cold climate, the LTECF is an effective treatment system for removal of PPCPs. These results suggest that treatment systems similar to the LTECF may be a good option for providing effective treatment of PPCPs in cold climates. CWs are often a viable and preferred alternative to conventional systems in the north, given the availability of landscape to accommodate these systems, and their low operational and maintenance costs (Verlicchi and Zambello, 2014). Therefore, this study provides guidance to design future CWs for effective removal of PPCPs, such as using multiple, large treatment cells and extended periods of HRT.

However, despite the effectiveness of the LTECF for PPCP removal, aquatic and terrestrial organisms that live within, or utilize the LTECF for various life-cycle stages, may be at risk of contamination and adverse health effects from consuming PPCPs within the facility. Recent research has found that birds consuming aquatic invertebrates from WWTPs, contaminated with PPCPs, have shown effects in brain development, growth rates, behaviour, reproduction, and immunocompetence (Dods et al., 2005; Park et al., 2009; Markman et al., 2011). This is of concern given that Whitehorse biologists have found that the LTECF has become among the most heavily-used summer moulting and fall staging areas for waterfowl in the Yukon Southern Lakes region (Jim Hawkings, pers. comm.), and is used extensively for breeding by many species of birds. Therefore, quantifying the occurrence and fate of PPCPs within the LTECF was the first step towards understanding the potential risk to waterfowl using the facility.

Results from this study, in conjunction with data found in the literature, suggest that among all PPCPs, triclosan and triclocarban may pose the greatest risk to waterfowl feeding at the LTECF. Triclosan and triclocarban were the PPCPs with the highest concentrations in *Daphnia* and algae. Furthermore, triclocarban was the only PPCP to exceed the threshold set in the Persistence and Bioaccumulation Regulations in the *Canadian Environmental Protection Act, 1999*. A review by Brausch and Rand (2011) found among all personal care products they studied, triclocarban and triclosan were the only two that presented an environmental hazard based on toxicity and environmental data. Likewise, GreenScreen®, a recognized tool for comparative chemical hazard assessment, classifies triclosan as a GreenScreen Benchmark 1 chemical of high concern,

and triclocarban as a GreenScreen Benchmark 2 chemical with very high aquatic toxicity (Clean Production Action, 2014).

Recently, the Government of Canada completed an assessment of triclosan under the *Canadian Environmental Protection Act, 1999*, to determine if it poses a risk to Canadians and their environment (Government of Canada, 2016). It was concluded that triclosan does not meet criteria 64(b) and 64(c), which state that a substance is toxic if it enters or may enter the environment in a quantity or concentration or under conditions that constitute or may constitute a danger to the environment on which life depends; or constitute or may constitute a danger in Canada to human life or health. However, it did meet criteria 64(a), which states a substance is entering or may enter the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity. Because triclosan meets one of the three criteria under section 64 of CEPA, it was defined as a toxic substance.

Therefore, it is recommended that a priority for future research at the LTECF should involve a risk assessment for triclosan and triclocarban. Studies have found adverse effects can occur even at low exposure levels found in the environment (Government of Canada, 2016). Both adults and juvenile birds at the LTECF are exposed to elevated levels of triclosan and triclocarban through the consumption of aquatic invertebrates or algae, and may be accumulating levels high enough to cause adverse effects on behaviour, growth rate, and reproduction. There is evidence of effects of triclosan on the endocrine system at environmentally relevant concentrations (Government of Canada, 2016). Furthermore, research has shown that birds feeding on aquatic invertebrates contaminated with PPCPs can display negative physiological and behavioural effects (Dods et al., 2005; Markman et al., 2008; Markman et al., 2011).

There has been only one toxicity study reported in the literature that examined triclosan in waterfowl and no toxicity data for triclocarban in birds. Results of the study found triclosan was "relatively nontoxic" to Mallard (median lethal dose [LD_{50}] $\geq$ 2,150 mg/kg bw for Mallard) based on a 14-day acute oral toxicity test to Mallard at 19 weeks of age (Government of Canada, 2016). It is possible that the toxicity of triclosan and triclocarban on birds at the LTECF may be

more severe, given the known increase in risk caused from cumulative effects and chronic exposure, as well as exposure to developing ducklings. Cumulative effects, caused from the interaction of multiple PPCPs, have been shown to have stronger effects than chemicals acting alone. Cleuvers et al. (2003) performed toxicity tests on *Daphnia* using low concentrations of various combinations of PPCPs. Their study showed that the effects caused from the combination of PPCPs were stronger than the effects caused from individual PPCPs. Furthermore, cumulative effects were also observed in organisms at concentrations in which no or only slight effects were observed from single compounds (Verlicchi et al., 2012). Therefore, cumulative effects should be considered in a future risk assessment at the LTECF.

