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Modeling the Loading and Fate of Estrogen

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Modeling the Loading and Fate of Estrogen

by

Michael Eugene Fleming

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
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Abstract

Endocrine disrupting compounds may produce infertility, nervous system disorders, and improper functioning of the immune system in humans and wildlife. Estrogens are classified as the most potent and common endocrine disrupting compounds, and the major point source for estrogen is municipal wastewater. Monitoring of estrogen is challenging, expensive, and intermittent; and therefore, the focus of this work is modeling estrone, 17β-estradiol, and 17α-ethynylestradiol concentrations from wastewater treatment plants in Calgary and Edmonton, Alberta, and Brandon, Manitoba.

Demographic groups, excretion rates, population estimates, average daily flows, calculated estrogen transformation, calibration, calculated influent-to-effluent reduction percentages, and a treatment unit removal matrix are used to determine loading estimations of estrogen. The results demonstrate reasonable accuracy against previous measurements, and findings are consistent with concentrations reported in the literature. Upon further calibration with additional local data, the model may be used as a risk assessment analysis tool for these contaminants of concern.
Acknowledgements

First, I would like to thank my supervisors, Dr. Gopal Achari and Dr. Quazi K. Hassan, for the opportunity, guidance, infinite patience, flexibility, and understanding. Second, I would also like to thank the City of Calgary for providing the required data in order to make this thesis possible; and Lastly, I would also like to thank my wife Leanne and my daughter Isabelle for their sacrifices in order to allow me the opportunity to achieve this accomplishment.
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<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRBC</td>
<td>Bow River Basin Council</td>
<td></td>
</tr>
<tr>
<td>BNR</td>
<td>Biological Nutrient Removal</td>
<td></td>
</tr>
<tr>
<td>$D_{EE2}$</td>
<td>Average daily dose of 17α-ethynylestradiol</td>
<td>(µg /d)</td>
</tr>
<tr>
<td>E1</td>
<td>Estrone</td>
<td>(µg /l or ng/l)</td>
</tr>
<tr>
<td>E2</td>
<td>17β-estradiol</td>
<td>(µg /l or ng/l)</td>
</tr>
<tr>
<td>E3</td>
<td>Estriol</td>
<td>(µg /l or ng/l)</td>
</tr>
<tr>
<td>EE2</td>
<td>17α-ethynylestradiol</td>
<td>(µg /l or ng/l)</td>
</tr>
<tr>
<td>$E_c$</td>
<td>Internal generation of estrogen from other estrogens</td>
<td></td>
</tr>
<tr>
<td>EDC</td>
<td>Endocrine disrupting compounds</td>
<td>(µg /d)</td>
</tr>
<tr>
<td>EPEA</td>
<td>Environmental Protection and Enhancement Act</td>
<td></td>
</tr>
<tr>
<td>$F_{E1}$</td>
<td>The amount of estrogen excreted in the Feces per capita for each defined demographic group</td>
<td>(µg /d)</td>
</tr>
<tr>
<td>HRT</td>
<td>Hormone Replacement Therapy</td>
<td></td>
</tr>
<tr>
<td>ML/day</td>
<td>Million Liters per day</td>
<td>(l)</td>
</tr>
<tr>
<td>m$^3$</td>
<td>Cubic Metres</td>
<td>(m$^3$)</td>
</tr>
<tr>
<td>OC</td>
<td>Oral contraceptives</td>
<td></td>
</tr>
<tr>
<td>Q</td>
<td>WWTP influent Flow Rate</td>
<td>l/d</td>
</tr>
<tr>
<td>$R_T$</td>
<td>The overall fraction of steroid lost in transit through the sewer network.</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
<td></td>
</tr>
<tr>
<td>$T_E$</td>
<td>The total concentration of estrogen in all forms.</td>
<td>(µg/l)</td>
</tr>
<tr>
<td>$T_{E1}$</td>
<td>The total concentration of E1</td>
<td>(µg/l)</td>
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<td>Acronym</td>
<td>Description</td>
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<tr>
<td>T&lt;sub&gt;E2&lt;/sub&gt;</td>
<td>The total concentration of E2 (µg/l)</td>
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<tr>
<td>T&lt;sub&gt;EE2&lt;/sub&gt;</td>
<td>The total concentration of EE2 (µg/l)</td>
<td></td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
<td></td>
</tr>
<tr>
<td>USDHHS</td>
<td>US Department of Health and Human Services</td>
<td></td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
<td></td>
</tr>
<tr>
<td>WP</td>
<td>WorleyParsons</td>
<td></td>
</tr>
<tr>
<td>WWTP</td>
<td>Wastewater Treatment Plant</td>
<td></td>
</tr>
</tbody>
</table>
Chapter 1 - Introduction

1.1 Background

Globally, there are growing health concerns regarding contaminants entering our water systems that provide us safe drinking water, irrigation water, food, and recreational opportunities (Wise et al., 2010). Estrogen, a major class of unregulated pollutants (Umali et al., 2012), is of considerable interest because trace amounts may lead to infertility, developmental disorders, disorders of the nervous system, and improper functioning of the immune system in humans and wildlife (USDHHS, 2010). In fact, recent examples exist of intersex fish in Alberta waterways (Evans et al., 2012). In large cities, such as Calgary, wastewater treatment plants (WWTPs) can be considered a major point source for contaminants entering our waterways such as the Bow River (Drewes et al., 2003; Belfroid et al., 1999b; Ternes et al., 1999a). Provincial and federal regulators do not require monitoring and managing of estrogen associated with WWTPs. As such, limited data exist regarding estrogens in wastewater influent and effluent. Furthermore, cities in Canada are expanding and growing exponentially. The city of Calgary, for example, is expected to double to over 2.4 million residents by 2041 (Wright, 2014). The growing population will add increasing pressure on water quality and quantity in the region, including the Bow River (Chu et al., 2009).

Measuring estrogen compounds in wastewater is challenging, high priced, and intermittent due to its extremely low concentrations (Johnson and Williams, 2004; Umali et al., 2012). For example, solid phase extraction, liquid-liquid extraction, or freeze-drying are usually used with solvents such as methanol, combinations of acetone/methanol, or acetonitrile; samples are dried, solvent is added again, and enzymes are commonly added to transform conjugated-to-unconjugated compounds. Concentrated samples commonly undergo further analysis by
chromatography together with mass spectrometry (Umali et al., 2012). Some studies indicate, however, that analytical methods prevented the recognition of conjugated compounds in wastewater influent, suggesting the absence of the enzyme conversion step (Lee et al., 2005a; Nelson et al., 2007; Fernandez et al., 2007b).

It has been previously acknowledged that monitoring estrogen concentrations in treated effluent is a requirement to complete accurate risk assessments (Jones et al., 2001). In Alberta, for example, the city of Calgary Environmental Protection and Enhancement Act (EPEA) approval (#95-MUN-317) permits the city of Calgary to continue releasing treated effluent into the Bow River with the requirement for a total loading management plan. The total loading management plan includes TSS, phosphorus, nitrate, ammonia, dissolved oxygen, temperature, and density of aquatic vegetation (Golder, 2004b). However, estrogens, a major category of unregulated pollutants (Umali et al., 2012), are subsequently not part of today's city of Calgary total loading management plan.

Modelling estrogen is required in order to 1) allow for accurate estimation of estrogen while avoiding high costs and time associated with laboratory analysis; 2) better formulate a citywide risk estimate for these contaminants of concern; 3) better predict which WWTP poses the greatest endocrine disruption due to steroid estrogens; and 4) developing management strategies that focus on the contaminant(s) of most concern.
1.2 Objectives

Given that humans excrete estrogen on a daily basis (Johnson and Williams, 2004), and the major point source for estrogen are WWTPs (Drewes et al., 2003; Belfroid et al., 1999b; Ternes et al., 1999a), it should be practical to estimate the concentrations of estrogen that enter and leave WWTPs. Previous work by Johnson and Williams (2004) and others (Atkinson et al., 2012; Umali et al., 2012) suggest that estrogen concentrations can be estimated using demographic group excretion rates, census statistics, flow rates, and WWTP operational parameters. As such, the objective of this research is to model the wastewater concentrations of estrone (E1), 17β-estradiol (E2), and 17α-ethynylestradiol (EE2) in order to predict the range of concentrations that can be expected to occur at the Fish Creek and Bonnybrook WWTPs located in Calgary, Alberta; the Goldbar WWTP located in Edmonton, Alberta; and the WWTP located in Brandon, Manitoba. The model will also include a treatment unit removal matrix to further quantify the amount of estrogen removed during each treatment unit process. Concentrations will be predicted for WWTP influent and effluent. Note: for the purpose of this study, Pine Creek WWTP will not be included in the model due to a lack of available data.

1.3 Chapter Overview

The following five chapters arrange this paper. The introductory chapter, Chapter 1, provides an overview of the separate chapters and integrates the general themes of the research and paper included. The literature review chapter, Chapter 2, provides an overview of estrogenic effects, measured concentrations of estrogen in Canada, sources of estrogens, characteristics and properties of estrogens, mechanisms for estrogen removal, fate of estrogen in treatment plants, and operating variables on estrogen removal. The study area and data description chapter, Chapter 3, provides an overview of the study area including the sampling
site locations, WWTP description and comparison, the type of data, and the procedures used to collect the data. The modeling chapter, Chapter 4, provides an overview of the methodology used to revise the demographic profile parameter ratios, calculation of the sewer biological transformation rate using local data, calculation of the removal matrix, influent-to-effluent percentage removal, model equations, loading parameters, assumptions, and model simulation. The results and discussion chapter, Chapter 5, provides an analysis of the model simulation for the Calgary, Edmonton, and Brandon facilities, including model calibration, estrogen transformation, estimated influent and effluent estrogen results in graphs and tables, estimated removal matrix results, estrogen composition, sensitivity analysis, and a discussion section. Finally, the Summary, Contribution to Science and Technology, and Further Recommendations chapter, Chapter 6, provides a summary of conclusions, contributions, and recommendations.
Chapter 2 - Literature Review

2.1: Introduction

2.1.1 Effects of Estrogen

Prior to approximately 15 years ago, water quality studies concentrated on conventional pollutants such as pesticides. The discovery of estrogen in wastewater (Purdom et al., 1994) directed the focus towards physiologic effects of these compounds in water (Daughton and Ternes, 1999), and a new category of contaminants, classified as endocrine disrupting compounds (EDCs), began to draw scientific interest. The growing interest is due to the possible health risks associated with EDCs (Diamanti-Kandarakis et al., 2009)—EDCs may interfere with the body’s endocrine system leading to infertility, developmental disorders, disorders of the nervous system, and improper functioning of the immune system in humans and wildlife (USDHHS, 2010). A broad variety of compounds, both anthropogenic and non-anthropogenic, are believed to induce endocrine disruption, such as estrogens, drugs or medications, dioxin, bisphenol A, and polychlorinated biphenyls (USDHHS, 2010). However, researchers suggest that estrogens are among the most potent and common EDCs of immediate concern in sewage effluent (Desbrow et al., 1998; Matsui et al., 2000; Korner et al., 2000).

Studies have shown that wastewater containing estrogen can raise estrogen levels in receiving water and negatively affect aquatic organisms. For example, in the U.K. Purdom et al. (1994) found that male trout (Oncorhynchus mykiss) subjected to estrogenic wastewater effluent contained an egg yolk precursor protein, i.e. vitellogenin, normally present in higher concentrations in females. Additional studies in the U.K. also discovered oocytes in the testes of wild roach (Rutilus rutilus), and believe the species was subjected to wastewater effluent containing estrogen (Johnson and Williams, 2004). In Alberta, Evans et al. (2012) reported that fish (Longnose dace—Rhinichthys cataractae) exposed to estrogen within the Oldman River
displayed distorted gene regulation and high female-to-male ratios. In another Alberta study, Jeffries et al. (2010) detected vitellogenin in adult male longnose dace exposed to estrogen and other EDCs in the South Saskatchewan River Basin. In Ontario, Tetreault et al. (2011) discovered that populations of fish (male darters—*Etheostoma blennioides* and *E. caeruleum*) exposed to wastewater effluent within the Grand River Watershed experienced up to 60% rates of intersex—suggesting potential health impacts to fish populations due to EDCs such as estrogen. In one long-term study over three years, estrogens containing a concentration of approximately 5 ng/L killed the entire fish population by male feminization in a Canadian lake. Estrogen concentrations leading to vitellogenin in fish have been observed as low as 0.5 ng/L of EE2 (Purdom et al., 1994; Hansen et al., 1998), 1 ng/L of E2 (Routledge et al., 1998) and 25 ng/L of E1 (Routledge et al., 1998) [Table 2-1].

<table>
<thead>
<tr>
<th>Estrogen Steroid</th>
<th>Lowest observable effects level</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrone</td>
<td>25-50 ng/L&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;sup&gt;a&lt;/sup&gt;Routledge et al. (1998)</td>
</tr>
<tr>
<td>17β-oestradiol</td>
<td>1-10 ng/L&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;sup&gt;b&lt;/sup&gt;Purdom et al. (1994); Hansen et al., 1998; &lt;sup&gt;c&lt;/sup&gt;Jobling et al. (2003)</td>
</tr>
<tr>
<td>17α-ethynylestradiol</td>
<td>0.5&lt;sup&gt;b&lt;/sup&gt;-1&lt;sup&gt;c&lt;/sup&gt; ng/L</td>
<td></td>
</tr>
</tbody>
</table>

Although estrogen has been shown to have adverse effects on fish, there is also suspicion of estrogenic effects on plant physiology. For example, Shore et al. (1992) reported that E1 and E2 concentrations of 5-500 ng/l within irrigation water yielded higher plant growth; in contrast, concentrations from 50-500 µg/l inhibited plant growth (Shore et al., 1992). In addition, estrogenic wastewater used for irrigation has been shown to raise the levels of phytoestrogens
within plants such as alfalfa (Shore et al., 1995). There is also suspicion that estrogen from birth control pills (i.e. EE2) is associated with the global rise of prostate cancer for men (Margel, 2011). For example, in one study that analyzed different forms of birth control used in 87 countries, researchers found a high correlation between the use of birth control pills containing EE2 and the percentage of prostate cancer and death resulting from prostate cancer (Margel, 2011). Studies have also shown that mammals, reptiles, and birds exposed to estrogen can have negative reproductive consequences. Even worms exposed to estrogen are known to accumulate estrogen leading to transfer and bioaccumulation in other species.

2.2.2 Measured Influent and Effluent Estrogen Concentrations

To date, limited and sporadic WWTP influent and effluent sampling has been completed in Canada. In a survey of 18 municipal WWTPs across Canada, Servos et al. (2005) reported raw wastewater influent concentrations for estrogen in the range of 0.019-0.078 µg/l for E1 and 0.0024-0.026 µg/l for E2; effluent values ranged from 0.001-0.096 µg/l for E1 and 0.0002-0.0147 µg/l for E2. In another survey of 12 municipal WWTPs along the Thames River in Ontario, Lishman et al. (2006) reported influent values in the range of 0.016 to 0.049 µg/L for E1 and 0.006 to 0.0139 µg/l for E2, and effluent values of <0.005-0.038 µg/l for E1 and <0.005 µg/l for E2. In the Toronto area, Lee et al. (2005b) reported raw wastewater influent concentrations for estrogen in the range of 0.008-0.052 µg/l for E1 and 0.003-0.022 µg/l for E2; effluent values ranged from <0.001- 0.054 µg/l for E1 and <0.001-0.002 µg/l for E2. In western Canada, Fernandez et al. (2007a) reported EE2 Influent concentrations from 4 municipal WWTPs ranging from <0.001-0.002 µg/l, and effluent concentrations ranging from <0.001 - 0.005 µg/l. Maximum reported values measured in Canada for wastewater effluent was 0.370 µg/l for E1 (Atkinson et al., 2012), 0.064 µg/l for E2, and 0.042 µg/l for EE2 (Ternes et al., 1999a). Common average
removal percentages for estrogens in Canadian tertiary WWTP effluent range from 66.7-99.9% for E1 (Lishman et al., 2006; Servos et al., 2005), 69.4-99.9% for E2 (Lishman et al., 2006; Servos et al., 2005), and 70.2-87.5% for EE2 (Cicek et al., 2007).

2.2.3 Sources of Estrogens

Agriculture, landfills, industry, and humans represent the major sources for steroid estrogens that enter our water systems (Wise et al., 2010). Municipal WWTPs are recognized as a principle point source of estrogen in heavily populated areas (Drewes et al., 2003; Belfroid et al., 1999b; Ternes et al., 1999a), and although WWTPs remove a considerable amount of EDCs through numerous operational processes, the compounds can still persist in wastewater effluent. As such, EDCs continue to be released into aquatic environments (Sosiak and Hebben, 2005). Researchers suggest that the primary contributors to estrogenic activity in municipal wastewater are the steroidal estrogens E1, E2, and EE2 (Johnson and Williams, 2004).