Furthermore, a risk assessment at the LTECF should consider the effects of chronic exposure. Current risk assessments of PPCPs are mainly based on acute toxicity data rather than chronic, although it is widely accepted that chronic exposure has higher risk (Verlicchi et al., 2012). Breeding and juvenile ducks may be particularly vulnerable to chronic effects, due to their prolonged exposure to PPCPs through the consumption of contaminated prey (i.e., aquatic invertebrates). Until fledging, the majority of the diet of a duckling hatched at the LTECF consists of contaminated prey. Aquatic invertebrates serve as a critical protein source for female ducks during egg production, and for early development of ducklings (Swanson, 1977; Mini et al., 2014). Therefore, if the diet used for early development of ducklings is contaminated, and the contaminants are known to bioaccumulate, there may be negative impacts on duckling growth and development. An aspect of the risk assessment should involve collecting tissue samples (i.e., blood/feather/egg shell) from selected species and age classes of ducks to quantify levels of accumulation.

Future research on PPCPs at the LTECF should also consider the occurrence of methyl-triclosan. Studies have recently identified methyl-triclosan, a methyl derivative of triclosan, to be present in WWTP effluent, surface water, and fish tissue (Government of Canada, 2016). Methyl-triclosan has similar properties to triclosan, such as high toxicity and bioaccumulation potential (Government of Canada, 2016). Methyl-triclosan is relatively stable and lipophilic and thus is able to bioaccumulate in biota (Brausch and Rand, 2011). In fact, some studies have found that methyl-triclosan was more bioaccumulative than triclosan. For example, Boehmer et al. (2004)

measured triclosan concentrations up to 3.4 ng/g ww in the muscle of fish sampled in numerous rivers in Germany. Corresponding concentrations of methy-triclosan in the same samples were up to about 90 times higher than the triclosan concentrations. The occurrence of methyl-triclosan was not analyzed for in this study because the laboratory did not have the capacity; however, it seems prudent to monitor for the occurrence of methyl-triclosan at the LTECF in any future studies.

Another recommendation for future research includes sampling other WWTP in Yukon to compare occurrence and removal efficiencies. For example, a small portion of the Whitehorse population utilizes a separate lagoon treatment system from the LTECF. A comparative study at this lagoon would contribute an additional data set to understanding the effectiveness of using constructed wetlands in northern communities for removal of PPCPs. A study such as this may help reduce knowledge gaps in aspects of PPCP removal mechanisms in CW, such as the influence of configuration design, hydraulic retention time, vegetation, and water chemistry parameters. Moreover, a comparative study at the WWTP in Dawson City, a city of 1,300 people located 530 km north of Whitehorse, may help reduce knowledge gaps regarding the effectiveness of constructed wetlands versus conventional systems in northern climates. Dawson City has recently installed an aerobic activated sludge system for treating their municipality's waste. A study on the occurrence and removal of PPCPs in Dawson City may provide evidence to guide future development of WWTP in the north.

Literature Cited

Aurelien, B.H., Sylvie, B., Alain, D., Jerome, G., Yves, P., 2013. Ecotoxicological risk assessment linked to the discharge by hospitals of bio-accumulative pharmaceuticals into aquatic media: The case of mitotane. Chemosphere 93, 2365-2372.

Boehmer, W., Ruedel, H., Wenzel, A., Scroeter-Kermani, C., 2004. Retrospective monitoring of triclosan and methyl-triclosan in fish: results from the German Environmental Specimen Bank. Organohalogen Compounds 66, 1516-1521.

Brausch, J.M., Rand, G.M., 2011. A review of personal care products in the aquatic environment: Environmental concentrations and toxicity. Chemosphere 82, 1518-1532.

Brun, G.L., Bernier, M., Losier, R., Doe, K., Jackman, P., Lee, H., 2006. Pharmaceutically active compounds in Atlantic Canadian sewage treatment plant effluents and receiving waters, and potential for environmental effects as measured by acute and chronic aquatic toxicity. Environmental Toxicology and Chemistry 25, 2163-2176.

Camacho-Muñoz, D., Martin, J., Santos, J.L., Aparicio, I., Alonso, E., 2012. Effectiveness of conventional and low-cost wastewater treatments in the removal of pharmaceutically active compounds. Water, Air & Soil Pollution 223, 2611-2621.

Canadian Environmental Protection Act, 1999. S.C. 1999, c. 33. Retrieved from http://laws-lois.justice.gc.ca/eng/acts/c-15.31/. Accessed on February 13, 2016.

Carranza-Diaz, O., Schultze-Nobre, L., Moeder, M., Nivala, J., Kuschk, P., Koeser, H., 2014. Removal of selected organic micropollutants in planted and unplanted pilot-scale horizontal flow constructed wetlands under conditions of high organic load. Ecological Engineering 71, 234-245.

Chen, Y., Yu, G., Cao, Q., Zhang, H., Lin, Q., Hong, Y., 2013. Occurrence and environmental implications of pharmaceuticals in Chinese municipal sewage sludge. Chemosphere 93, 1765-1772.

City of Whitehorse, 2014. Water Use License. MN93-001: 2014 Annual Report. City of Whitehorse, Department of Water and Waste Services, pp. 18.

Clean Production Action, 2014. Chemicals in consumer products are draining trouble into the Great Lakes ecosystem. Prepared for the Canadian Environmental Law Association. CELA Publication # - 997, pp. 16.

Conkle, J.L., White, J.R., Metcalfe, C.D., 2008. Reduction of pharmaceutically active compounds by a lagoon wetland wastewater treatment system in Southeast Louisiana. Chemosphere 73, 1741-1748.

Coogan, M.A., Edziyie, R.E., La Point, T.W., Venables, B.J., 2007. Algal bioaccumulation of triclocarban, triclosan and methyl-triclosan in a North Texas wastewater treatment plant receiving stream. Chemosphere 67, 1911-1918.

Coogan, M.A., La Point, T.W., 2008. Snail bioaccumulation of triclocarban, triclosan and methyl-triclosan in a North Texas, USA, stream affected by wastewater treatment plan runoff. Environmental Toxicology and Chemistry 27, 1788-1793.

Crisp, T.M., Clegg, E.D., Cooper, R.L., Wood, W.P., Anderson, D.G., Baetcke, K.P., et al., 1998. Environmental Endocrine Disruption: An effects assessment and analysis. Environmental Health Perspectives 106, 11-56.

Dods P.L., Birmingham, E.M., Williams, T.D., Ikonomou, M.G., Bennie, D.T., Elliott, J.E., 2005. Reproductive success and contaminants in tree swallows (*Tachycineta bicolor*) breeding at a wastewater treatment plant. Environmental Toxicology and Chemistry 24, 3106-3112.

Duebbert, H.F., Lokemoen, J.T., 1980. High duck nesting success in a predator-reduced environment. Journal of Wildlife Management 44, 428-437.

Gatermann, R., Biselli, S., Hühnerfuss, H., Rimkus, G., Hecker, M., Karbe, L., 2002. Synthetic musks in the environment. Part 1: Species-dependent in bioaccumulation of polycyclic and nitro musk fragrances in freshwater fish and mussels. Archives of Environmental Contamination and Toxicology 42, 437-446.

Gobas, F.A., 2001. Assessing bioaccumulation factors of persistent organic pollutants in aquatic food-chains, in: Harrad, S. (Eds.), Persistent Organic Pollutants. Springer US, New York, pp. 145-165.

Gollop, J.B., Marshall, W.H., 1954. A guide for aging duck broods in the field. Northern Prairie Wildlife Research Centre. U.S. Dept. of the Interior, Bureau of Sport Fisheries and Wildlife, Minneapolis, pp. 14.

Government of Canada., 2016. Assessment Report: Triclosan. Environment and Climate Change Canada, Health Canada. Retrieved from http://ec.gc.ca/ese-ees/65584A12-2B7D-4273-9F7A-38EDF916ECAF/EN%20FSAR%20Triclosan%20with%20ISBN.pdf. Accessed on December 1, 2016.

Hamilton, A.J., Robinson, W., Taylor, I.R., Wilson, B.P., 2005. The ecology of sewage treatment gradients in relation to their use by waterbirds. Hydrobiologia 534, 91-108.

Hijosa-Valsero, M., Matamoros, V., Martin-Villacorta, J., Bécares, E., Bayona, J.M., 2010. Assessment of full-scale natural systems for the removal of PPCPs from wastewater in small communities. Water Resources 44, 1429-1439.

Hirsch, R., Ternes, T., Haberer, K., Kratz, K.L., 1999. Occurrence of antibiotics in the aquatic environment. Science of the Total Environment 225, 109-118.

Horne, A.J., Goldman, C.R., 1994. Limnology, second edition. McGraw-Hill Co., New York.

Hu, Z., Shi, Y., Cai, Y., 2011. Concentrations, distribution, and bioaccumulation of synthetic musks in the Haihe River of China. Chemosphere 84, 1630-1635.

Hydromantis Inc., 2010. Emerging substances of concern in biosolids: Concentrations and effects of treatment processes. Submitted to Canadian Council of Ministers of the Environment, pp. 255. Retrieved from http://www.ccme.ca/files/Resources/waste/biosolids/pn_1440_contam_invt_rvw.pdf. Accessed on March 3, 2016.

Imfeld, G., Braeckevelt, M., Kuschk, P., Richnow, H.H., 2009. Monitoring and assessing processes of organic chemicals removal in constructed wetlands. Chemosphere 74, 349-362.

Jelic, A., Gros, M., Ginebreda, A., Cespedes-Sánchez, R., Ventura, F., Petrovic, M., et al., 2011. Occurrence, partition and removal of pharmaceuticals in sewage water and sludge during wastewater treatment. Water Resources 45, 1165–1176.