2.2.4 Human Endogenous Estrogen

Humans of either sex produce and excrete three endogenous steroid estrogens, i.e. E1, E2, and estriol (E3) [Wise et al. 2010]. Endogenous estrogens originate primarily within the ovaries and testis of the human body, and exit the body according via urine and feces. Enzymes within the body are responsible for transforming the estrogens to a point where they become hydrophilic due to the addition of esters to the hydroxyl groups such as sulphates or glucuronides. E1 has two conjugates while E2 has eight conjugates due to two hydroxyl groups. The commonly reported conjugates of E1 are estrone-3-glucuronide and estrone-3-sulphate; the commonly reported conjugates of E2 are estradiol-17-glucuronide, 17β-estradiol-3-glucuronide, and 17β-estradiol-3-sulphate. When estrogens are conjugated, they usually do not display estrogenic effects that are evident when the additional group lies on the C3 position. Urine
mainly contains glucuronide verses sulphate, and the majority of estrogen leaving the body is found in the urine while the remaining concentrations are found in the feces. However, estrogen in the feces is unconjugated due to the presence of bacteria within the feces (De Mes, 2007).

2.2.5 Human Exogenous Estrogen

The human body can also contain and excrete exogenous estrogen in the form of EE2. Oral contraceptives, such as the birth control pill, contain synthetic estrogen EE2. These pills are commonly prescribed and used around the world (Williams and Stancel, 1996). The amount of EE2 prescribed per pill is approximately 35 µg (Johnson and Williams, 2004). Similar to other hormones, enzymes within the body are responsible for transforming EE2 to a point where they become hydrophilic. The transformation is due to the addition of esters to the hydroxyl groups such as sulphates or glucuronides. Similar to E2, eight conjugates exist for EE2 due to two hydroxyl groups (De Mes, 2007). However, EE2 is also highly persistent due to the ethinyl group that prevents oxidation to E1; and therefore, degrades slowly over an extended period of time. Approximately 20-48% of the prescribed EE2 is absorbed and approximately 52-80% of the prescribed EE2 is excreted from the body (Wise et al., 2010). EE2 excreted from the body is mostly in the conjugated form; however, it is common for EE2 glucuronides to unconjugate to the free form of EE2 in WWTPs. As such, it is assumed in this study that EE2 glucuronides unconjugate to the original form in WWTPs.

2.2.6 Characteristics of Estrogen

The presence of estrogen in WWTPs and the environment can be related to its specific properties, attributes, and molecular formulas (Table 2-2). The structures of estrogen differ by the quantity of attachments and the positioning in relation to the carbon ring chains that shape the estrogen core structure (Figure 2.0). 17β-estradiol is commonly referred as E2 because its
structure is made up of two hydroxyl groups; Estrone, a metabolite of E2, is commonly referred as E1 due to one hydroxyl group (De Mes, 2007).

All estrogens are generally stable due to their low Henry’s constant and vapor pressure, (Lai et al., 2000); however, since the values for EE2 are the lowest, it can be classified as the most stable of all estrogens. Estrogens are also classified as water-soluble; however, endogenous estrogens with a water solubility rating of 13 mg/l is more water-soluble than exogenous estrogen of 4.8 mg/l. In addition, the K_{ow} values of EE2 are the highest in comparison to other estrogens, and suggest that EE2 is hydrophobic and very likely to attach to sludge and sediment (Lai et al., 2000).

<table>
<thead>
<tr>
<th>Property</th>
<th>Estrone (E1)</th>
<th>17(\beta)-estradiol (E2)</th>
<th>17(\alpha)-ethynylestradiol (EE2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula</td>
<td>C_{18}H_{22}O_{2}</td>
<td>C_{18}H_{24}O_{2}</td>
<td>C_{20}H_{24}O_{2}</td>
</tr>
<tr>
<td>Molecular Weight</td>
<td>270.37</td>
<td>272.39</td>
<td>296.4</td>
</tr>
<tr>
<td>Log K_{ow}</td>
<td>3.4</td>
<td>3.9</td>
<td>4.2</td>
</tr>
<tr>
<td>Vapour Pressure (Pa)(^{b})</td>
<td>(3.0 \times 10^{-8})</td>
<td>(3.0 \times 10^{-8})</td>
<td>(6.0 \times 10^{-9})</td>
</tr>
<tr>
<td>Water Solubility at 20(^\circ)C (mg/l)(^{c})</td>
<td>13</td>
<td>13</td>
<td>4.8</td>
</tr>
<tr>
<td>Henry’s Constant at 25(^\circ)C (atm-m(^3) mol(^{-1}))(^{c})</td>
<td>(3.8 \times 10^{-10})</td>
<td>(3.64 \times 10^{-11})</td>
<td>(7.94 \times 10^{-12})</td>
</tr>
<tr>
<td>Half-life (days)(^{d})</td>
<td>(0.8^{e}; 2-3^{f})</td>
<td>(1.5^{e}; 2-3^{f}; 0.2-9^{g})</td>
<td>(0.9-2^{e}; 4-6^{f})</td>
</tr>
</tbody>
</table>

\(^{a}\)Lai et al. (2000); \(^{b}\)Yu et al. (2004); \(^{c}\)Bodzek and Dudziak (2006); \(^{d}\)River Water; \(^{e}\)Briciu et al., 2009; \(^{f}\)Ying et al., 2002; \(^{g}\)Ju"rgens et al., 2002.
2.2.7 Estrogenic Potency

The relative potency of each estrogen can be shown as EE2 > E2 > E1 > E3 (De Mes, 2007). Wise et al. (2010) reported that vitro assays in fish determined that EE2 is twice as potent as E2, E2 is two to five times more potent than E1, and E1 is eight times more potent than E3. Since E3 is the least potent endocrine disrupter to aquatic life, it is excluded from this study and is consistent with the approach taken by other researchers (Atkinson et al., 2012; Umali et al., 2012; Johnson and Williams, 2004). Although the concentrations of EE2 are generally low in comparison to the other estrogens, it’s potency and resilience in WWTPs is much greater than endogenous estrogens (Ternes et al., 1999a/b); as such, EE2 is included in this study.

2.2.8 Transformation of Hormones

Most of the human estrogens are excreted in the conjugated forms or inactive forms. However, estrogen de-conjugation and reactivation can occur in sewer systems and during the wastewater treatment process. For example, researchers in a German WWTP have shown that more non-conjugated E1 and E2 effluent from the de-nitrification treatment unit can be observed than the influent amount; in contrast, EE2 influent and effluent were generally the same (Andersen et al. 2003). The results support the theory that E1 and E2 glucuronide and sulfate
conjugates not detected in the primary influent were deconjugated in the denitrification tank. However, there are some cases, where the effluent can still contain conjugates, particularly EE2; and conjugate persistence, and the analytical techniques that measure de-conjugate forms, can help explain why in some cases elevated measurements of EE2 have been reported in the effluent (Andersen et al. 2003).

2.2.9 Mechanisms for Estrogen Removal

Biodegradation and sorption can be classified as the two major mechanisms that determine the outcome and percentage of estrogen remaining in WWTPs (Racz and Goel, 2009).

2.2.9.1 Absorption and Adsorption

Absorption can be defined as a physical or chemical process where a substance is assembled throughout the bulk of the solid or liquid (McMurray, 2003); adsorption can be defined as a physical or chemical process where a substance accumulates at the surface of an adsorbent (Oxford Dictionaries, 2014). As estrogen is hydrophobic and contains a solid water partition coefficient upwards of 3.0, estrogen can be considered highly attracted to solids (Carballa et al. 2008). However, limited work has been completed to date to quantify adsorption as a significant step in removing estrogen. Researchers have developed Freundlich sorption isotherms for both natural and synthetic estrogen, and discovered that n is close to 1, implying linear adsorption (Clara et al. 2004). In fact, the calculated adsorption distribution coefficients of 2.6 to 2.8 are in agreement with the values where sorption can occur between 2.1 and 2.9 (Carballa et al. 2008). Researchers who completed batch studies on highly concentrated solutions of estrogen discovered significant adsorption potential for estrogen on sludge given that no saturation occurred during the experiments. Researchers who studied the effects of sorption on natural estrogen discovered that in both aerobic activated and non-activated sludge, estrogen
removal occurred by sorption onto the solid phase (Zeng et al. 2009). However, anaerobic conditions lead to higher sorption rates than aerobic conditions (Lai et al. 2000). Andersen et al. (2005) predicted that as high as 75% sorption of estrogen can occur within a typical activated sludge treatment process using adsorption isotherms. Ren et al. (2007) determined that estrogen within heat-activated sludge can reach adsorption equilibrium within approximately ten minutes. In another study, sorption of estrogen to other compounds was found to be directly associated with the amount of total organic carbon (TOC): the more TOC the greater the amount of sorption (Racz and Goel, 2010). Researchers have determined that the sorption coefficients of estrogen are independent of log $K_{ow}$, and the interaction between the estrogen phenolic moieties and dissolved organic matter is the main reason for sorption (Racz and Goel, 2010).

**2.2.9.2 Biodegradation**

Evidence of steroid biodegradation by soil microorganisms (e.g. Proactinomyces) has been known as early as 1944 (Turfitt, 1944). Estrogen is a cholesterol derivative that can supply micro-organisms with a suitable carbon source for biodegradation; however, its complex structure prevents efficient or timely biodegradation. As such, the longer estrogens are subjected to micro-organisms in an aerobic environment the higher the percentage of estrogen removal (Racz and Goel, 2010). In some cases, the evidence can be contradictory for biodegradation in an anaerobic environment. For example, in one study researchers found that under different anaerobic digestion conditions such as methanogenesis and sulphate and nitrate reducing conditions, with an incubation time of approximately 400 days, resulted in little to no change in estrogen concentrations (Czajka and Londry, 2006). Similarly, other researchers who completed a study on the effects of anaerobic digestion in an animal waste treatment plant discovered that estrogen concentrations also remained constant demonstrating no effects of anaerobic digestion
on biodegradation (Furuichi et al. 2006). Studies have also shown that no estrogen degradation occurs under anaerobic conditions in different sludge types such as granular, floculent, biological, and digested swine waste (de Mes et al. 2008). Researchers suggest that estrogens resistance to biodegrade under anaerobic conditions may lead to accumulation of estrogen. In a full scale WWTP in Germany, researchers concluded that natural and synthetic estrogen degradation did not occur under methanogenic conditions with temperatures of approximately 33°C, SRT of approximately 20 days, and primary and secondary sludge feed (Andersen et al. 2003). In another study, researchers found that the estrogenic activity of the biosolids formed from anaerobic digestion doubled under temperatures of 35°C, HRT of 10 days, and primary and activated sludge (Holbrook et al. 2002). In contrast, other researchers reported that natural estrogen degradation did occur under anaerobic digestion conditions (Lee and Liu 2002; Carballa et al. 2004; Carballa et al. 2006). For example, Carballa et al. (2004; 2006) reported that approximately 80% of all estrogen was removed under anaerobic digestion with temperatures that ranged from 37°C to 55°C and SRTs that ranged from 6 to 20 days.

Overall, detailed control studies are needed on the effects of anaerobic digestion on estrogen removal due to the inconclusive results from previous studies.

2.3.0 Fate of estrogens in treatment plants

WWTPs are not specifically designed to remove estrogen, and little data has been collected to determine the efficiency of each WWTP processing unit for removing estrogen. Trickling filter systems have been compared to conventional activated sludge processes, and researchers have determined that conventional activated sludge systems had greater rates of estrogen removal due to greater rates of biodegradation—greater rates of biodegradation is believed to be attributed to greater aeration (Ternes et al. 1999b).
In one study, that examined 47 WWTPs in Canada, researchers determined that the lowest concentrations of estrogen effluent were observed in extended aeration activated sludge plants; these plants removed more than 95% for both E2 and EE2 (Lee et al. 2004). Observations of UV effects on E1, E2, and EE2 removal have shown that the lower the concentration of estrogen entering the UV facility, the higher percent removal: 30 to 100% removal for lower concentrations entering the facility verses 4-24% removal for higher concentrations entering the facility (Lee et al. 2004).

Researchers have also shown positive results in estrogen removal under aerobic biodegradation within activated sludge taken from plants that employ BOD. For example, in one study—under 20°C, initial estrogen concentrations of approximately 1.0 µg/l, and TSS of 0.52 g—researchers discovered a 50% decrease of E2 within 0.2 hours, 50% of E1 within 1.5 hours, and no significant degradation of EE2 after 48 hours (Ternes et al. 1999b). In another study, complete mineralization of E2 occurred within approximately 2 hours, little to no change occurred in E1, and approximately 40% EE2 was removed within 24 hours (Layton et al. 2000).

Studies have shown, within five WWTPs in the United States, that estrogen taken from the raw influent and primary settling effluent showed little concentration change. However, in some cases researchers have discovered that the estrogen concentration was higher in the primary settling effluent versus the raw influent, and suggest that the increase from raw influent to primary effluent is likely due to the oxidation of conjugated estrogens in the primary clarifier. In fact, researchers have shown the presence of conjugated estrogen in some German WWTPs where approximately 60% of E1, 50% of E2, and 25% of EE2 were conjugated in raw influent (Adler et al. 2001).

In Japan, Matsui et al. (2000) reported that the estrogenic activity measured by yeast
estrogen screening decreased after each processing unit within the WWTP, and denitrification within the activated sludge treatment process showed the greatest decrease. Similarly, in Germany, Andersen et al. (2003) reported lower concentrations of estrogen with each successive treatment unit within the Wiesbaden WWTP, and the greatest reduction occurred at the denitrification stage. Andersen et al. (2003) noted that the concentration of E1 within the first denitrification tank decreased by 50%, EE2 concentration decreased by approximately 70%, and little change occurred with E2 concentration. Researchers explained that denitrification and dilution—with the return sludge from the secondary clarifier and the internal recirculation containing little estrogen from the last nitrification tank—contributed to the reduced concentrations of E1 and EE2, while deconjugation by sludge bacteria resulted in the similar concentration measurements of E2. In the same study, it was also observed that E1 and E2 concentration was reduced further in the second de-nitrification tank and the following aerated reactor by approximately 94%, while EE2 was reduced by 20% (Andersen et al. 2003). The reductions suggest that de-nitrification and aerobic biological degradation appears to play a role in reducing the amount of E1/E2 and EE2 respectively. Furthermore, the reduced concentration between the first and second reactor may also suggest slow sorption kinetics and no equilibrium between the sorbed and dissolved estrogens.

The type of activated sludge system employed with a WWTP also appears to influence the amount of estrogen that is removed. For example, effluent estrogen concentration measurements taken in a WWTP in Germany employing a activated sludge system with a mixed reactor system and BOD removal were much higher (24 ng/l for E1, 5 ng/l for E2, and 2 ng/l for EE2) when compared to the same plant that was later upgraded to all year nitrification and denitrification (all estrogen effluent measurements were below the detection level of 1 ng/l)
2.3.1 Operating Variables on Estrogen Removal

Researchers have shown that the removal of estrogen in wastewater can be influenced by variables such as temperature, pH, salinity, suspended solids, initial influent concentration, hydraulic retention time, and sludge retention time (Birkett and Lester, 2003). For example, in one study, researchers found that high rates of estrogen removal were correlated to high solids content (Lee et al. 2004). The affect of HRT and SRT on estrogen removal appears to be estrogen specific. For example, in one study that examined the affects of SRT and HRT on the removal of E1, E2, and EE2 showed that most of the E1 and E2 were removed at high SRT and HRT while results varied with low SRT and HRT; In contrast, SRT and HRT appeared to show no affect on EE2 removal (Henrik et al. 2003).

Henrik et al. (2003) reported that minor estrogen reductions were observed using activated sludge taken from a plant with a sludge retention time of approximately 4 days compared to sludge taken from the same plant with a sludge retention time of 11 to 13 days; this study also suggests that a increase in sludge retention time increases the amount of microorganisms that are able to degrade estrogen. Activated sludge oxidation-reduction potential may also influence the removal of estrogen in WWTPs. Researchers suggest that aerobic activated sludge systems are more efficient in removing estrogen than a plant employing biological phosphorus removal due to the fact that estrogen breaks down more under aerobic conditions verses anaerobic (Johnson et al. 2000).