Jjemba, P.K., 2008. Pharma-ecology: The occurrence and fate of pharmaceuticals and personal care products in the environment. John Wiley & Sons Inc., Hoboken.

Kannan, K., Reiner, J.L., Yun, S.H., Perrotta, E.E., Tao, L., Johnson-Restrepo, B., et al., 2005. Polycyclic musk compounds in higher trophic level aquatic organisms and humans from the United States. Chemosphere 61, 693-700.

Kümmerer, K. (Editor)., 2008. Pharmaceuticals in the environment: Sources, fate, effects and risks, third edition. Springer-Verlag, Berlin, Heidelberg.

Li, X., Zheng, W., Kelly, W.R., 2013. Occurrence and removal of pharmaceutical and hormone contaminants in rural wastewater treatment lagoons. Science of the Total Environment 445-446, 22-28.

Li, Y., Zhu, G., Ng, W.J., Tan, S.K., 2014. A review on removing pharmaceutical contaminants from wastewater by constructed wetlands: Design, performance and mechanism. Science of the Total Environment 468-469, 908-932.

Lin, Y.C., Lin, C.F., Tsai, Y.T., Lin, H.H., Chen, J., Wang, X.H., et al., 2010. Fate of selected pharmaceuticals and personal care products after secondary wastewater treatment processes in Taiwan. Water Science Technology 62, 2450-2458.

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

Llorens, E., Matamoros, V., Domingo, V., Bayona, J.M., Garcia, J., 2009. Water quality improvement in a full-scale tertiary constructed wetland: effects on conventional and specific organic contaminants. Science of the Total Environment 407, 2517-2524.

MacLeod, S.L., Wong, C.S., 2010. Loadings, trends, comparisons, and fate of achiral and chiral pharmaceuticals in wastewaters from urban tertiary and rural aerated lagoon treatments. Water Resources 44, 533-544.

Markman, S., Leitner, S., Catchpole, C., Barnsley, S., Müller, C.T., Pascoe, D., et al., 2008. Pollutants increase song complexity and the volume of the brain area HVC in a songbird. PLoS ONE 3, 1-6.

Markman, S., Müller, C.T., Pascoe, D., Dawson, A., Buchanan, K.L., 2011. Pollutants affect development in nestling starlings *Sturnus vulgaris*. Journal of Applied Ecology 48, 391-397.

Matamoros, V., Garcia, J., Bayona, J.M., 2008. Organic micropollutant removal in a full-scale surface flow constructed wetland fed with secondary effluent. Water Resources 42, 653-660.

McClellan, K., Halden, R.U., 2010. Pharmaceuticals and personal care products in archived U.S. biosolids from the 2001 EPA national sewage sludge survey. Water Research 44, 658-668.

Melvin, S., Leusch, F., 2016. Removal of trace organic contaminants from domestic wastewater: A meta-analysis comparison of sewage treatment technologies. Environment International 92, 183-188.

Miege, C., Choubert, J.M., Ribeiro, L., Coquery, M.. 2009. Fate of pharmaceuticals and personal care products in wastewater treatment plants – Conception of a database and first results. Environmental Pollution 157, 1721-1726.

Mini, A.E., Harrington, E.R., Rucker, E., Dugger, B.D., Mowbray, T.B., 2014. American Wigeon. The Birds of North America (P. G. Rodewald, Ed.) Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America: https://birdsna.org/Species-Account/bna/species/amewig

Mossop, D.H., 1991. Initial survey of the waterbird populations on the Needlerock wetland, Yukon 1985-87 -- Stratified helicopter counts. Whitehorse: Department of Renewable Resources.

Osemwengie, L.I., Gerstenberger, S.L., 2004. Levels of synthetic musk compounds in municipal wastewater for potential estimation of biota exposure in receiving waters. Journal of Environmental Monitoring 6, 533-539.

Park, K.J., Muller, C.T., Markman, S., Swinscow-Hall, O., Pascoe, D., Buchanan, K.L., 2009. Detection of endocrine disrupting chemicals in aerial invertebrates at sewage treatment works. Chemosphere 77, 1459-1464.

Piest, L.A., Sowls, L.K., 1985. Breeding duck use of a sewage marsh in Arizona. Journal of Wildlife Management 49, 580-585.

Qiang, Z., Dong, H., Zhu, B., Qu, J., Nie, Y., 2013. A comparison of various rural wastewater treatment processes for the removal of endocrine-disrupting chemicals (EDCs). Chemosphere 92, 986-992.

Reyes-Contreras, C., Hijosa-Valsero, M., Sidrach-Cardona, R., Bayona, J.M., Becares, E., 2012. Temporal evolution in PPCP removal from urban wastewater by constructed wetlands of different configuration: a medium-term study. Chemosphere 88, 161-167.

Rivera-Utrilla, J., Sánchez-Polo, M., Ferro-García, M.Á., Prados-Joya, G., Ocampo-Pérez, R., 2013. Pharmaceuticals as emerging contaminants and their removal from water: A review. Chemosphere 93, 1268-1287.

Shi, W., Wang, L., Rousseau, D.P., Lens, P.N., 2010. Removal of estrone, 17 α-ethinylestradiol, and 17 β-estradiol in algae and duckweed-based wastewater treatment systems. Environmental Science Pollution Research 17, 824-833.

Sinclair, P.H., Nixon, W.A., Eckert, C.D., Hughes, N.L. (Eds.)., 2003. Birds of the Yukon Territory. University of British Columbia Press, Vancouver.

Smith, C.A., Meikle, J.C., Roots, C.F. (Eds.)., 2004. Ecoregions of the Yukon Territory: Biophysical properties of Yukon landscapes. Canada: Agriculture and Agri-Food Canada, Summerland.

Staicer, C.A., Freedman, B., Srivastava, D., Dowd, N., Jennifer, K., Hayden, J., et al., 1994. Use of lakes by black duck broods in relation to biological, chemical, and physical features. Hydrobiologia 279-280, 185-199.

Swanson, G.A., 1977. Diel food selection by Anatinae on a waste-stabilization system. Journal of Wildlife Management 41, 226-231.

United States Environmental Protection Agency (U.S. EPA)., 2000. Bioaccumulation testing and interpretation for the purpose of sediment quality assessment. EPA-823-R-00-001. Bioaccumulation Analysis Workgroup, Washington, pp. 136.

Verlicchi, P., Aukidy, M.A., Zambello, E., 2012. Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment – A review. Science of the Total Environment 429, 123-155.

Verlicchi, P., Zambello, E., 2014. How efficient are constructed wetlands in removing pharmaceuticals from untreated and treated urban wastewaters? A review. Science of the Total Environment 470-471, 1281-1306.

Vieno, N., Tuhkanen, T., Kronberg, L., 2005. Seasonal variation in the occurrence of pharmaceuticals in effluents from a sewage treatment plant and in the recipient water. Environmental Science Technology 39, 8220-8226.

Wang, J., Wang, S., 2016. Removal of pharmaceuticals and personal care products (PPCPs) from wastewater: A review. Journal of Environmental Management 182, 620-640.

Whitley, G., Thirumurthi, D., 1992. Field monitoring and performance evaluation of the Whitehorse sewage lagoon. Canadian Journal of Civil Engineering 19, 751-759.

Wilson, B.A., Smith, V.H., Denoyelles, J.F., Larive, C.K., 2003. Effects of three pharmaceutical and personal care products on natural freshwater algal assemblages. Environmental Science and Technology 37, 1713-1719.

Xu, X., Lu, Y., Zhang, D., Wang, Y., Zhou, X., Xu, H., Mei, Y., 2015. Toxic assessment of triclosan and triclocarban on *Artemia salina*. Bulletin of Environmental Contamination and Toxicology 95, 728-733.

Ying, G., Kookana, R.S., Kumar, A., 2008. Fate of estrogens and xenoestrogens in four sewage treatment plants with different technologies. Environmental Toxicology and Chemistry 27, 87-94.

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

Zhang, D.Q., Tan, S.K., Gersberg, R.M., Sadreddini, S., Zhu, J., Tuan, N.A., 2011. Removal of pharmaceutical compounds in tropical constructed wetlands. Ecological Engineering 37, 460–464.

Zhang, D., Gersberg, R., Ng, W., Tan, S., 2014. Removal of pharmaceuticals and personal care products in aquatic plant-based systems: A review. Environmental Pollution 184, 620-639.

Zimmerling, J.R., 2006. Why birders and birds flock to sewage lagoons. BirdWatch Canada. 6 pg. Retrieved from http://foreveryoungnews.com/system/ckeditor_assets/attachments/74/BWCsu06.pdf?1347552088. Accessed on October 10, 2010.

Appendix 1. Complete set of water sample results, including duplicate, collected in the primary, secondary and tertiary stages of treatment, in the spring, summer and fall of 2013 at the Livingstone Trail Environmental Control Facility, Yukon, Canada (n=6).

	Primary	Secondary				Tertiary
	Influent	Influent	Influent	Influent	Influent	Effluent
Units = ng/L	Spring	Summer	Summer - duplicate	Summer	Fall	Fall
Gemfibrozil	190	78	150	130	110	87
Ibuprofen	10,000	100	170	88	<11	180
Naproxen	5,900	230	410	140	<11	<11
Triclosan	610	110	120	17	18	22
Triclocarban	31	13	13	8.1	<11	<11
Estrone (E1)	3.0	2.0	2.4	<1.5	<1.3	<1.6
17 α-estradiol (E2)	4.2	1.8	4.6	16.0	14.0	8.0
17 α-ethinylestradiol (EE2)	<2.0	<2.0	<2.0	3.8	<2.0	4.0
Galaxolide	213	17	19	12	22	<2.4
Tonalide	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4
Traseolide	68	8.2	8.2	8.0	<2.4	3.4
Cashmeran	<2.4	<2.4	n.d	<2.4	3.7	4.1
Celestolide	<2.4	5.2	5.3	4.7	<2.4	3.5
Phantolide	26	<2.4	<2.4	<2.4	<2.4	<2.4
Musk Ketone	n.d	<2.4	<2.4	<2.4	<2.4	<2.4
Acetaminophen	150,000	<8.0	<8.0	<8.0	<8.0	34
Caffeine	100,000	48	120	51	6.5	360
Carbamazapine	360	210	380	290	350	200
Citalopram	1,600	880	1,200	290	360	98
Cotinine	30	108	83	140	7.1	10
Venlafaxine	810	260	450	130	240	130
Sulfamethoxazole	150	n/a	n/a	n/a	n/a	n/a