It has also been reported that adsorption of both the natural and synethic estrogen is also dependent on variables including pH. For example, in one study researchers discovered no
effects of estrogen sorption to sludge on pH of 9 or less; however, approximately 50% desorption occurred on pH over 9 (Clara et al. 2004). While some researchers have shown that many variables may influence the concentration of estrogen in WWTPs, others researchers have also discovered that some of the same variables such as temperature, SRT, and pretreatments have no effect on estrogen sorption (Carballa et al. 2008).
Chapter 3 - Study Area and Data Description

3.1: Study Area

3.1.1 WWTP Model Simulation

The WWTPs selected in this study to run the model are located within the city of Calgary and Edmonton, Alberta and in Brandon, Manitoba (Figure 3-1). The Calgary facilities are distributed located along the east banks of the Bow River, the Edmonton facility is located on the city's eastern outskirts on the south bank of the North Saskatchewan River, and the Brandon facility is located adjacent to the Assiniboine River.
3.1.1.1 City of Calgary

The city of Calgary area is approximately 825 km$^2$ and contains approximately over 1.1 million residents (City Data, 2014 a). The Bow River flows through the city of Calgary and is well known in Alberta, for supporting economic development, providing recreational opportunities, and maintaining high environmental standards (BRBC, 2010). However, water quality varies along the river, and the most significant changes of water quality occurs downstream of the city of Calgary (BRBC, 2010). The city of Calgary relies on three wastewater
treatment facilities to protect public health and the environment: Bonnybrook WWTP, Fish Creek WWTP, and Pine Creek WWTP (City of Calgary, 2013). The city of Calgary releases over 150,000,000 m$^3$ of treated effluent to the Bow each year (City of Calgary, 2010). Calgary’s wastewater treatment also supports the surrounding communities of Cochrane, Chestermere, Airdrie and the Tsuu T’ina Nation; these communities pipeline their wastewater to Calgary for treatment (BRBC, 2005). Note: Pine Creek WWTP will be excluded from this study due to limited data.

3.1.1.2 City of Edmonton

The city of Edmonton area is approximately 684 km$^2$ and contains over 800,000 thousand residents (City Data, 2014 b). The North Saskatchewan River flows through the city of Edmonton and supplies Edmonton’s drinking water and is heavily used for recreation including canoeing, kayaking, jet skiing, boating, fishing, etc. (City of Edmonton, 2014). The city of Edmonton relies on the Gold bar wastewater treatment facility to protect public health and the environment. The city of Edmonton releases over 90,000,000 m$^3$ of treated effluent to the North Saskatchewan River each year (Fernandez et al. 2008).

3.1.1.3 City of Brandon

The city of Brandon area is approximately 77 km$^2$ and contains approximately 46,000 thousand residents (City Data, 2014 c). The Assiniboine River, a tributary of the Red River, flows through the city of Brandon and is also well known in Manitoba, for supporting economic development, providing recreational opportunities, and its historic significance in establishing the fur trade. The city of Brandon relies on the two WWTP’s to protect public health and the environment: one industrial WWTP that treats wastewater generated by the Maple Leaf Pork Processing Plant and one municipal WWTP that treats all of the waste water generated by the
city of Brandon. The city of Brandon municipalWWTP releases over 6,000,000 m$^3$ of treated effluent to the Assiniboine River each year. *Note: since this study will focus on municipal WWTPs, the industrial WWTP located in Brandon will be excluded from this study.*

### 3.1.2 WWTP Comparison

The WWTPs chosen to simulate the model all employ tertiary technologies, they have available data to use, and are in Western Canadian cities that are in relative close proximity to each other (Table 3-1). The proceeding sections provide a detailed description for each WWTP process.

<table>
<thead>
<tr>
<th>WWTP</th>
<th>Plant Class</th>
<th>Primary Treatment</th>
<th>Secondary Treatment</th>
<th>N., P., C. Removal$^d$</th>
<th>Disinfection$^e$</th>
<th>Lagoon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish Creek$^a$</td>
<td>Tertiary</td>
<td>Yes</td>
<td>Activated Sludge</td>
<td>P.C.</td>
<td>UV (High)</td>
<td>Yes</td>
</tr>
<tr>
<td>Bonnybrook$^a$</td>
<td>Tertiary</td>
<td>Yes</td>
<td>Activated Sludge</td>
<td>Yes</td>
<td>UV (High)</td>
<td>Yes</td>
</tr>
<tr>
<td>Brandon$^b$</td>
<td>Tertiary</td>
<td>Yes</td>
<td>Activated Sludge</td>
<td>When needed</td>
<td>UV (High)</td>
<td>Yes</td>
</tr>
<tr>
<td>Goldbar$^c$</td>
<td>Tertiary</td>
<td>Yes</td>
<td>Activated Sludge</td>
<td>Yes</td>
<td>UV (High)</td>
<td>Yes</td>
</tr>
</tbody>
</table>

$^a$ obtained from the City of Calgary through personal communication; $^b$ Cicek et al. (2007); $^c$ Fernandez et al. (2008); $^d$ N=nitrogen, P=phosphorus, C=carbon; $^e$ UV=ultraviolet;

### 3.1.3 Calgary's Wastewater Treatment

The city of Calgary relies on three wastewater treatment facilities to protect public health and the environment: Bonnybrook WWTP, Fish Creek WWTP, and Pine Creek WWTP (City of Calgary, 2013). *Note: Pine Creek WWTP will be excluded from this study due to limited data.*
3.1.3.1 Bonnybrook

The Bonnybrook WWTP currently serves a population of approximately 880,000 and is located upstream of Fish Creek and Pine Creek WWTPs. In 2012, the facility treated over 133,300,000 m$^3$ of total wastewater with an average daily flow of approximately 364,000 m$^3$/day (Appendix A). The Bonnybrook WWTP is classified as a tertiary plant that employs sludge fermentation, biological nutrient removal (BNR), i.e. nitrogen and phosphorus removal, and effluent ultraviolet-light (UV) disinfection. The WWTP effluent is continuously discharged to the Bow River. Sludge is coagulated in dissolved air flotation and gravity thickeners, pumped to digesters for anaerobic digestion, and transported to the Shepard Sludge Lagoons for settlement (Do, 2004).

3.1.3.2 Fish Creek

The Fish Creek WWTP currently serves a population of approximately 104,000 and is located downstream of the Bonnybrook facility. In 2012, the facility treated over 11,900,000 m$^3$ of total wastewater with an average daily flow of approximately 32,500 m$^3$/day (Appendix A). The Fish Creek WWTP is also a tertiary treatment plant with chemical phosphorus removal and UV disinfection. In contrast to the Bonnybrook WWTP, the Fish Creek WWTP does not employ BNR. Furthermore, the hydraulic retention time is also shorter in Fish Creek verses Bonnybrook WWTP (Chen et al., 2006).

3.1.4 Calgary’s General Wastewater Process

Calgary’s wastewater treatment consists of various stages and a series of complex processes prior to its release back into the environment (City of Calgary, 2013).
3.1.4.1 Headworks

The wastewater first enters the sewer system from residential dwellings, school facilities, businesses, hospitals, etc. Wastewater is either gravity fed or pumped through the sewer system to the Fish Creek or Bonnybrook WWTP. Once the wastewater arrives at the WWTP, it flows and is filtered through screens; these screens filter coarse debris (e.g. plastic, paper, etc.). The screened wastewater then flows into grit tanks. Grit tanks allow for dense debris to settle at the bottom of the tanks where it is collected and transported to the landfill. The remaining water is classified as Primary Influent. The Primary influent is gravity fed to the Primary Clarifiers for further processing (City of Calgary, 2013).

3.1.4.2 Primary Clarifiers

Primary clarifiers are aerobic settling tanks. Wastewater retention in the Primary Clarifiers is approximately three hours. The retention time allows for further material settling at the bottom of the tank. The bottom sludge and top waste (i.e. scum and grease) are collected and pumped to the anaerobic digesters. These digesters allow for further decomposition to occur as part of the sludge treatment process. The remaining wastewater from the primary clarifiers or primary effluent moves into the secondary liquid treatment process (City of Calgary, 2013).

3.1.4.3 Liquid-treatment Processing

3.1.4.3.1 Air Activated Sludge Process / Bioreactors

The bioreactors are aerobic tanks containing a mixture of large amounts of natural microorganisms with the primary effluent from the clarifiers. Air is released towards the bottom of the bioreactors and aeration tanks ensuring direct contact between organic material and microorganisms. The combination of oxygen and organic matter provides for a suitable environment
allowing the micro-organisms to reproduce and consume dissolved nutrients such as phosphorous and ammonia. The bioreactors have a retention time of approximately seven hours. The mixed liquor, or combination of activated sludge and primary effluent, then moves to the secondary clarifiers for further processing (City of Calgary, 2013).

3.1.4.3.2 Secondary Clarifiers

Similar to the primary clarifiers, secondary clarifiers are also aerobic tanks that allow for settling and skimming. The secondary clarifier has a seven-hour retention time that allows the activated sludge to settle at the bottom while the secondary effluent flows to the disinfection process. The majority of the activated sludge is pumped to the bioreactors and aeration tanks for further processing while the remaining sludge requires thickening. Thickening occurs in five dissolved-air flotation tanks. The top waste is also skimmed in the secondary clarifiers and transported to the anaerobic digesters (City of Calgary, 2013).

3.1.4.3.3 Disinfection (U.V.)

The disinfection process consists of a UV facility. Secondary clarifier effluent flows through the facility and subjected to high intensity UV light. The UV light interferes with the microbial community genetics and subsequently prevents their ability to reproduce and prevent disease. After disinfection, the effluent flows directly into the Bow River. The final effluent is considered clear, high in oxygen, low in organic matter, ammonia, phosphorus, and microorganisms (City of Calgary, 2013).
3.1.4.4 Sludge-handling Processing

3.1.4.4.1 Digesters

The digesters hold the primary and activated sludge for 25 days in the absence of oxygen. During this period the anaerobic bacteria transforms complex organic material to simple substances that are also more stable (e.g. carbon dioxide). Micro-organisms that cause disease and odor are reduced in the digester. Efficiency is improved by using digester gas for mixing and heating to ensure that the optimal temperature is maintained at 35 degrees centigrade (City of Calgary, 2013).

3.1.4.4.2 Shepard Lagoon Facility

The final sludge from the digesters is transported to the sheppard sludge lagoons. The lagoon system contains a total of 10 cells, a holding capacity of 743,000 m3, and occupies 20 hectares of land in Southeast Calgary. Water from the lagoons is further processed in the WWTPs prior to its release into the Bow River. The remaining biosolids from the lagoons are used in fields for fertilizer as part of the CALGRO program (City of Calgary, 2013).

3.1.5 Edmonton's Wastewater Treatment

The city of Edmonton relies on the Gold bar wastewater treatment facility to protect public health and the environment. The city of Edmonton releases over 90,000,000 m³ of treated effluent to the North Saskatchewan River each year (Fernandez et al. 2008).

3.1.5.1 Gold bar WWTP

The Gold bar WWTP currently serves a population of approximately 750,000 and is one of Canada’s largest Class IV WWTP (Fernandez et al. 2008). It has a current design treatment Capacity of 310 million litres per day, a peak primary treatment capacity of 910 million litres per
day, and a peak secondary/tertiary treatment capacity of 420 million litres per day. The Gold bar WWTP is classified as a tertiary plant that employs sludge fermentation, BNR, i.e. nitrogen and phosphorus removal, and effluent UV disinfection. Full tertiary treatment takes approximately 18 hours with the final effluent being continuously discharged to the North Saskatchewan River (EPCOR, 2015).

3.1.6 Edmonton General Wastewater Process

Edmonton’s wastewater treatment consists of various stages and a series of complex processes prior to its release back into the environment.

3.1.6.1 Pre-treatment

Similar to Calgary’s WWTP, the wastewater arriving at the WWTP flows through filtered through screens; these screens filter coarse debris (e.g. plastic, paper, etc.). The screened wastewater then flows into grit tanks. Screening the large inorganic material is important in preventing equipment damage and optimizing the removal of organic material and contaminants. The grit and large inorganic material is shipped to the Waste Management Centre in Edmonton (EPCOR, 2015).

3.1.6.2 Enhanced Primary Treatment (for wet weather flows)

There are two types of collection systems in Edmonton, one is a combined system where storm water and sewer are contained in one line, in the older part of the city, and in the newer part of the city separate lines for the storm water and sewer (EPCOR, 2015).

Periods of high precipitation can exceed the capacity of the combined line with sewer/storm-runoff and the overflows are directed to the River in order to avoid sewer backup in households.
It is estimated that approximate 15% of all of the sewer lines are sewage/storm runoff combined. However, the amount of flow directed at the river is rare as the enhanced primary treatment clarifiers within the Goldbar facility allows the plant to handle peak flows that are common in the spring and in summer storms (EPCOR, 2015).

3.1.6.3 Primary Treatment

Normally, wastewater moves from pre-treatment stage to the primary treatment clarifiers. The primary treatment clarifiers can remove up to about half of the common traditional wastewater contaminants. In the clarifiers, heavy materials settle at the bottom of the tank while lighter material floats at the surface. The bottom sludge and top waste (i.e. scum and grease) are collected and pumped to the fermenters and anaerobic digesters. These digesters allow for further decomposition to occur as part of the sludge treatment process. The remaining wastewater from the primary clarifiers or primary effluent moves into the secondary liquid treatment process (EPCOR, 2015).

3.1.6.4 Solids Handling

3.1.6.4.1 Fermenters

Sludge collected from the primary clarifiers is transported to the four fermenters where it’s stored under an anaerobic environment for approximately 7 days allowing the sludge to thicken. The sludge is then mixed with secondary sludge in blend tanks prior to moving to the digesters. The liquid extract from the fermenters contains volatile fatty acids and is used in the BNR within the secondary treatment process as discussed further (EPCOR, 2015).
3.1.6.4.2 Digesters

Sludge from the fermenters moves to the anaerobic digesters where it is further treated under high temperatures of 37°C. The digester breaks down and stabilizes the organic sludge removed during the treatment processes. The byproducts from the digesters such as methane is retained and used as fuel to heat the Goldbar WWTP. Also, approximately 5% of the digested sludge is shipped over 10 km to lagoons at the Clover Bar site where it is further treated prior to its release to the environment (EPCOR, 2015).

3.3.1.6.4.3 Biosolids

Liquid separated from the digested sludge within the lagoons returns to the Gold Bar plant for further treatment while the biosolids are applied on farm land or used in co-composters. The nitrogen and phosphorus within the biosolids are used to amend the soil and as fertilizers (EPCOR, 2015).

3.1.6.5 Secondary Treatment

At the secondary treatment stage, all of the existing organic matter in the wastewater can be classified as dissolved solids that cannot be removed by settling alone. At this stage, microorganisms are used to further treat the wastewater in the secondary treatment process by consuming the dissolved organic matter and contaminants. Large amounts of air is pumped into the bioreactor tanks increasing the microorganism consumption (EPCOR, 2015).
3.1.6.6 Biological Nutrient Removal

BNR is also employed in the secondary bioreactors where microorganisms remove nutrients such as phosphorus and ammonia. High concentrations of phosphorus and ammonia can also be classified as contaminants to the environment. After this process, the wastewater moves to the final clarification stage for further treatment (EPCOR, 2015).

3.1.6.7 Final Clarification

Similar to the primary clarifiers, secondary clarifiers are also aerobic tanks that allow for settling and skimming. The secondary clarifier allows the floc to settle at the bottom while the secondary effluent flows to the disinfection process. The majority of the settled floc is returned to the bioreactors while the rest is pumped to the digesters for solids handling (EPCOR, 2015).

3.1.6.8 UV Disinfection

The wastewater moves from the secondary clarifiers to the high intensity UV disinfection facility prior to its release to the North Saskatchewan River. The UV light interferes with the microbial community genetics and subsequently prevents their ability to reproduce and prevent disease (EPCOR, 2015).

3.1.6.9 Membrane Filtration

Approximately 5% of the wastewater from the secondary clarifier bypasses the UV disinfection facility and moves to the membrane filtration system creating industry process water. The filters contain porous synthetic strands with microscopic pore openings. The filters allow water to pass
while capturing the bacteria. The filtration system disinfects the wastewater similar to the UV treatment facility (EPCOR, 2015).

3.1.7 Brandon Wastewater Treatment

The city of Brandon relies on the two WWTP’s to protect public health and the environment: one industrial WWTP that treats wastewater generated by the Maple Leaf Pork Processing Plant and one municipal WWTP that treats all of the waste water generated by the city of Brandon. The city of Brandon municipal WWTP releases over 6,000,000 m$^3$ of treated effluent to the Assiniboine River each year (City of Brandon, 2014).

3.1.8 Brandon WWTP Process

The Brandon WWTP consists of the following stages: primary treatment, a sequencing batch reactor, a disinfection facility and a lagoon system (City of Brandon, 2014).

3.1.8.1 Primary Treatment

Wastewater from the city of Brandon enters the WWTP through the Main Lift Station where bar screens collect the waste and is disposed in the City landfill. The wastewater moves to the primary treatment building that removes the grit by a gravity vortex grit removal unit. It is further treated with fine screens with openings of 4 mm. All of the solid waste removed from the primary treatment unit is disposed in the City’s landfill site. The wastewater moves from the primary treatment facility to the bioreactors for further treatment (City of Brandon, 2014).
3.1.8.2 Sequencing Batch Reactor

The bioreactors at the Brandon facility are classified as Sequencing Batch Reactors (SBR)s. SBRs are activated sludge reactors where basins are filled with wastewater during a period and operated in a batch treatment mode. Through a timed sequence, SBR can produce aerobic, anaerobic, or anoxic conditions that allows for certain microorganisms to multiply. The operating flexibility allows one to create the conditions suited for particular organisms while preventing the growth of unsuitable microorganisms. The reactor contains five stages including the Fill, React, Settle, Decant and Idle stages (City of Brandon, 2014).