Sulfapyridine	170	n/a	n/a	n/a	n/a	n/a
Trimethoprim	87	n/a	n/a	n/a	n/a	n/a
Atenolol	850	n/a	n/a	n/a	n/a	n/a
Metaprolol	440	n/a	n/a	n/a	n/a	n/a
Propanolol	64	n/a	n/a	n/a	n/a	n/a
Sotalol	29	n/a	n/a	n/a	n/a	n/a
Perfluorooctane sulfonate (PFOS)	<5	n/a	n/a	n/a	n/a	n/a
Perfluorooctanoate (PFOA)	75	n/a	n/a	n/a	n/a	n/a
Bisphenol A	220	n/a	n/a	n/a	n/a	n/a
Octylphenol	19	n/a	n/a	n/a	n/a	n/a
Nonylphenol	n.d	n/a	n/a	n/a	n/a	n/a

n.d: not detected; n.a: not available

Appendix 2. Complete set of water sample results, including field blank, collected in the influent, middle and effluent locations in the primary, secondary and tertiary stages of treatment, in the spring of 2014 at the Livingstone Trail Environmental Control Facility, Yukon, Canada (n=10).

	Primary			Secondary			Tertiary			Field
Units = ng/L	Influent	Middle	Effluent	Influent	Middle	Effluent	Influent	Middle	Effluent	Blank
Gemfibrozil	180	99	110	70	34	21	110	85	110	<1
Ibuprofen	320	340	245	480	346	310	200	270	250	<1
Naproxen	44	87	72	n.d	n.d	n.d	18	27	n.d	<4
Triclosan	10	11	<1	<1	<1	<1	<1	<1	<1	<1
Triclocarban	11	45	16	<1	<1	<1	<1	<1	<1	<1
Estrone (E1)	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3
17 α-estradiol (E2)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
17 α-ethinylestradiol (EE2)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Galaxolide	310	370	360	300	160	180	53	51	45	n.d
Tonalide	18	17	16	7.6	5.6	7.6	4.8	3.0	7.7	<2.4
Traseolide	18	15	20	16	12	9.1	n.d	n.d	n.d	n.d
Cashmeran	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4
Celestolide	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Phantolide	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Musk Ketone	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d

n.d: not detected; n/a: not available

Appendix 3. Complete set of water sample results, including field blank, collected in the influent, middle and effluent locations in the primary, secondary and tertiary stages of treatment, in the summer of 2014 at the Livingstone Trail Environmental Control Facility, Yukon, Canada (n=10).

	Primary			Secondary			Tertiary			Field
Units = ng/L	Influent	Middle	Effluent	Influent	Middle	Effluent	Influent	Middle	Effluent	Blank
Gemfibrozil	260	280	270	200	160	180	110	110	99	<1
Ibuprofen	11,300	13,800	12,700	83	110	100	320	330	270	<1
Naproxen	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Triclosan	1,260	1,910	1,990	93	92	100	332	231	273	<1
Triclocarban	69	130	140	<1	<1	<1	38	23	23	<1
Estrone (E1)	7.6	7.3	7.2	4.6	9.9	4.6	5.8	6.2	5.9	4.6
17 α-estradiol (E2)	<3	<3	<3	<3	12	<3	<3	68	<3	<3
17 α-ethinylestradiol (EE2)	9.1	15	11	13	4.3	5.6	11	<3	5.0	<3
Galaxolide	400	260	350	38	41	33	<2.4	<2.4	<2.4	<2.4
Tonalide	32	37	28	9.7	12	11	<2.4	<2.4	<2.4	< 2.4
Traseolide	3.2	6.8	3.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4
Cashmeran	110	90	75	7.2	6.0	8.8	3.8	<2.4	<2.4	< 2.4
Celestolide	9.4	3.5	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4
Phantolide	10	4.1	3.5	7.6	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4
Musk Ketone	<2.4	<2.4	<2.4	n.d	n.d	n.d	n.d	n.d	n.d	n.d

n.d: not detected

Appendix 4. Complete set of water sample results, including field blank, collected in the influent, middle and effluent locations in the primary, secondary and tertiary stages of treatment, in the fall of 2014 at the Livingstone Trail Environmental Control Facility, Yukon, Canada (n=10).