3.1.8.2.1 Fill Stage

During the fill stage, the reactor containing the wastewater can be aerobic, anaerobic or a combination of both. Biodegradation is achieved in this stage during the anaerobic fill. Influent is spread along the settled sludge through a distribution manifold and is not diluted by mixing, making BNR more effective (City of Brandon, 2014).

3.1.8.2.2 React Stage

Once the wastewater leaves the fill phase it then moves to another reactor where aeration allows for the continuation of biodegradation. In this stage, the mixed liquor is removed from the aeration header and used in the jet aerator (City of Brandon, 2014).

3.1.8.2.3 Settle Stage

During the settle stage, the air supply is removed allowing for the organic matter to settle while the treated supernatant remains on top (City of Brandon, 2014).
3.1.8.2.4 Decant Stage

In the decant stage, the treated wastewater liquid is removed while excluding the sludge (City of Brandon, 2014).

3.1.8.2.5 Idle/Waste Sludge Stage

In the idle/waste sludge stage, the settled sludge is removed and pumped to the holding cells located in the City’s lagoon system. The idle/waste sludge stage is similar to the traditional secondary clarifier units seen elsewhere (City of Brandon, 2014).

3.1.8.3 Disinfection

The wastewater moves from the idle/waste sludge stage to the high intensity UV disinfection facility prior to its release to the Assiniboine River or further treatment in the lagoon system. As written previously, the UV light interferes with the microbial community genetics and subsequently prevents their ability to reproduce and prevent disease (City of Brandon, 2014).

3.1.8.4 Lagoon System

The lagoon system contains numerous sludge receiving cells and sludge isolation cells. The facility also contains aerobic lagoon cells used during peak flow events that exceed the Brandon WWTP wastewater capacity. The lagoon facility is used primary for excessive flows and acts as a clarifier for the final effluent from the UV facility (City of Brandon, 2014).
3.2 Data Description

3.2.1 Data Collection

The estrogen data used for comparison and validation to the simulated model were obtained from WWTPs located in Alberta and Manitoba (Figure 3-1). The estrogen data collected to calculate the influent-to-effluent removal rates were collected in various provinces across Canada (Table 3-2); estrogen data used to calculate the removal matrix for each WWTP individual treatment stage were collected in Brandon, Manitoba. Note: due to world-wide limited information, supplemental data was included from one WWTP in Italy for the EE2 influent-to-effluent removal rates and one WWTP in Germany for the removal matrix calculations.

3.2.1.1 Estrogen Data used for Model Comparison and Validation

The Calgary steroid estrogen data used in this study, for comparison to the simulated model, was previously measured using 24-hour composite samples within the effluent at Fish Creek and Bonnybrook WWTPs in 2003 and 2012 (Appendix B). The detection limits for steroid estrogen data collected in 2003 ranged from 0.0005-0.0081µg/l, and the reportable detection limits for steroid estrogen collected in 2012 ranged from 0.004 to 0.005 µg/l (Appendix B). The detailed data collection methods and procedures are fully described in Appendix C.

The Brandon WWTP estrogen data was collected in 2003, using a combination of grab and 24-hour composite samples for the primary influent and final effluent, respectively. The detailed data collection methods and procedures are fully described in Appendix C. The estrogen data collected from the Edmonton WWTP in 2006 measures the total estrogenic activity or RYA measured E2-eq data for the initial influent using 24-hour composite samples.
Although, the RYA measured E2-eq data measures the total estrogenic activity, E1 and E2 have been identified as the major contributors to RYA measured activity (Fernandez et al., 2008); however, since RYA measures the total estrogenic activity and lacks compound specificity, it will be compared to the total estrogen concentration from the model or E1 + E2 + EE2. The detailed data collection methods and procedures are fully described in Appendix C. Figure 3-2 shows the locations where the primary influent and final effluent data was sampled within the WWTPs.

Figure 3-2: Primary influent (PI) and final effluent (FE) grab and composite (C.) sample locations.

3.2.1.2 Estrogen Data used for Model Calibration and Validation

The model calibration data set included half of the EE2 influent and effluent measurements taken within the Brandon WWTP in 2003; the model validation data set included the remaining EE2 measurements within the Brandon facility and also data from the Calgary facilities as fully described in Appendix B and Appendix C. Note: due to limited data, estrogen that was not detected during the sampling analysis in the Calgary WWTPs, are assumed to be present but below the applicable detection limits; and, these concentrations below the detection limits were also used to validate the calibrated model—albeit with an additional level of error.
For example, if a sampling analysis showed no presence of estrogen with a detection level of <0.005 (μg/l), it is assumed that there is estrogen present and its concentration is below the detection limits; the predictions from the calibrated model should therefore coincide with concentrations below the detection limits verses concentrations above.

### 3.2.1.3 WWTP Estrogen Removal Rate Data

The mean percent estrogen influent-to-effluent removal values were calculated with 95% confidence limits using measured raw data collected from other WWTPs throughout Canada. Data from Canadian WWTPs were targeted in this study due to similarities in technologies employed and geographical reference, both of which may reduce variances due to operational processes employed, weather, and climate. The detailed sampling analysis, methods, and procedures used to collect and measure the data can be found in detail within each listed reference (Table 3-2).

<table>
<thead>
<tr>
<th>Reference</th>
<th>E1(n)</th>
<th>E2(n)</th>
<th>EE2(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baronti et al., 2000</td>
<td></td>
<td></td>
<td>30c</td>
</tr>
<tr>
<td>Cicek et al., 2007a</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Lee et al., 2003</td>
<td>11</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>Lee et al., 2005</td>
<td>6</td>
<td>8</td>
<td>No data</td>
</tr>
<tr>
<td>Pauwels et al., 2008</td>
<td>6</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Servos et al., 2005</td>
<td>9</td>
<td>9</td>
<td>No data</td>
</tr>
<tr>
<td>Total:</td>
<td>40</td>
<td>37</td>
<td>51</td>
</tr>
</tbody>
</table>

* the raw data related to this reference were obtained from N. Cicek, University of Manitoba through personal communication; * (n) raw data sample size used; * EE2 data supplemented from a WWTP in Italy due to limited data within Canada; Note: data showing an influent-to-effluent concentration increase within WWTPs were not considered due to reported limitations in analytical methods preventing the detection of conjugated estrogen compounds commonly present in raw influent (Lee et al., 2005a; Fernandez et al., 2007b).

The mean excretion values used in the model accounts for both conjugated and unconjugated forms of estrogen. However, as discussed previously, analytical methods can
prevent the detection of conjugated estrogen compounds present in raw influent and detected the transformed unconjugated compounds during the WWTP process (Lee et al., 2005a; Nelson et al., 2007; Fernandez et al., 2007b). These cases led to an increase in effluent over influent values due to analytical methods; therefore, measurements showing an increase in effluent concentration were not considered.

3.2.1.4 Removal Matrix Data used to build the Model

The Wiesbaden and Brandon WWTP estrogen data was collected in 2001 (Anderson et al. 2003) and 2003 (Cicek et al. 2007) respectively. All of the data sampled at each individual treatment stage in the Wiesbaden WWTP were 24-hour proportional flow composite samples; the Brandon WWTP data sampled at the initial influent stage were grab samples, while all remaining samples were 24-hour proportional flow composite samples. Samples of E1, E2, and EE2 in both the Brandon and Wiesbaden WWTPs were measured prior and post each individual treatment process. The data collection methods and procedures are fully described in detail in Appendix C.

3.2.1.5 WWTP Data

The Calgary wastewater flow data and historical population served by each WWTP between 2002 and 2012 was collected and provided by the city of Calgary (Appendix A). The Brandon Manitoba WWTP parameters were obtained from Cicek et al. (2007), while the Edmonton WWTP parameters were obtained from Fernandez et al. (2008) [Table 3-3].
Table 3-3: Comparison among the operational parameters of the wastewater treatment plants (WWTPs)\(^a\)

<table>
<thead>
<tr>
<th>WWTP name/location</th>
<th>Pop. served</th>
<th>Average daily flow rate (m(^3)/d)</th>
<th>Wastewater Temperature</th>
<th>Wastewater Source</th>
<th>SRT(^e) (days)</th>
<th>HRT(^f) (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish Creek(^b)</td>
<td>168,951</td>
<td>66,240</td>
<td>N/A</td>
<td>Domestic</td>
<td>N/A</td>
<td>10</td>
</tr>
<tr>
<td>Bonnybrook(^b)</td>
<td>794,227</td>
<td>384,870</td>
<td>N/A</td>
<td>Domestic</td>
<td>N/A</td>
<td>23</td>
</tr>
<tr>
<td>Brandon(^c)</td>
<td>43,020</td>
<td>16,523</td>
<td>10 - 12</td>
<td>Domestic</td>
<td>1.2</td>
<td>6</td>
</tr>
<tr>
<td>Edmonton(^d)</td>
<td>750,000</td>
<td>250,000</td>
<td>15.2 – 21.6</td>
<td>Domestic</td>
<td>4-8</td>
<td>6-7</td>
</tr>
</tbody>
</table>

\(^a\) parameters are averages for the following years: Edmonton 2006; BB 2003, FC 2003; Brandon 2003; \(^b\) obtained from the City of Calgary through personal communication; \(^c\) Cicek et al. (2007); \(^d\) Fernandez et al. (2008); \(^e\) SRT=sludge retention time; \(^f\) HRT=hydraulic retention time

3.2.1.6 City of Calgary Demographic Data

The city of Calgary collects census data for age and gender distribution comparisons twice in a five year period (City of Calgary 2011); therefore, city of Calgary census data collected in 2001, 2004, 2006, 2009, and 2011 was used to define the demographic profile served by each WWTP in the city of Calgary. The model assumes that the age and gender percentages calculated from the city of Calgary census data matches the surrounding communities that transport wastewater to the city i.e. Cochrane, Chestermere, Airdrie, and the Tsuu T’ina Nation. The model runs for the Edmonton and Brandon facilities also assumes the same demographic profiles calculated from the census data from Calgary.
Chapter 4: Modeling

4.1 Methodology

The load estimation model for this study provided influent and effluent concentration estimates of prescription and natural occurring estrogens in WWTPs. The model can be considered simplistic by only requiring minimal data inputs from the user, and accounts for the demographic profile of the population served by each WWTP. It is based on a previous model developed by Johnson and Willaims (2004). However, this study improves the previous model estimation model by including 1) estrogen excretion rates for pubescent males versus assuming constant rates for all males, 2) revisions in all demographic profile parameters, 3) calculated 95% confidence limits for estrogen removal rates using measured data collected from WWTPs throughout Canada, 4) calculated biological transformation rate of E2 to E1, 5) estimated estrogen removal matrix for each WWTP process unit, 6) model calibration, and 7) a completed sensitivity analysis for defining model output sensitivity to changes in its input parameters. The sensitivity analysis will act as a check on the model logic and robustness of the simulation as well as defining the importance of model parameters; and therefore, the effort required in further data acquisition for different parameters.

A schematic diagram illustrating the methods employed in this study is shown in Figure 4-1. The diagram consists of three main components: 1) revision of the demographic profile parameter ratios and calculation of the sewer biological transformation rate using local data, 2) Calculation of the removal matrix and the influent-to-effluent percentage removal using data from various WWTPs, and 3) influent and effluent model simulations and comparisons. Brief descriptions of the three major components are provided in the following paragraphs.
The model accounts for estrogen excreted in both urine and feces, and considers the lost of estrogen on transit through the sewers. WWTP influent concentrations are estimated per year using revised demographic profile parameters, previously defined per capita mean excretion rates, sewer transformation rate of estrogen, and flow rates. The demographic profile parameters will be revised by using local data in Canada and the United States. The estimated sewer biological transformation rate of E2 to E1 (i.e. $R_T$) for this study was calculated by 1) running the
model for the Brandon WWTP due to available compound specific influent data, 2) assessing whether the model agreed with the data when analyzing E1+E2 together against E1+E2 measurements, and 3) if in agreement, calculate the difference between the modeled E2 and the measured E2. The difference between the modeled E2 and the measured E2 would therefore constitute the transformation rate of E2 to E1. The preliminary model simulation results show agreement with the measurements as all of the daily mean measurements for E1+ E2 fell within the predicted intervals, and the estimated biological sewer transformation rate of E2 to E1 was therefore calculated to be 27% based on the percent change equation:

\[ R_T = \frac{(E_{2,a} - E_{2,b})}{E_{2,a}} \times 100 \]  

(1)

where \( E_{2,a} \) and \( E_{2,b} \) represents the mean modeled value and mean measurements respectively.

The WWTP final effluent concentrations are estimated using calculated influent concentrations and mean predicted removal rates. The mean percent estrogen influent-to-effluent removal values will be calculated with 95% confidence limits using measured raw data collected from other WWTPs mainly throughout Canada. For example, to determine the highest predicted effluent concentration, with 95% confidence limits, the upper standard error value for EE2 excretion concentration and the lowest predicted removal performance are used; to determine the lowest predicted effluent concentration, the lower standard error value for EE2 excretion concentration and the highest predicted removal performance are used.

The WWTP concentrations estimated to be found after each individual treatment process will be calculated using data from the Brandon and Wiesbaden WWTPs. Since some components of the Brandon and Wiesbaden treatment process differs from Bonny Brook and Fish Creek, only the data obtained from relevant process units will be included in the calculations. For example,
the Brandon WWTP employs a high intensity UV disinfection unit similar to Fish Creek and Bonnybrook, whereas the Wiesbaden WWTP do not employ UV disinfection. As a result, UV disinfection data from the Brandon facility was used. In contrast, the Brandon WWTP employs a gravity vortex grit removal unit as part of its primary treatment whereas Wiesbaden employs a primary clarifier (i.e. open-air tanks used for settling and skimming) similar to Bonnybrook and Fish Creek. As a result, data from the Wiesbaden WWTP Primary treatment unit was used verses the vortex grit removal process found in Brandon.

Estimated values in this study are compared against measured data for E1, E2, and EE2. Due to limited amount of data for both Calgary facilities, supplemental measurements from similar size facilities were used for comparison and validation, i.e. Fish Creek WWTP measurements were supplemented with the Brandon WWTP measurements and the Bonnybrook WWTP measurements were supplemented with measurements from the Goldbar facility. The supplemental data used from the above noted WWTPs were mainly chosen because they both employ tertiary technologies, they have available data to use, and are in Western Canadian cities that are in relative close proximity to each other (Table 3-1).

4.2 Model Equations:

The Johnson and Williams (2004) model will form the basis used to determine the total amount of estrogen arriving at the WWTP:

\[ T_{E1} = T_{E1} + T_{E2} + T_{EE2} \]  

(2)

where \( T_E (\mu g/l) \) is the total estrogen influent loading concentration, \( T_{E1} (\mu g/l) \) represents the total E1 influent loading concentration, \( T_{E2} (\mu g/l) \) represents the total E2 influent loading concentration, and \( T_{EE2} (\mu g/l) \) represents the total EE2 loading concentration.
For E2:

\[ T_{E2} = \frac{\sum_{i=0}^{n} \left( P_{G,i}(U_{E,i} + F_{E,i}) \right)}{Q} - R_T \]  \hspace{1cm} (3)

For E1:

\[ T_{E1} = \frac{\sum_{i=0}^{n} \left( P_{G,i}(U_{E,i} + F_{E,i}) \right) + E_c}{Q} \]  \hspace{1cm} (4)

For EE2:

\[ T_{EE2} = \frac{\sum_{i=0}^{n} \left( P_{G,i}(U_{E,i} + F_{E,i}) \right)}{Q} \]  \hspace{1cm} (5)

where \( R_T \) represents the percentage of E2 removed in the sewer system, \( P_{G,i} \) is the number of people within the demographic group of the WWTP served population, \( U_{E,i} (\mu g / d) \) is the estrogen excretion rate in urine per individual of a particular demographic profile, \( F_{E,i} (\mu g / d) \) is the estrogen excretion rate in feces per individual of a particular demographic profile, and \( E_c \) is the concentration of E1 transformed from E2 during the sewer transit (i.e. the equivalent concentration of E2 removed in the sewer system represented as \( R_T \)), and \( Q \) is the WWTP average daily flow rate (l/d).