	Primary			Secondary			Tertiary			
Units = ng/L	Influent	Middle	Effluent	Influent	Middle	Effluent	Influent	Middle	Effluent	Field Blank
Gemfibrozil	140	155	200	250	240	290	120	120	110	<1
Ibuprofen	14,900	15,700	14,300	620	720	780	180	210	170	<1
Naproxen	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Triclosan	1,400	1,400	970	260	260	300	82	86	56	<1
Triclocarban	95	110	55	<1	<1	<1	<1	<1	<1	<1
Estrone (E1)	<3	<3	<3	15	14	15	6.0	7.8	4.9	<3
17 α-estradiol (E2)	<3	<3	<3	84	92	90	13	<3	<3	<3
17 α-ethinylestradiol (EE2)	3.5	<3	<3	28	13	15	13	9	<3	<3
Galaxolide	400	300	300	13	14	14	<2.4	<2.4	<2.4	<2.4
Tonalide	5.4	12	22	17	16	13	10	3.1	<2.4	<2.4
Traseolide	5.1	4.3	5.2	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4
Cashmeran	110	86	85	3.8	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4
Celestolide	35	42	51	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4
Phantolide	9.3	3.9	4.5	2.7	3.3	3.4	<2.4	<2.4	<2.4	n.d
Musk Ketone	<2.4	<2.4	<2.4	n.d	n.d	n.d	n.d	n.d	n.d	<2.4

n.d: not detected

Appendix 5. Complete set of sludge sample results collected in the primary, secondary and tertiary stages of treatment, in the spring, summer and fall of 2013 at the Livingstone Trail Environmental Control Facility, Yukon, Canada (n=4).

	Primary		Secondary		Tertiary
	Influent	Influent	Influent	Influent	Effluent
Units = ng/g	Spring	Summer	Summer	Fall	Fall
Gemfibrozil	123	<4.0	<4.0	<4.0	<4.0
Ibuprofen	720	7.8	4.6	6.4	<4.2
Naproxen	82	<4.2	<4.2	27	170
Triclosan	93,000	9,000	3,400	200	100
Triclocarban	31,000	1,200	660	110	36
Estrone (E1)	<1.0	1.6	1.9	1.9	2.6
17 α-estradiol (E2)	4.8	1.8	<1.0	<1.0	<1.0
17 α-ethinylestradiol (EE2)	18.0	7.6	4.9	<2.0	2.9
Galaxolide	1,450	7.1	8.1	4.4	<4.0
Tonalide	<4.0	<4.0	<4.0	<4.0	<4.0
Traseolide	89	<4.0	<4.0	<4.0	n.d
Cashmeran	15	<4.0	<4.0	<4.0	<4.0
Celestolide	29	<4.0	<4.0	<4.0	<4.0
Phantolide	2,050	<4.0	<4.0	<4.0	<4.0
Musk Ketone	n.d	n.d	n.d	n.d	n.d
Acetaminophen	127	<4.2	<4.2	<4.2	<4.2
Caffeine	830	5.4	<4.2	<4.1	4.3
Carbamazapine	77	80	86	<4.4	<4.4
Citalopram	6,200	250	2,100	<4.4	<4.4
Cotinine	40	5.4	<4.1	<4.1	<4.1
Venlafaxine	270	41	35	6.2	9.9
Sulfamethoxazole	n.d	n/a	n/a	<3.2	<3.2
Sulfapyridine	4	n/a	n/a	<3.2	<3.2

Trimethoprim	8	n/a	n/a	<3.2	<3.2
Atenolol	45	n/a	n/a	<4.0	<4.0
Metaprolol	88	n/a	n/a	4.2	6.1
Propanolol	220	n/a	n/a	76	75
Sotalol	12	n/a	n/a	6.0	6.0
Perfluorooctane sulfonate (PFOS)	8	n/a	n/a	n/a	n/a
Perfluorooctanoate (PFOA)	<4	n/a	n/a	n/a	n/a
Bisphenol A	110	n/a	n/a	6.8	9.1
Octylphenol	n.d	n/a	n/a	<4.1	<4.1
Nonylphenol	n.d	n/a	n/a	9.1	51

n.d: not detected; n/a: not available

Appendix 6. Complete set of sludge sample results collected in the influent, middle and effluent of secondary cell 2, in the summer of 2014 at the Livingstone Trail Environmental Control Facility, Yukon, Canada (n=3).