\( R_T \) is estimated to be 27% based on the results of the Brandon model test as discussed further below. \( E_c \) for equation (3) will be the amount of degraded E2 (i.e. 27%). Excretion value rates used account for the free, glucuronide, and sulfate forms of the compound (Johnson and Williams, 2004). For EE2, the excretion value rates used the following average daily dose (i.e. \( D_{EE2} \)) of OC equation based on a cycle of 21 days usage per month:

\[ D_{EE2} = \frac{[\text{Recommended Dose} \times 21 \text{ days per month} \times 12 \text{ months}]}{365 \text{ days}} \]  \hspace{1cm} (6)

The following equation describes the removal efficiency calculation based on the raw measured influent and effluent data:
\[ R_E = \left( \frac{E_{infl} - E_{Effl}}{E_{infl}} \right) \times 100 \]  

(7)

where \( R_E \) is the percentage of the WWTP influent load removed, \( E_{infl} \) is the influent load (\( \mu g/l \)), and \( E_{Effl} \) is the effluent load (\( \mu g/l \)).

The following equation describes the final effluent calculation based on the estimated influent concentration and calculated removal percentage efficiency:

\[ F_{EEffl} = E_{infl} - (E_{infl} \times R_E) \]  

(8)

where \( F_{EEffl} \) (\( \mu g/l \)) is the final estimated effluent concentration value for a particular estrogen compound.

The following equation describes how the 95% confidence limits were calculated using the mean percent estrogen influent-to-effluent removal values:

\[ CL_{95} = X \pm [Margin of Error] \]  

(9)

\[ CL_{95} = X \pm \{1.96 [SD/(\sqrt{n})]\} \]  

(10)

where \( CL_{95} \) is the 95% confidence limit, \( X \) is the mean percent estrogen influent-to-effluent removal values, \( SD \) is the standard deviation, and \( n \) is the sample size.
4.3 Model Parameters

4.3.1 Demographic Profile

The percentage of males and females used in the model, as calculated using the city of Calgary census data as described previously, shows that the mean population percentage of males within the city of Calgary is 50.2% (SD 0.12), while the mean population percentage of females within the city of Calgary is 49.8% (SD 0.12). These above percentages were applied to the total historical population served by each WWTP. Note: the model assumes that the age and gender percentages calculated from the city of Calgary census data matches the surrounding communities that transport wastewater to the city i.e. Cochrane, Chestermere, Airdrie, and the Tsuu T’ina Nation. The model runs for the Edmonton and Brandon facilities also assumes the same demographic profiles calculated from the census data from Calgary.

Demographic profiles for the model are divided into six categories due to differences in excretion rates of estrogen: menopausal females, menstruating females, females taking hormone replacement therapy (HRT), pregnant females, females on birth control, and males. The excretion rates for the six demographic profiles used in this study were taken from Johnson and Williams (2004) from two Standard Errors to give 95% confidence limits (Table 4-1).

4.3.1.1 Menopausal Females (E1 and E2)

The model uses a revised estimated age of menopause derived from a study where researchers analyzed data collected from 4694 premenopausal women enrolled in the New York University Women Study. In an average of 5.4 years of observation, there were 2035 incidences of menopause, with the mean age of 51.3 years (Kato et al., 1998). Using the city of Calgary
census data (City of Calgary, 2011) and the mean age of 51 categorizes approximately 25% of the total female population as menopausal females (Table 4-1).

4.3.1.2 Menstruating females (E1 and E2)

The model uses a revised menstruating age group derived from two studies (Al-Sahab et al., 2010; Kato et al., 1998). In one study, researchers analyzed data collected from 1403 individuals to determine the estimated mean age of menarche in Canada. The study showed the estimated mean and median of age at menarche was 12.72 years (SD = 1.05) and 12.67 years, respectively (Al-Sahab et al., 2010). As described previously, the mean age for menopause used in the model is 51 years based on a study of 4694 premenopausal women during a 5.4 year period (Kato et al. 1998). Since menstruation last until menopause, the model assumes the age group from 13 to 51 years of age. Since pregnant woman excrete E1 and E2 at different rates, and woman that use birth control continue to excrete endogenous estrogen (Williams et al., 2003), the population of menstruating females was therefore estimated by subtracting the number of pregnant women from the total number of women between the ages of 13 and 51. This will represent 58% of the total female population or 29% of the total population (Table 4-1).

4.3.1.3 Women on HTR (E1 and E2)

The model uses a revised HRT usage value based on the age-standardized rate of estrogen HRT use in Alberta for 2006-2007 of 5.4% (CIHI, 2008). Therefore, for the purpose of this study, 5.4% of the total female population is assumed to be using estrogen-only HRT (Table 4-1).

4.3.1.4 Pregnant Women (E1 and E2)

The model uses a revised pregnancy rate based on the average crude birth rate in Alberta
from 2001 to 2010: 13.3/1000 persons or 1.33% per person (GoA, 2011). Since 49.8% of the total population for the city of Calgary is estimated to be female, based on the city of Calgary census age and gender distribution data (City of Calgary, 2011), 2.67% of the female population is therefore estimated to be pregnant (Table 4-1).

4.3.1.5 Males (E1 and E2)

It has been shown that estrogen and urine production in children of both sexes are extremely low until puberty (Winter et al., 1978 as reported in Johnson and Williams, 2004). The results of one study in Demark showed that the average age for male puberty was 13 years (Jorgensen et al., 1991). Therefore, this study will exclude estrogen production from prepubescent males of 13 years of age and younger. As a result, E1 and E2 excretion rates for 84% of the total male population was accounted for and likewise 16% of the male population (or 8% of the total population) was not accounted for (Table 4-1). In contrast, other studies such as Johnson and Williams (2004) assumed excretion rates were constant over the entire male population. However, it is important to note that the mean estimates for males and females will be slightly low since prepubescent males and females were not included in this study.

4.3.1.6 Women on Birth Control - EE2

Fisher and Boroditsky (1999) reported that 28% of all women in Canada used OC as the dominant method of contraception. Therefore, for the purpose of this study, 28% of all women are assumed to be excreting EE2 (Table 4-1). Furthermore, and consistent with the dosage used by Johnson and Williams (2004), a dose of 35 µg/d was chosen.
Table 4-1: Estrogen excretion rates and corresponding group size

<table>
<thead>
<tr>
<th>Estrogen Compound</th>
<th>Demographic Group</th>
<th>Per Capita Mean (Range) Excretion (µg/day)</th>
<th>Group Size (%)^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>Males</td>
<td>2.6 (1.4-2.9)</td>
<td>84 (42) ^c</td>
</tr>
<tr>
<td></td>
<td>Menstruating females</td>
<td>11.7 (7.5-15.4)</td>
<td>58 (29) ^d</td>
</tr>
<tr>
<td></td>
<td>Menopausal females</td>
<td>1.8 (0.0-5.7)</td>
<td>25 (13) ^e</td>
</tr>
<tr>
<td></td>
<td>Women on HRT</td>
<td>28.4 (24.0-33.0)</td>
<td>5.4 (2.7) ^f</td>
</tr>
<tr>
<td></td>
<td>Pregnant Women</td>
<td>550 (432-668)</td>
<td>2.7 (1.1) ^g</td>
</tr>
<tr>
<td>E2</td>
<td>Males</td>
<td>1.8 (1.3-2.4)</td>
<td>84 (42) ^c</td>
</tr>
<tr>
<td></td>
<td>Menstruating females</td>
<td>3.2 (1.7-4.6)</td>
<td>58 (29) ^d</td>
</tr>
<tr>
<td></td>
<td>Menopausal females</td>
<td>1.0 (0.0-3.5)</td>
<td>25 (13) ^e</td>
</tr>
<tr>
<td></td>
<td>Women on HRT</td>
<td>56 (51.5-61.5)</td>
<td>5.4 (2.7) ^f</td>
</tr>
<tr>
<td></td>
<td>Pregnant Women</td>
<td>393 (340-445)</td>
<td>2.7 (1.3) ^g</td>
</tr>
<tr>
<td>EE2</td>
<td>Women on Birth Control</td>
<td>10.5 (9.6-11.3)</td>
<td>28 (14) ^h</td>
</tr>
</tbody>
</table>

^a the mean excretion rate and upper and lower values derived by Johnson and Williams (2004) to give 95% confidence limits; ^b Group size percent is derived from the total male population and total female population. The group size percent is also derived from the total population as shown in brackets; ^c (Jorgensen et al., 1991); ^d (Al-Sahab et al., 2010; Kato et al., 1998); ^e (Kato et al., 1998); ^f (CIHI, 2008); ^g (GoA, 2011); ^h Fisher and Boroditsky (1999).

4.3.2 Estrogen Transformation Rate

As discussed further in this paper, the estimated sewer transformation rate of E2 to E1 is calculated based on the results of the preliminary model simulation for the WWTP in Brandon Manitoba. The estimated sewer rate used in the model simulation for Bonnybrook and Fish Creek WWTP’s is 27%.

4.3.3 WWTP Estrogen Removal Rate

The mean percent estrogen influent-to-effluent removal values used in the model were calculated with 95% confidence limits using measured raw data collected from other WWTPs throughout Canada (Table 4-2).
Table 4-2: Data sources and calculated Canadian WWTP Mean Estrogen Removal Percentage

<table>
<thead>
<tr>
<th>Reference</th>
<th>E1(n)</th>
<th>E2(n)</th>
<th>EE2(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baronti et al., 2000</td>
<td>-</td>
<td>-</td>
<td>30&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cicek et al., 2007&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Lee et al., 2005a</td>
<td>11</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>Lee et al., 2005b</td>
<td>6</td>
<td>8</td>
<td>No data</td>
</tr>
<tr>
<td>Pauwels et al., 2008</td>
<td>6</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Servos et al., 2005</td>
<td>9</td>
<td>9</td>
<td>No data</td>
</tr>
</tbody>
</table>

Mean Removal %

|               | 85 (±6)<sup>c</sup> | 86 (80-93) | 75 (±7) |

<sup>a</sup> reference provides estrogen sample averages only, the raw data related to this reference and used in this study was obtained directly from the author but unpublished; <sup>b</sup> (n) raw data sample size used; <sup>c</sup> ( ) calculated 95% confidence limits; <sup>d</sup>EE2 data supplemented from a WWTP in Italy due to limited data within Canada; Note: data showing an influent-to-effluent concentration increase within WWTPs were not considered due to reported limitations in analytical methods preventing the detection of conjugated estrogen compounds commonly present in raw influent (Lee et al., 2005a; Nelson et al., 2007; Fernandez et al., 2007b).

The mean excretion values used in the model accounts for both conjugated and unconjugated forms of estrogen. However, as discussed previously, analytical methods can prevent the detection of conjugated estrogen compounds present in raw influent and detected the transformed unconjugated compounds during the WWTP process (Lee et al., 2005a; Nelson et al., 2007; Fernandez et al., 2007b). These cases led to an increase in effluent over influent values due to analytical methods; therefore, measurements showing an increase in effluent concentration were not considered.
4.3.4 Removal Matrix

The calculated removal matrix results used in the model are described in Table 4-3.

<table>
<thead>
<tr>
<th>Estrogen</th>
<th>Primary Clarifier</th>
<th>Activated Sludge Bioreactor&lt;sup&gt;a,b&lt;/sup&gt;</th>
<th>Secondary Clarifier&lt;sup&gt;c&lt;/sup&gt;</th>
<th>UV Disinfection&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>*</td>
<td>87 (86-89)</td>
<td>9 (4-10)</td>
<td>3 (1-7)</td>
</tr>
<tr>
<td>E2</td>
<td>30 (25-35)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>56 (53-59)</td>
<td>9 (7-11)</td>
<td>2 (1-3)</td>
</tr>
<tr>
<td>EE2</td>
<td>38 (31-44)</td>
<td>20 (17-22)</td>
<td>25 (15-31)</td>
<td>4 (2-6)</td>
</tr>
</tbody>
</table>

<sup>a</sup> n = 2 (Andersen et al. 2003); <sup>b</sup> percentages for the activated sludge bioreactor accounts for dissolved and sorbed estrogen; percentages do-not account for nitrification which is consistent with the facilities in this study; <sup>c</sup> n = 8 (Cicek et al. 2007); <sup>c</sup> brackets indicate the range; “*” = data available but not used as this study assumes that any increase in estrogen concentration between treatment units is due to the analytical methods employed that researchers suggest do-not account for the initial presence of conjugated estrogen that commonly unconjugates during the treatment process (Lee et al., 2005a; Nelson et al., 2007; Fernandez et al., 2007b).

4.4 Model Assumptions
The model contains numerous assumptions that are listed below:

1) The demographic profiles used in the E1 and E2 model will assume the following:
   a) The age and gender percentages calculated using the city of Calgary census data matches the cities of Edmonton and Brandon.
   b) The model also assumes the demographic profiles parameters are consistent among all cities.

2) The steroid estrogen transformation during sewer transit will assume the following:
   a) The model will assume that the calculated transformation of E2 to E1 in the Brandon simulation will hold true for the Calgary simulations;
   b) It will be assumed that E1 & EE2 will not be transformed during sewer transit based on previous observations of E1 and EE2 in activated sludge microcosms.
(Johnson and Williams, 2004).

3) The EE2 model assumes the following:

a) 28% of the total female population consumes the contraceptive pill based on the findings of Fisher and Boroditsky (1999);

b) For the purposes of this study, and consistent with the dosage used by Johnson and Williams (2004), a dose of 35 µg/d was chosen. It was assumed that British medical practice would not differ greatly from other industrialized Western countries.

4) The estrogen model will assume the following:

a) All major sources of estrogens have been accounted: E1, E2, and EE2.

b) Only estrogen excreted from humans are entering and leaving the WWTP.

c) Where estrogen was not detected during the sampling analysis in the WWTPs, it is assumed that estrogen is present but below the applicable detection limits.

d) This study assumes that any increase in estrogen concentration between treatment units is due to the analytical methods employed that researchers suggest do not account for the initial presence of conjugated estrogen that commonly unconjugates during the treatment process.

5) The model assumes a normal distribution curve for the 95% confidence limit calculations.

4.5 Model Limitations
The model contains limitations that are listed below:

1) The removal matrix was developed using limited data and further data is required to better corroborate the removal matrix model.
2) The 95% confidence limits were calculated using data from various WWTPs within Canada and additional local data is required to further corroborate the influent-to-effluent removal percentages.

3) The model do not account for estrogen accumulation in the lagoon sludge that is transported for use in agricultural fields.
Chapter 5 - Results and Discussion

5.1 Results

5.1.1 Model Simulation for WWTPs in Brandon, Manitoba and Edmonton, Alberta

The results of the model and actual measurements for the Brandon and Edmonton WWTPs are summarized in Figures 5-1, 5-2, 5-3, 5-4, and Tables 5-1, 5-2, and 5-3. Model estimations for E1 and E2 together are in agreement with actual measurements for the Brandon facility (i.e. 10% difference influent and <1% difference effluent). The mean E1 and E2 influent and effluent concentration measurements per day for the Brandon facility lie within the upper and lower confidence limits of the model; standard deviations between the measured and estimated means for the Brandon facility are similar. In contrast, the estimated EE2 for the Brandon facility is not supported by the actual measurements: 97% influent difference (189% influent change) and 162% effluent difference (843% effluent change). As such, the model for EE2 was subsequently calibrated, through trial and error, to ensure a good fit between the limits of the model and the EE2 measurements prior to simulating the model for the Edmonton and Calgary facilities. Model calibration was achieved by adjusting the upper and lower limits to a point that included all of the measurements for half of the Brandon data set. For EE2 influent, model calibration was achieved by increasing the upper limits by factor of 6.4, increasing the mean estimated values by a factor of 2.9, and decreasing the lower limits by a factor of 1.4. For the influent-to-effluent EE2 percent removal, model calibration was achieved by decreasing the upper removal percentage from 87.2% to 62%, the estimated mean from 80.5% to 40%, and the lower removal percentage from 73.8% to 0%. Overall, the initial simulation of the model appears to be in agreement with the measurements for E1 and E2, and the model required calibration in order to achieve a good fit for EE2. Note: the cutoff for model agreement with actual
measurements was based on the model covering 90% of all daily mean estrogen measurements—a similar approach used by Ram and Gillett (1993).

The Edmonton simulation for estrogen showed agreement with the mean E2-eq measurements for the Edmonton facility (i.e. 4% influent difference). However, the standard deviations between measurements and the model showed large variance. A slight improvement in the standard deviations can be seen when the EE2 calibrated version of the model, based on the Brandon simulation calibration results for EE2, is used. However, the standard deviations and the means are very similar and comparable when sample number 29, a potential outlier or anomaly, is removed and no calibration is applied: twenty-five of the 30 E2-eq estrogen influent measurement samples for the Edmonton facility lie within the upper and lower confidence limits of the model. Note: the threshold to determine outliers in this study includes measurements that are more than twice the standard deviation away from the mean; this approach is common and consistent with previous data analysis (Hellerstein, 2008). The potential outlier identified for the Edmonton data set was approximately four standard deviations away from the mean.

Figure 5-1: Estimated Brandon and Edmonton WWTP estrogen influent
Note 1: B. represents the Brandon WWTP; Note 2: Ed. Represents the Edmonton WWTP; Note 3: C. represents calibration; Note 4: O. represents sample 29 outlier/anomaly removed; Note 5: The error bars represent the standard deviation from the mean.