		Secondary	
Units = ng/g	Influent	Middle	Effluent
Gemfibrozil	1.8	9.6	8.5
Ibuprofen	8.2	10	37
Naproxen	<1	<1	<1
Triclosan	380	900	1,900
Triclocarban	712	1,100	880
Estrone (E1)	3.6	4.3	3.9
17 α-estradiol (E2)	<2	<2	<2
17 α-ethinylestradiol (EE2)	<2	2.3	3.3
Galaxolide	140	150	86
Tonalide	11	10	13
Traseolide	<4.0	4.1	<4.0
Cashmeran	n.d	n.d	n.d
Celestolide	n.d	n.d	n.d
Phantolide	n.d	n.d	n.d
Musk Ketone	n.d	n.d	n.d

n.d: not detected

Appendix 7. Complete set of aquatic invertebrate sample results collected in secondary cell 2, in the summer of 2013 at the Livingstone Trail Environmental Control Facility, Yukon, Canada (n=10).

Units = ng/g	*Daphnia*							Chironomid		Tipulidae
Gemfibrozil	<3.7	<3.7	<3.7	n.d	n.d	<3.7	<3.7	<3.7	<3.7	<3.7
Ibuprofen	<3.9	<3.9	<3.9	<3.9	<3.9	<3.9	<3.9	<3.9	<3.9	<3.9
Naproxen	n.d	<3.9	<3.9	<3.9	n.d	n.d	<3.9	n.d	n.d	<3.9
Triclosan	31	12	33	4.0	6.2	36	10	14	<3.7	5.4
Triclocarban	23	7.5	25	<4.1	10	9.6	18	29	<4.1	8.5
Estrone (E1)	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0
17 α-estradiol (E2)	<4.4	<4.4	<4.4	<4.4	<4.4	<4.4	<4.4	<4.4	<4.4	<4.4
17 α-ethinylestradiol (EE2)	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0
Galaxolide	n/a	6.5	5.2	<4.0	<4.0	<4.0	n/a	n/a	n/a	n/a
Tonalide	n/a	<4.0	<4.0	<4.0	<4.0	<4.0	n/a	n/a	n/a	n/a
Traseolide	n/a	<4.0	<4.0	<4.0	<4.0	<4.0	n/a	n/a	n/a	n/a
Cashmeran	n/a	<4.0	<4.0	<4.0	<4.0	<4.0	n/a	n/a	n/a	n/a
Celestolide	n/a	n.d	n.d	n.d	n.d	n.d	n/a	n/a	n/a	n/a
Phantolide	n/a	<4.0	<4.0	<4.0	<4.0	<4.0	n/a	n/a	n/a	n/a
Musk Ketone	n/a	5.8	<4.0	<4.0	<4.0	8.13	n/a	n/a	n/a	n/a
Acetaminophen	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0
Caffeine	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0
Carbamazapine	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0
Citalopram	<4.2	<4.2	<4.2	<4.2	<4.2	<4.2	<4.2	<4.2	<4.2	<4.2
Cotinine	<3.8	<3.8	<3.8	<3.8	<3.8	<3.8	<3.8	<3.8	<3.8	<3.8
Venlafaxine	<3.9	n.d	<3.9	<3.9	<3.9	<3.9	<3.9	<3.9	<3.9	<3.9

n.d: not detected; n/a: not available

Appendix 8. Complete set of aquatic invertebrate sample results collected in secondary cell 2, in the summer of 2014 at the Livingstone Trail Environmental Control Facility, Yukon, Canada (n=3).

		Secondary	
Units = ng/g	Influent	Middle	Effluent
Gemfibrozil	7.3	<2	2.7
Ibuprofen	7.8	3.1	<2
Naproxen	n.d	n.d	n.d
Triclosan	19	5	6.1
Triclocarban	8	3	3.8
Estrone (E1)	<2	<2	<2
17 α-estradiol (E2)	<2	<2	<2
17 α-ethinylestradiol (EE2)	4.4	<2	2.4
Galaxolide	<4.0	<4.0	<4.0
Tonalide	<4.0	<4.0	<4.0
Traseolide	<4.0	<4.0	<4.0
Cashmeran	n.d	n.d	n.d
Celestolide	n.d	n.d	n.d
Phantolide	<4.0	<4.0	<4.0
Musk Ketone	<4.0	5.5	4.3

n.d = not detected

Appendix 9. Complete set of algae sample results collected in the long-term storage pond, in the fall of 2014 at the Livingstone Trail Environmental Control Facility, Yukon, Canada (n=3).

		Tertiary	
Units = ng/g	Influent	Middle	Effluent
Gemfibrozil	9.7	34	46
Ibuprofen	19	46	15
Naproxen	n.d	n.d	n.d
Triclosan	61	210	140
Triclocarban	4.7	19	47
Estrone (E1)	<2	<2	<2
17 α-estradiol (E2)	3.3	<2	4.2
17 α-ethinylestradiol (EE2)	<2	<2	<2
Galaxolide	<4.0	<4.0	<4.0
Tonalide	<4.0	<4.0	<4.0
Traseolide	<4.0	<4.0	<4.0
Cashmeran	n.d	n.d	n.d
Celestolide	n.d	n.d	n.d
Phantolide	11	<4.0	<4.0
Musk Ketone	n.d	n.d	n.d

n.d = not detected