Table 5-1: Brandon WWTP predicted and actual estrogen influent concentration

<table>
<thead>
<tr>
<th>Influent</th>
<th>Model (μg/l)</th>
<th>Actual (μg/l)</th>
<th>Mean % D&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
<td>Avg</td>
</tr>
<tr>
<td>95% L.L.</td>
<td>0.038</td>
<td>0.037</td>
<td>0.037</td>
</tr>
<tr>
<td>E1+ E2</td>
<td>Mean</td>
<td>0.059</td>
<td>0.059</td>
</tr>
<tr>
<td></td>
<td>95% U.L.</td>
<td>0.073</td>
<td>0.072</td>
</tr>
<tr>
<td>EE2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Mean</td>
<td>0.0038</td>
<td>0.0038</td>
</tr>
<tr>
<td></td>
<td>95% L.L.</td>
<td>.0035</td>
<td>.0035</td>
</tr>
<tr>
<td></td>
<td>95% U.L.</td>
<td>.0041</td>
<td>.0041</td>
</tr>
<tr>
<td>EE2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Mean</td>
<td>.0211</td>
<td>.0020</td>
</tr>
<tr>
<td></td>
<td>95% U.L.</td>
<td>.0263</td>
<td>.0261</td>
</tr>
</tbody>
</table>

<sup>a</sup> EE2 simulation without calibration; <sup>b</sup> EE2 simulation with calibration; <sup>c</sup> Percent difference calculated as \[\frac{(V_1-V_2)}{(V_1+V_2)/2}] \times 100; <sup>d</sup> Percent change calculated as \[\frac{(V_2-V_1)}{V_1}] \times 100]
Table 5-2: Edmonton WWTP total predicted and actual estrogen influent concentration

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Influent</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model (μg/l)</td>
<td>Actual (μg/l)</td>
<td>Mean % D&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Estrogen</td>
<td>Mean</td>
<td>0.070</td>
<td>0.073 (0.060)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4% (15%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>95% L.L.</td>
<td>0.047</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% U.L.</td>
<td>0.086</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> brackets represents the value when sample no. 29 is removed as a potential outlier; <sup>b</sup> Percent difference calculated as 
\[ \frac{(V_1-V_2)/(V_1+V_2/2)}*100; \]

Figure 5-2: Estimated total estrogen for the Edmonton WWTP
Figure 5-3 Estimated total estrogen for the Edmonton WWTP using EE2 calibration

Figure 5-4: Estimated estrogen effluent for Brandon WWTP

Note: The error bars represent the standard deviation from the mean.
### Table 5-3: Brandon WWTP predicted and actual estrogen effluent concentration

<table>
<thead>
<tr>
<th>Effluent</th>
<th>Model (μg/l)</th>
<th>Actual (μg/l)</th>
<th>Mean %D&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
<td>Avg</td>
</tr>
<tr>
<td>E1+ E2</td>
<td>.0022</td>
<td>.0022</td>
<td>.0022</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% U.L.</td>
<td>.056</td>
<td>.055</td>
<td>.016</td>
</tr>
<tr>
<td>95% L.L.</td>
<td>.0004</td>
<td>.0004</td>
<td>.0004</td>
</tr>
<tr>
<td>EE2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% U.L.</td>
<td>.0011</td>
<td>.0011</td>
<td>.0011</td>
</tr>
<tr>
<td>95% L.L.</td>
<td>.0021</td>
<td>.0020</td>
<td>.0020</td>
</tr>
<tr>
<td>EE2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% U.L.</td>
<td>.010</td>
<td>.0099</td>
<td>.0099</td>
</tr>
</tbody>
</table>

<sup>a</sup> EE2 simulation without calibration; <sup>b</sup> EE2 simulation with calibration; <sup>c</sup> Percent difference calculated as \((V_1-V_2)/(V_1+V_2/2)\)*100; <sup>d</sup> Percent change calculated as \(((V_2-V_1)/V_1)\)*100

5.1.1.1 Edmonton and Brandon Model Validation

For the Brandon facility, 100% of the mean daily influent and effluent values predicted for E1 and E2 fell within the 95% confidence intervals for the model while 100% of the mean daily influent and effluent values predicted for EE2 fell within the 95% confidence intervals using calibration. For the Edmonton WWTP, 79% of the mean daily influent values predicted for total estrogen or E1+E2+EE2 lies within the 95% confidence intervals for the model. Note: as previously written, only total estrogen values can be validated for the Edmonton WWTP due to type of data collected from the facility, i.e. E2-eq.
A regression analysis between influent measurements and predictions for the Brandon Model gave $R^2$ values of 0.81 for E1, 0.95 for E2, and 0.98 for EE2 (Figure 5-5). A regression analysis between effluent measurements and predictions for the Brandon Model gave $R^2$ values of 0.99 for E1, 0.80 for E2, and 0.80 for EE2 (Figure 5-5). A regression analysis between influent measurements and predictions for the Edmonton Model gave an $R^2$ value of 0.84 (Figure 5-6).

No regression analysis for estrogen effluent can be completed for the Edmonton model due to limited data.

Figure 5-5: Comparison between modelled and actual measurements for E1, E2, and EE2 at the Brandon WWTP site for both influent and effluents. Similar analyses were not conducted due to lack of actual measurements at other WWTP sites.
Figure 5-6: Comparison between modelled and actual measurements for the total estrogen influent at the Goldbar WWTP site. Similar analyses were not conducted due to lack of actual measurements at other WWTP sites.

5.1.2 Calculated WWTP Estrogen Transformation Rate

Since the results for the Brandon WWTP model simulation are in agreement with the actual measurements when analyzing E1+E2 together, the estimated transformation rate of E2 to E1 is therefore calculated to be 27%; thus, 27% will be the assumed transformation rate of E2 to E1 used for the Calgary facility model analysis.

5.1.3 Fish Creek WWTP Estimated Influent

The influent results for Fish Creek show estimated values between 2002 and 2012 ranging from 0.028-0.061 µg/l for E1, 0.013-0.024 µg/l for E2, 0.0034-0.0050 µg/l for EE2, and 0.045-0.090 µg/l for the total estrogen (Figure 5-7; Figure 5-8; Figure 5-9 and Figure 5-10). When the EE2 calibrated version of the model is applied, based on the previous Brandon simulation, the EE2 values range from 0.002-0.032 µg/l, and the total estrogen from 0.043-0.12 µg/l (Figure 5.11 and Figure 5.12). Although there are no Fish Creek influent measurements for comparison, the estimated E1, E2, and EE2 influent values are within the range of other commonly reported Canadian values of 0.008-0.078 µg/l for E1, of 0.0024-0.026 µg/l for E2.
(Lee et al., 2005b; Lishman et al., 2006; Servos et al., 2005), and <0.001-0.039 µg/l for EE2 (Baronti et al., 2000; Cicek et al., 2007; Fernandez et al., 2007). Also, comparing the modeled influent for Fish Creek WWTP against the Brandon WWTP measurements, two Western Canadian facilities serving generally small populations, show that E1, E2, and EE2 mean measurements at the Brandon facility are within the estimated upper and lower limits of the Fish Creek facility (Figure 5-13). Only the EE2 calibrated version of the model allows for the EE2 mean measurement to lie within the limits of the model; calibration also moves the total estrogen mean measurement closer to the estimated mean verses non-calibration.

The trend-line \( R^2 = 0.79475 \) results in an annual relative growth rate of 0.0008 µg/l for E1, 0.0003 µg/l for E2, 0.00008 µg/l for EE2, 0.0002 µg/l for EE2 using calibration, 0.0012 µg/l for the total estrogen, and 0.0013 µg/l for the total estrogen using calibration (Table 5-4).

Projecting the influent concentrations to the year 2020 shows a mean annual projected high of approximately 0.054 µg/l (0.040 – 0.067 µg/l range) for E1, 0.022 µg/l (0.018-0.027 µg/l range) for E2, 0.0053 µg/l (±0.0005) for EE2, 0.015 µg/l (0.0025-0.036 µg/l range) for EE2 calibration, 0.08 µg/l (±0.02) for the total estrogen, and 0.091 µg/l (0.06-0.13 µg/l range) for total estrogen with calibration (Table 5-5). Estrogen composition can be described as E1>E2>EE2: E1 66% (63-68% range), E2 27% (26-29% range), and EE2 7% (6-8% range) [Figure 5-14]. Estrogen composition, using the EE2 calibrated version of the model, can be also be described as E1>E2>EE2: E1 59% (52-65% range), E2 24% (21-30% range), and EE2 17% (5-28% range); however, as expected the EE2 composition increases significantly [Figure 5-15].
Figure 5-7: Estimated Fish Creek WWTP E1 influent from 2002 to 2012

\[ y = 0.0003x + 0.0155 \]
\[ r^2 = 0.79 \]

Figure 5-8: Estimated Fish Creek WWTP E2 influent from 2002 to 2012

\[ y = 0.0008x + 0.0376 \]
\[ r^2 = 0.79 \]
Figure 5-9: Estimated Fish Creek WWTP EE2 influent from 2002 to 2012

\[ y = 8 \times 10^{-5}x + 0.0036 \]

\[ r^2 = 0.79 \]

Figure 5-10: Estimated Fish Creek WWTP total estrogen influent from 2002 to 2012

\[ y = 0.0013x + 0.0568 \]

\[ r^2 = 0.79 \]
Figure 5-11: Estimated Fish Creek EE2 influent using the EE2 calibrated version of the model

Figure 5-12: Estimated Fish Creek total estrogen using the EE2 calibrated version of the model
Figure 5-13: Comparison of Fish Creek influent model versus actual measurements

Figure 5-14: Estimated Fish Creek WWTP total estrogen influent composition from 2002 to 2012
5.1.4 Bonnybrook WWTP Estimated Influent

Bonnybrook estimated influent values between 2002 - 2012 range from 0.018-0.036 µg/l for E1, 0.010-0.019 µg/l for E2, 0.0027-0.0038 µg/l for EE2, and 0.03-0.06 µg/l for the total estrogen (Figure 5-16; Figure 5-17; Figure 5-18; Figure 5-19). When the EE2 calibrated version of the model is applied, based on the previous Brandon simulation, the EE2 values range from 0.0016-0.024 µg/l, and the total estrogen from 0.030-0.079 µg/l (Figure 5-20 and Figure 5-21). Similar to Fish Creek, there are also no direct Bonnybrook influent measurements for comparison; however, the estimated E1 influent values lie within the range of other commonly reported Canadian values of 0.008-0.078 µg/l for E1, 0.0024-0.026 µg/l for E2 (Lee et al., 2005b; Lishman et al., 2006; Servos et al., 2005), and values of <0.001-0.039 µg/l for EE2 (Baronti et al., 2000; Cicek et al., 2007; Fernandez et al., 2007). Furthermore, comparing the Bonnybrook estimated influent values against the city of Edmonton mean measurements, two Western facilities serving larger populations, show that the mean influent total estrogen measurements for 2006 are within the estimated upper and lower range of the model when the EE2 calibrated version of the model is applied (Figure 5-22). The predicted E1, E2, and EE2 concentration dips
in 2005 and 2011 due to higher than average daily flows. In fact, 2005 was the highest average daily flow reported, i.e. 406,977 m³/d, from 2002 to 2012. The trend-line ($R^2 = 0.77328$) results in an annual relative growth rate of 0.0005 µg/l for E1, 0.0003 µg/l for E2, 0.00006 µg/l for EE2, 0.0002 µg/l for EE2 using calibration, 0.00086 µg/l for the total estrogen, and 0.001 µg/l for total estrogen using calibration (Table 5-4). Projecting the influent concentrations to the year 2020 shows a mean annual projected high of approximately 0.033 µg/l (0.025-0.041 µg/l range) for E1, 0.018 µg/l (0.014-0.021 µg/l range) for E2, 0.004 µg/l (±0.0003) for EE2, 0.012 µg/l (0.002-0.028 µg/l range) for EE2 using calibration, estrogen total of 0.055 µg/l (±0.0012), and estrogen total using calibration of 0.063 µg/l (0.04-0.09 µg/l range) [Table 5-5]. Similarly to Fish Creek, the total estrogen composition for Bonnybrook can also be described as E1>E2>EE2: E1 60% (58-61% range), E2 32% (31-32% range), and EE2 8% (7-9% range) [Figure 5-23]. Estrogen composition, using the EE2 calibrated version of the model, can be also be described as E1>E2>EE2: E1 53% (45-60% range), E2 28% (24-34% range), and EE2 19% (5-31% range); however, and similar to Fish Creek, the EE2 composition increases significantly [Figure 5-24].

Figure 5-16: Estimated Bonnybrook WWTP E1 influent from 2002 to 2012. Note: no influent measurements are available for comparison.
Figure 5-17: Estimated Bonnybrook WWTP E2 influent from 2002 to 2012. Note: no influent measurements are available for comparison.

Figure 5-18: Estimated Bonnybrook WWTP EE2 influent from 2002 to 2012. Note: no influent measurements are available for comparison.
Figure 5-19: Estimated Bonnybrook WWTP total estrogen influent from 2002 to 2012

Figure 5-20: Estimated Bonnybrook EE2 influent using EE2 calibrated version of the model. Note: no influent measurements are available for comparison.
Figure 5-21: Estimated Bonnybrook total estrogen influent using the EE2 calibrated model

\[ y = 0.001x + 0.0442 \]
\[ r^2 = 0.77 \]

Figure 5-22: Comparison of Bonnybrook influent model verses actual measurements
5.1.5 Fish Creek WWTP Estimated Effluent

Fish Creek estimated E1 effluent values between 2002 - 2012 range from 0.0035-0.013
µg/l, 0.0009-0.0049 µg/l for E2, 0.0006-0.0016 µg/l for EE2, and 0.0062-0.019 µg/l for the total estrogen (Figure 5-25; Figure 5-26; Figure 5-27; Figure 5-28). When the EE2 calibrated version of the model is applied, based on the previous Brandon simulation, the EE2 values range from 0.002-0.012 µg/l, and the total estrogen from 0.005-0.024 µg/l (Figure 5-29 and Figure 5-30). As expected, the effluent values are lower than influent concentrations. Fish Creek measurements on January 15, 2003 and July 09, 2012 both fall below the detection levels of 0.0068 µg/l and 0.004 µg/l for E1, and 0.005 µg/l and 0.004 µg/l for E2 respectively (Figure 5-25; Figure 5-26). Although exact concentrations are not known, the estimated values in the model support E1 and E2 concentrations that lie below the detection limits; however, these concentrations are mostly towards the lower limits of the model. The EE2 measurements taken at the Fish Creek facility in 2003 also fits well within the upper and lower limits of the calibrated model.

Comparing the Fish Creek WWTP model results against the Brandon WWTP measurements, two Western Canadian facilities serving generally small populations, show that the E1, E2, and EE2 mean measurements taken at the Brandon facility are within the estimated upper and lower limits of the Fish Creek facility (Figure 5-31). Similarly to the EE2 measurement taken at Fish Creek in 2003, the Brandon measurement of EE2 only fits within the calibrated version of the model. Also, the estimated effluent values lie within the range of other commonly reported Canadian values of <0.001-0.096 µg/l for E1, <0.001-0.015 µg/l for E2 (Lee et al., 2005b; Lishman et al., 2006; Servos et al., 2005), and <0.001-0.005 µg/l for EE2 (Baronti et al., 2000; Cicek et al., 2007; Fernandez et al., 2007). However, sporadic effluent values of EE2 have been reported up to 0.042 µg/l in Canada (Ternes et al., 1999a). The trend-line ($R^2$ =0.79475) results in an annual relative growth rate of 0.0001 µg/l for E1, 0.00004 µg/l for E2, 0.00002 µg/l for EE2, 0.0001 µg/l for EE2 using the calibrated model, 0.00016 µg/l total
estrogen, and 0.00024 µg/l for the total estrogen using the calibrated model (Table 5-4).

Projecting the effluent concentrations to the year 2020 shows a mean annual projected high of approximately 0.008 µg/l (range: 0.004-0.016 µg/l) for E1, 0.0024 µg/l (range: 0.0008-0.0049 µg/l) for E2, 0.0011 µg/l (range: 0.0006-0.0014 µg/l) for EE2, 0.0089 µg/l (range: 0.003-0.014 µg/l) for EE2 using calibration, 0.012 µg/l (range: 0.0050-0.0220 µg/l) for the total estrogen, and 0.019 µg/l (range: 0.0080-0.034 µg/l) for the total estrogen using the calibrated model (Table 5-5). Similar to the influent results, the total estrogen composition of effluent can be described as E1>E2>EE2: E1 71% (70-74% range), E2 21% (14-22% range), and EE2 9% (7-11% range) [Figure 5-32]. However, the estrogen composition changes with the calibrated version of the model—E1>EE2>E2: E1 45% (41-52% range), and EE2 40% (38-46% range), and E2 13% (10-14% range) [Figure 5-33].

Figure 5-25: Estimated Fish Creek WWTP E1 effluent from 2002 to 2012
Figure 5-26: Estimated Fish Creek WWTP E2 effluent from 2002 to 2012

Figure 5-27: Estimated Fish Creek WWTP EE2 effluent from 2002 to 2012
Figure 5-28: Estimated Fish Creek WWTP total estrogen effluent from 2002 to 2012

![Graph showing estimated total estrogen effluent from 2002 to 2012 with regression line y = 0.0002x + 0.0081 and r² = 0.79]

Figure 5-29: Estimated Fish Creek EE2 effluent using the EE2 calibrated model

![Graph showing estimated EE2 effluent from 2002 to 2012 with regression line y = 0.0001x + 0.0063 and r² = 0.79]
Figure 5-30: Estimated Fish Creek total estrogen effluent using the EE2 calibrated model

Figure 5-31: Comparison of Fish Creek effluent model verses actual measurements
5.1.6 Bonnybrook WWTP Estimated Effluent

Bonnybrook estimated effluent values between 2002 - 2012 range from 0.0013-0.008 µg/l for E1, 0.0004-0.0033 µg/l for E2, 0.0003-0.0010 µg/l for EE2, 0.002-0.012 µg/l for EE2 using the calibrated model, 0.002-0.013 µg/l for the total estrogen, and 0.003-0.021 µg/l for total
estrogen using the calibrated model (Figure 5-34; Figure 5-35; Figure 5-36; Figure 5-37, Figure 5-38, Figure 5-39). Similar to Fish Creek, the effluent values are lower than influent concentrations. E1 Measurements in 2003 and 2012 of 0.0026 µg/l and 0.007 µg/l respectively, both lie within the estimated values in the model (Figure 5-34; Figure 5-40). Similarly, the 2003 E2 measurement of 0.0015 µg/l is also within the estimated range of concentrations; the 2012 E2 measurement is below the detection limit of 0.004 µg/l, and concentrations below these limits are supported by the model (Figure 5-35; Figure 5-40). In addition, measurements for EE2 in 2003 and 2012 also lie below the detection levels of 0.0039 µg/l and 0.005 µg/l respectively, and are supported by the model (Figure 5-36 and 5-37). Similar to Fish Creek, the estimated values for Bonnybrook lie within the typically reported Canadian concentrations between <0.001-0.096 µg/l for E1, <0.001-0.015 µg/l for E2 (Lee et al., 2005b; Lishman et al., 2006; Servos et al., 2005), and <0.001-0.005 µg/l for EE2 (Baronti et al., 2000; Cicek et al., 2007; Fernandez et al., 2007).

Consistent with E1 influent fluctuations, the predicted E1 effluent concentration dips in 2005 and 2011 due to the higher than average daily flows. The trend-line \( R^2 = 0.77328 \) results in an annual relative growth rate of 0.00008 µg/l for E1, 0.00003 µg/l for E2, 0.00001 µg/l for EE2, 0.0001 µg/l for EE2 using the calibrated model, 0.00012 µg/l for the total estrogen, and 0.00021 µg/l for the total estrogen under calibration (Table 5-4). Projecting the effluent concentrations to the year 2020 shows a mean annual projected high of approximately 0.0050 µg/l (range: 0.002-0.099 µg/l) for E1, 0.0019 µg/l (range: 0.0006-0.0038 µg/l) for E2, 0.0008 µg/l (±0.0003) for EE2, 0.007 µg/l (range: 0.002-0.011 µg/l) for EE2 under calibration, 0.008 µg/l (range: 0.003-0.015 µg/l) for the total estrogen, and 0.014 µg/l (range: 0.004-0.024 µg/l) for the total estrogen under calibration (Table 5-5). Consistent with Fish Creek, the Bonnybrook total estrogen
composition can be also described as E1>E2>EE2: E1 64% (62-65% range), E2 25% (20-27% range), and EE2 11% (8-16% range) [Figure 5-41]. Also, similar to Fish Creek, estrogen composition changes with the calibrated version of the model—EE2>E1>E2: EE2 49% (45-51% range), E1 35% (33-39% range), and E2 14% (13-16% range) [Figure 5-42].

Figure 5-34: Estimated Bonnybrook WWTP E1 effluent from 2002 to 2012
Figure 5-35: Estimated Bonnybrook WWTP E2 effluent from 2002 to 2012

![Graph showing estimated E2 effluent concentrations from 2002 to 2012. The graph includes a trend line with the equation $y = 3 \times 10^{-5}x + 0.0014$ and an $r^2$ value of 0.77.](image)

Figure 5-36: Estimated Bonnybrook WWTP EE2 effluent from 2002 to 2012

![Graph showing estimated EE2 effluent concentrations from 2002 to 2012. The graph includes a trend line with the equation $y = 1 \times 10^{-5}x + 0.0006$ and an $r^2$ value of 0.77.](image)
Figure 5-37: Estimated Bonnybrook WWTP EE2 effluent using the EE2 calibrated model

Figure 5-38: Estimated Bonnybrook WWTP total estrogen effluent from 2002 to 2012
Figure 5-39: Estimated Bonnybrook total estrogen effluent using the EE2 calibrated model

Figure 5-40: Comparison of Bonnybrook effluent model verses actual measurements
Figure 5-41: Estimated Bonnybrook WWTP total estrogen effluent composition 2002 to 2012

Figure 5-42: Bonnybrook total estrogen effluent composition using the EE2 calibrated model
5.1.7 Fish Creek and Bonnybrook Model Validation

No direct influent measurements are available for validation for the Fish Creek and Bonnybrook facilities. However, 100% of the mean daily effluent values measured for E1 and E2 lies within the 95% confidence intervals for the Bonnybrook WWTP model. The mean daily effluent value measured for EE2 within the Fish Creek facility also is within the 95% confidence intervals for the model. Comparing measurements taken from the Brandon and Goldbar WWTP’s against predictions with Fish Creek and Bonnybrook respectively, due to similarities among facilities and limited available data, shows 100% of the E1, E2, and EE2 mean daily influent and effluent measurements taken within the Brandon WWTP lies within the predicted intervals for the Fish Creek WWTP. The mean total estrogen measurement taken from the Edmonton WWTP—a larger and comparable facility to Bonnybrook—lies within the predicted intervals for the Bonnybrook WWTP. Note: as previously written, only total estrogen measurements from the Edmonton WWTP are included due to type of data collected from the facility, i.e. E2-eq.

A regression analysis was completed with a) measurements from the Brandon WWTP and predictions from the Fish Creek WWTP and b) measurements with the Goldbar WWTP and predictions with Bonnybrook WWTP. The influent regression analysis for the Fish Creek gave $R^2$ values of 0.84 for E1, 0.98 for E2, and 0.99 for EE2; the influent regression analysis for the total estrogen within Bonnybrook gave an $R^2$ value of 0.98 (Figure 5-43). The effluent regression analysis for the Fish Creek gave $R^2$ values of 0.97 for E1, 0.76 for E2, and 0.97 for EE2 (Figure 5-43). Note: individual grab sample data was used from the Brandon Facility and 24-hr composite sample data was used from the Edmonton facility. No regression analysis for estrogen effluent can be completed for the Bonnybrook WWTP due to limited data.
Figure 5-43: Regression analysis for Fish Creek and Bonnybrook WWTPs.

- **Fish Creek WWTP (E1 Influent)**
  - Equation: $y = 0.4935x + 0.0193$
  - Correlation: $r^2 = 0.84$
  - RMSE: 0.0092

- **Fish Creek WWTP (E2 Influent)**
  - Equation: $y = 0.8964x + 0.0019$
  - Correlation: $r^2 = 0.98$
  - RMSE: 0.0020

- **Fish Creek WWTP (EE2 Influent)**
  - Equation: $y = 0.9871x + 0.0001$
  - Correlation: $r^2 = 0.99$
  - RMSE: 0.0002

- **Bonnybrook WWTP (Total Estrogen)**
  - Equation: $y = 0.8119x + 0.0016$
  - Correlation: $r^2 = 0.97$
  - RMSE: 0.0012

- **Fish Creek WWTP (E1 Effluent)**
  - Equation: $y = 0.4424x + 0.001$
  - Correlation: $r^2 = 0.76$
  - RMSE: 0.0014
5.1.8 Fish Creek and Bonnybrook WWTP Summary Comparisons

In comparison, the estimated influent and effluent concentrations for E1, E2, and EE2 are consistently higher in Fish Creek versus Bonnybrook (Figure 5-44, 5-45, 5-46, and 5-47). In addition, the estimated annual concentration rate of increase, in most cases, is greater in the Fish Creek compared to Bonnybrook (Table 5-4). In contrast, Bonnybrook, in most cases, had the highest percent of increase to the year 2020 due to lower initial concentrations compared to Fish Creek (Table 5-5).

Figure 5-44: Fish Creek and Bonnybrook WWTP influent comparison
Figure 5-45: Fish Creek and Bonnybrook influent comparison using the EE2 calibrated model

Figure 5-46: Fish Creek and Bonnybrook WWTP effluent comparison
Figure 5-47: Fish Creek and Bonnybrook effluent comparison using the EE2 calibrated model

Table 5-4: Annual estimated increase of estrogen (ng/l) for Fish Creek & Bonnybrook WWTPs

<table>
<thead>
<tr>
<th>WWTP</th>
<th>E1</th>
<th>E2</th>
<th>EE2</th>
<th>Total Estrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L.</td>
<td>M.</td>
<td>H.</td>
<td>L.</td>
</tr>
<tr>
<td>F.C.</td>
<td>0.3</td>
<td>0.8</td>
<td>1.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Inf.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B.B.</td>
<td>0.4</td>
<td>0.5</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Inf.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F.C.</td>
<td>0.06</td>
<td>0.1</td>
<td>0.2</td>
<td>0.01</td>
</tr>
<tr>
<td>Eff.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B.B.</td>
<td>0.03</td>
<td>0.08</td>
<td>0.1</td>
<td>0.009</td>
</tr>
<tr>
<td>Eff.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: italic values represent the values generated by the EE2 calibrated version of the model as calculated from the Brandon WWTP.
Table 5-5: Projected increase of WWTP estrogen (ng/l) to the year 2020

<table>
<thead>
<tr>
<th>WWTP</th>
<th>E1</th>
<th>E2</th>
<th>EE2</th>
<th>Total Estrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12a</td>
<td>20b</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>F.C. Infl.</td>
<td>48</td>
<td>54</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>B.B. Infl.</td>
<td>29</td>
<td>33</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>F.C. Effl.</td>
<td>7.2</td>
<td>8</td>
<td>11</td>
<td>2.2</td>
</tr>
<tr>
<td>B.B. Effl.</td>
<td>4.3</td>
<td>5</td>
<td>15</td>
<td>1.7</td>
</tr>
</tbody>
</table>

aYear 2012; bYear 2020; Note: italic values represent the values generated by the EE2 calibrated version of the model as calculated from the Brandon WWTP.

5.1.9 Removal Matrix Results

The calculated removal matrix results are described in Table 5-6.

Table 5-6: The removal percent difference for each pre and post treatment unit process

<table>
<thead>
<tr>
<th>Estrogen</th>
<th>Primary Clarifier a</th>
<th>Activated Sludge Bioreactor a,b</th>
<th>Secondary Clarifier c</th>
<th>UV Disinfection c</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>*</td>
<td>87 (86-89)</td>
<td>9 (4-10)</td>
<td>3 (1-7)</td>
</tr>
<tr>
<td>E2</td>
<td>30 (25-35) c</td>
<td>56 (53-59)</td>
<td>9 (7-11)</td>
<td>2 (1-3)</td>
</tr>
<tr>
<td>EE2</td>
<td>38 (31-44)</td>
<td>20 (17-22)</td>
<td>25 (15-31)</td>
<td>4 (2-6)</td>
</tr>
</tbody>
</table>

a n = 2 (Andersen et al. 2003); b percentages for the activated sludge bioreactor accounts for dissolved and sorbed estrogen; percentages do not account for nitrification which is consistent with the facilities in this study; c n = 8 (Cicek et al. 2007); c brackets indicate the range; “*” = data available but not used as this study assumes that any increase in estrogen concentration between treatment units is due to the analytical methods employed that researchers suggest do not account for the initial presence of conjugated estrogen that commonly unconjugates during the treatment process (Lee et al., 2005a; Nelson et al., 2007; Fernandez et al., 2007b).

The results of applying the calculated average, upper, and lower removal percentages against the average, upper, and lower modeled influent values for Fish Creek and Bonnybrook
are shown in Figures 5-48 and 5-49. The activated sludge bioreactors and the UV disinfection process show the greatest decrease in estrogen in both facilities while the primary and secondary clarifiers show the least amount removed. For both facilities, E1 and E2 show the highest percentage removal followed by EE2. The results of the removal matrix simulation show that the amount of estrogen is subsequently reduced following each successive treatment unit.

Figure 5-48: Example of modelled WWTP removal matrix at the Fish Creek site. The upper and lower bars represent the: (i) upper limit influent value with the lower limit removal percentage; and (ii) lower limit influent value with the higher limit removal percentage, for the estrogen of interest.

Note 1: the graph uses the modeled influent data for Fish Creek in 2012. Note 2: The upper bar represents the upper limit influent value with the lower limit removal percentage; the lower bar represents the lower limit influent value with the higher limit removal percentage.
Figure 5-49: Example of modelled WWTP removal matrix at the Bonnybrook site. The upper and lower bars represent the: (i) upper limit influent value with the lower limit removal percentage; and (ii) lower limit influent value with the higher limit removal percentage, for the estrogen of interest Estimated removal matrix for the Bonnybrook WWTP

\[\text{Note 1: the graph uses the modeled influent data for Bonnybrook in 2012. Note 2: The upper bar represents the upper limit influent value with the lower limit removal percentage; the lower bar represents the lower limit influent value with the higher limit removal percentage.}\]

5.2 Sensitivity Analysis

The Sensitivity Index (SI), developed by Hoffman and Gardner (1983), will account for the possible values when determining parameter sensitivity. The SI will calculate the output percent difference when varying one input parameter from its minimum value to its maximum value. The upper and lower range limits, or maximum and minimum values for each parameter, were determined by information collected from the literature and data provided by the city of Calgary. As such, the sensitivity index for each demographic profile and associated excretion rate variables were completed within the upper and lower range of the given value (Table 5-7).
The Sensitivity Index (SI), developed by Hoffman and Gardner (1983), will account for the possible values when determining parameter sensitivity:

\[
\text{Sensitivity Index} = \frac{\max(P_i) - \min(P_i)}{\max(P_i)}
\]  

(11)

where, \(\max(P_i)\) and \(\min(P_i)\) are maximum and minimum output values respectively from the range of input values used.

The sensitivity index results are shown in Figure 5-50. The sensitivity analysis was completed by calculating the estrogen influent loading concentration for E1, E2, and EE2 while
varying each individual variable to its upper and lower range (Table 5-7); these calculations represent the maximum and minimum output values \( \max (Pi) \) and \( \min (Pi) \) used in the SI equation (Equation 10) to determine the output % difference. The results for E1 can be expressed as follows: pregnant excretion rate > pregnant females > menstruating female excretion rate > daily treated flow of wastewater > females on HRT > menstruating female excretion rate > male excretion rate > HRT excretion rates > menstruating females > menopausal females > males; the results for E2 can be expressed as follows: pregnant females > pregnant excretion rate > daily treated flow of wastewater > females on HRT > menstruating female excretion rate > male excretion rate > menopausal female excretion rate > HRT excretion rates > menstruating females > menopausal females > males; and the results for EE2 can be expressed as females taking birth control pill > daily treated flow of wastewater > EE2 excretion rate. The results of E1 and E2 are generally consistent and the noted deviations can be explained by variances in the excretion limits. Overall, the percentage of females taking the BCP shows the greatest sensitivity variance of approximately 40%; and therefore, is a variable of importance when modeling EE2.
Figure 5-50: Example of sensitivity analysis for E1 (a), E2 (b), and EE2 (c) at the Bonnybrook WWTP site.

Note: P = pregnant females; D.F. = daily flow; HRT = hormone replacement therapy; MS = menstruating females; MP = menopausal females; BCP = birth control pill

5.3 Discussion

Modelling exercises in the past has been confounded by conflicting results. In one modelling study using data from five Italian and three Dutch WWTPs, Johnson et al. (2000) produced reasonable influent predictions for E1 (R^2 value of 0.50) and E2 (R^2 value of 0.47) while EE2 (R^2 value of 0.149) proved to be less accurate. In another study, Johnson and Williams (2004) modelled estrogen for six Roman and one German WWTP, and produced reasonably good results for predicting influent concentrations with R^2 values of 0.70 (p<0.001)
for E1, 0.66 (P<0.001) for E2, and 0.53 (p=0.06) for EE2. In addition, effluent predictions for E2 and EE2 all fell within the ranges of observed values while E1 proved difficult to predict. Umali et al. (2012) modelled E2 influent loading for WWTPs in Bethlehem and Allentown, and produced good results that varied 1.2 to 2.5% from observed values for Bethlehem and Allentown respectively. In contrast, Atkinson et al. (2012) found that measured E1 and E2 concentrations in wastewater influent for WWTPs in Ottawa and Cornwall were higher than predicted estimates—by a factor of 2 for E1.

In this study, the model produced good results for the Brandon WWTP facility with influent $R^2$ values of 0.81 for E1, 0.95 for E2, and effluent values of 0.99 for E1, 0.81 for E2. All of the E1 and E2 influent and effluent mean measurements per day fell within the upper and lower confidence limits of the model. In contrast, the initial model results for EE2 in the Brandon WWTP appear to be inconclusive as both the influent and effluent measurements proved to be much higher then predicted by a factor of 2 and 8 respectively. The discrepancy between the predicted and measured concentration may be due to underestimating the number of people taking the oral contraceptive pill and deviations from the average daily flow (Johnson et al., 2000). In fact, the sensitivity analysis confirms that the percentage of individuals taking the pill and the average daily flow are two of the top five most sensitive variables for the model. Excluding conventional hormonal replacement therapy is also likely another cause for underestimating EE2. Today for example, HRT contains natural estrogens (CIHI, 2008) and are accounted for in the model; however, in the past, prescribed conventional HRT contained EE2 (CIHI, 2008). According to CIHI (2008), the use of conventional HRT declined upwards of 90% by 2004 following the results of the 2002 WHI study. In 2006–2007, the age-standardized rate of conventional HRT use was only 1.1% in Alberta (CIHI, 2008). Only through extensive
calibration, does the model allow for all of the mean measurements of EE2 to fall within the upper and lower limits. The calibrated model produced good results with influent and effluent $R^2$ values of 0.99 and 0.80 respectively.

The model also produced reasonably good results for the Edmonton facility as 79% of the mean daily influent estrogen values predicted fell within the 95% confidence intervals for the model; the model produced an estrogen $R^2$ value of 0.79. The individual influent measurements that fell outside of the confidence limits, could be explained by the presence of other potential compounds producing estrogenic effects as well as competing estrogenic and anti-estrogenic substances, both of which E2-eq estrogen measurements are known to account for. Furthermore, the average annual parameters used in the model, such as flow rate, population served, consistent excretion rates, etc., are designed for averages and not daily fluctuations. Therefore, the results may vary and more detailed parameter information may need to be incorporated in order to capture the daily fluctuations. Using the calibrated version of the model increased the limits of the model and subsequently captured more individual measurements verses the non-calibrated model; however, it created a greater variance between the means and may suggest that calibration is WWTP specific.

Simulating the model for the Bonnybrook and Fish Creek WWTPs produced good results as all of the Bonnybrook effluent measurements for E1 and E2 and the Fish Creek measurement for EE2 fell within the 95% confidence intervals for the model. The model also produced good results when comparing the Brandon and Goldbar WWTP measurements against predictions with Fish Creek and Bonnybrook, respectively—Fish Creek produced influent $R^2$ values of 0.84 for E1, 0.98 for E2, and 0.99 for EE2, Bonnybrook produced an estrogen influent $R^2$ value of 0.98, and Fish Creek effluent $R^2$ values of 0.97 for E1, 0.76 for E2, and 0.97 for EE2. All of the
E1, E2, and EE2 mean daily influent and effluent measurements from the Brandon WWTP fell within the predicted intervals for the Fish Creek WWTP; the mean total estrogen measurement taken from the Edmonton WWTP—a larger and comparable facility to Bonnybrook—also fell within the predicted intervals for the Bonnybrook WWTP. The predicted concentration results of the model are also consistent with concentrations reported in the literature (Baronti et al., 2000; Fernandez et al., 2007; Lee et al., 2005b; Lishman et al., 2006; Servos et al., 2005). However, validating the model by using data from other facilities may increase the level of error and suggests that additional data is required to better corroborate the model. Similar to the Brandon simulation, only the calibrated version of the model supported the EE2 measurement for Fish Creek. The EE2 concentration in one measured effluent sample in Fish Creek of 2003 was higher than the model predicted by a factor of 8, and as discussed above, the discrepancy may likely be due to deviations in the number of individuals taking the oral contraceptive pill, variances from the average daily flow, and exclusion of EE2 present in HRT prescriptions during that time. The elevated effluent concentration of EE2 measured in 2003 may also be explained by sorption and desorption. As shown in Table 2-1, EE2 has a low Henry’s Law coefficient and high \( K_{ow} \); and therefore, EE2 is not volatile and is considered hydrophobic. As such, EE2 is believed to sorb onto solids during the activated sludge process, and also desorb from solids, due to its low binding energy, resulting in higher concentrations during sludge dewatering and higher effluent concentrations measurements. The EE2 calibrated version of the model seems to compensate for the above explanations as the Fish Creek 2003 measurement fits well within the upper and lower limits. Also, the calibrated model simulated for Fish Creek also supports EE2 concentrations within the Brandon facility. In addition, the calibrated version of the model
simulated for Bonnybrook supports the total estrogen mean influent measurement from the Edmonton facility in the same year, whereas the non-calibrated model does not.

The results of the model, both with and without calibration, show that the composition of influent estrogen can be expressed as $E_1 > E_2 > EE_2$. The composition breakdown results are consistent with the findings reported in the literature (Atkinson et al., 2012; Baronti et al., 2000; Fernandez et al., 2007; Lee et al., 2005b; Lishman et al., 2006; Servos et al., 2005), and results mainly from the model accounting for the calculated 27% degradation of $E_2$ to $E_1$ during sewer transit. Sewers contain native biofilms that have metabolic capabilities associated with biodegradation (Johnson and Williams, 2004). D’Ascenzo et al. (2003) for example, reported a depletion of glucuronide conjugates during sewer transit suggesting that biodegradation occurred. Ternes et al. (1999a) concluded, following aerobic batch experiments with diluted sludge, that $E_2$ readily biodegrades to $E_1$, its metabolite. De Mes (2007) reported cases where $E_2$ degraded to $E_1$ within minutes. In contrast, $EE_2$ degradation occurs at much slower rates and in some cases no degradation occurs (De Mes 2007). It is believed that the $EE_2$ ethinyl group is responsible for delaying enzyme expression, substrate-receptor binding, and metabolism (Racz and Goel, 2009). The greater persistence of $E_1$ in effluent can be explained by degradation of estrogens by sorption and desorption, heterotrophic degradation, cometabolism and deconjugation. In the case of deconjugation for example, bacterial enzymes commonly found in untreated wastewater influent and during the activated sludge process have been shown to readily convert excreted conjugates to active unconjugated estrogenic compounds such as $E_1$ (Racz and Goel, 2009). The results of the model show conflicting results for estrogen effluent composition when using the calibrated version verses the non-calibrated version: the non-calibrated model shows the effluent composition as $E_1 > E_2 > EE_2$ for the Brandon, Bonnybrook
and Fish Creek facilities, while the calibrated model shows the effluent composition as $E_1 > EE_2 > E_2$ for Fish Creek and $EE_2 > E_1 > E_2$ for both the Brandon and Bonnybrook facilities. The results may suggest that while the calibrated model limits showed positive results while capturing all of the measurements within the predicted intervals, the mean estimates may contain errors since the general composition findings are generally inconsistent with the literature.

As expected, the estimated levels of $E_1$, $E_2$, and $EE_2$ are higher in sewage influents than effluents. The modeled estrogen influent and effluent concentrations for $E_1$, $E_2$, and $EE_2$ for the Calgary facilities all show upward trends ($R^2$ values of 0.77 and 0.79) and annual growth rates that can be expressed as $E_1 > E_2 > EE_2$. In contrast, the effluent annual growth rates using the calibrated version of the model can be expressed as $E_1 = EE_2 > E_2$ (Table 5-4). As discussed above there are likely errors in the mean estimates using the calibrated model suggesting that the non-calibrated mean growth rates appear to be more plausible. In any case, concentrations to induce vitellogenin production in fish have been reported as low as 0.5 ng/l of $EE_2$ (Purdom et al., 1994; Hansen et al., 1998), 1 ng/l of $E_2$ (Routledge et al., 1998) and 25 ng/L of $E_1$ (Routledge et al., 1998) [Table 2-1]. The current and projected concentrations for $EE_2$ and $E_2$ for Fish Creek and Bonnybrook WWTPs both exceed the threshold concentrations to induce vitellogenin production in fish, and suggest that a potential risk to fish may exist. Furthermore, cumulative impacts of WWTPs, agriculture, landfills, industry, and humans also may contribute to the potential risk. However, in contrast, seasonal variations in temperature, hydrology, contaminant dispersion, and river dilution ratios would dramatically reduce concentrations and need to be taken into consideration to accurately assess risk. For example, in one study, Labadie and Budzinski (2005) reported that the concentrations of the estrogens found in the effluent and receiving rivers showed seasonal variations—in the summer, the degradation rate of estrogen
was greater than that of dilution, and the concentration of estrogen was reduced by half
approximately 2 km downstream of the WWTP outfall; in the winter, concentrations were
comparable 10 km downstream of the WWTP outfall. Fernandez et al. (2008) indicated that
during the summer months, more non-conjugated forms of estrogen are being released to the
receiving environment, while in the winter months, WWTP effluent contains much more
conjugated forms of estrogen verses non-conjugated forms. Conjugated forms of estrogen may
pose less of a risk to exposed fish populations.

As shown in the results section, E1, E2, and EE2 influent and effluent estimated
concentrations are higher in Fish Creek compared to Bonnybrook. The higher concentrations are
likely due to lower average daily treated flows in Fish Creek compared to Bonnybrook. For
example, the average daily treated flow range is 4-10 fold greater in Bonnybrook compared to
Fish Creek, and the average daily treated flow per person is 26% (SD 4.4%) higher in
Bonnybrook compared to Fish Creek. Shore et al. (1993), reported that wet periods, or high
precipitation events, results in lower influent concentrations with higher influent-to-effluent
removal percentages—the opposite occurs during dry periods. The findings from Shore et al.
(1993) first suggest that daily or yearly fluctuations in precipitation should be considered when
modelling estrogen as precipitation may influence volumes, concentrations, and removal
percentages; secondly, the findings may suggest that the higher amount of wastewater volume
treated per person in Bonnybrook and lower corresponding estimated concentrations may be due
to water infiltration from another source; and thirdly, the findings may also suggest that the
corresponding removal percentages may need to be adjusted slightly between both facilities in
order to account for the amount of treated waste water per person. It is also important to consider
seasonal variations in concentrations when modelling for estrogen. For example, higher estrogen
concentrations have been reported in the winter (Desbrow et al., 1998; Belfroid et al., 1999a) and higher influent-to-effluent removal percentages in the summer (Labadie and Budzinski, 2005) suggesting that lower temperatures during winter decrease the activity of biomass and corresponding degradation of estrogen. In fact, Atkinson et al. (2012) demonstrated that estrogen concentrations strongly correlated with ambient air temperature ($R^2=0.792$, $p=0.003$). In this case however, E1 increased with ambient air temperature due to increase microbial activity, oxidation of conjugates to unconjugates, and conversion of E2 to E1. WWTP operational parameters that may influence influent-to-effluent removal percentages, such as hydraulic retention time, should also be considered. In the case of hydraulic retention time, it has been reported consistently that longer retention times give higher removal efficiencies of E1, E2 and EE2 (Kirk et al., 2002; Svenson et al., 2003; Cargouet et al., 2004). Therefore, since Bonnybrook WWTP has longer hydraulic retention times compared to Fish Creek WWTP, the associated influent-to-effluent removal percentages should correspond accordingly.

The results of the removal matrix simulation show that the amount of estrogen is subsequently reduced within each successive treatment unit within the WWTP, with the activated sludge bioreactor showing the greatest reduction. The results are consistent with the literature. For example, Matsui et al. (2000) reported that the estrogenic activity measured by yeast estrogen screening decreased after each processing unit within the WWTP, and denitrification within the activated sludge treatment process showed the greatest decrease. Similarly, in Germany, Andersen et al. (2003) reported lower concentrations of estrogen with each successive treatment unit within the Wiesbaden WWTP, and the greatest reduction occurred at the denitrification stage. Researchers explain that denitrification and dilution—with the return sludge from the secondary clarifier and the internal recirculation containing little estrogen from
the last nitrification tank—contributed to the reduced concentrations of estrogen. The reductions also suggest that de-nitrification and aerobic biological degradation appear to play a role in reducing the amount of E1/E2 and EE2 respectively. Furthermore, the reduced concentration may also suggest slow sorption kinetics and no equilibrium between the sorbed and dissolved estrogens. The final effluent concentrations of the removal matrix are also supported by the model as all of the mean concentrations fall within the upper and lower limits of the model for the same year.
Chapter 6. Summary, Contribution to Science and Technology, and Further Recommendations

6.1 Summary and conclusions

EDCs such as estrogen are a growing environmental concern as trace amounts may lead to infertility, developmental disorders, disorders of the nervous system, and functioning of the immune system in humans and wildlife (USDHHS, 2010). Since WWTP effluents are considered a major point source for estrogen, more attention has recently been directed at better understanding WWTP processes and its relationship with estrogen.

Modeling estrogen loading concentrations and removal rates are beneficial considering the potential environmental risk and the difficulty and cost associated with analytical measurements due to low concentrations. Previous attempts to model estrogen loading and removal have resulted in mixed outcomes attributed to variability in loading parameters and WWTPs processes. Based on the results of this research, the following major findings are drawn on the basis of the study objective:

Objective: model the wastewater concentrations of estrone (E1), 17β-estradiol (E2), and 17α-ethynylestradiol (EE2) in order to predict the range of concentrations that can be expected to occur at the Fish Creek and Bonnybrook WWTPs located in Calgary, Alberta; the Goldbar WWTP located in Edmonton, Alberta; and the WWTP located in Brandon, Manitoba. This study provided reasonably good outcomes that are consistent with the literature and mean measurements taken within the Brandon, Edmonton, Fish Creek and Bonnybrook facilities; this analysis revealed reasonably good results for predicting E1 and E2 values and calibration was required to produce good results for EE2. When comparing the Calgary facilities, the results of the study indicate that the Fish Creek WWTP has the highest estrogen concentrations and the
highest predicted annual concentration rate of growth. Combining the results of the study with
the shorter hydraulic retention times may draw more focus to Fish Creek WWTP.

6.2 Contribution to Science and Technology

The contributions to Science and Technology include the following:

1) The model findings suggest that an accurate range of estrogen concentration can be
   predicted for Western Canadian Cities. The range of estrogen concentrations that can be
   expected to arrive and leave a specific WWTP may allow one to determine whether
   future comprehensive measurements are needed and may reduce the associated cost
   required to measure estrogen.

2) The calculated removal matrix suggest that the greatest reduction of estrogen can be seen
   in the activated sludge bioreactors and this may allow researchers to focus on this
   component of WWTP’s to better understand why and find ways for optimization in order
   to further reduce the concentration of estrogen effluent.

The contribution to modeling estrogen includes the following:

1) All of the parameters for the model, including menopausal females, menstruating
   females, females taking hormone replacement therapy (HRT), pregnant females, females
   on birth control, and males, have all been revised based on data from Canada. In addition,
   the male and female parameter definitions were revised to exclude prepubescent
   individuals due to extremely low estrogen output. The parameter revisions should
   improve model accuracy
2) According to the literature, although a simplistic approach was taken, it is the first time that the biological sewer estrogen transformation rate of E2 to E1 was calculated using data. It is also the first time that this model was calibrated.

3) The sensitivity analysis suggests that the two most important parameters to consider, in order to accurately predict estrogen, are the percentage of females taking birth control pill and the percentage of pregnant females. This may lead to strategies to accurately account for both in the general and specific populations.

6.3 Further Recommendations

The recommendations for future research work are given below:

1. Most studies account for the influent-to-effluent ratios for estrogen entering the plant and leaving the plant. However, little is known about the individual treatment unit. It is important to consider the input and output concentrations for each treatment unit in order to complete a estrogen mass balance, and allow one to examine and identify the processes that may need to be optimized within existing treatment plants.

2. As this study focuses on the concentration of estrogen entering and leaving the plant, there is a need to study how river dilution plays a role in reducing the concentration of estrogen and its subsequent effects on fish.

3. Since estrogens can transform to and from conjugated and non-conjugated compounds, it is also important for researchers to consider measuring both.

4. As daily fluctuations of estrogens can occur at the WWTP inlet, especially in the morning when urine inputs are the greatest, it is important for one to consider the sample time and analysis used, i.e. grab versus 24-hour composite.
5. Since estrogen data is very limited, random, and sporadic in WWTP’s across Canada, there is a need for consistent and comprehensive estrogen datasets in order to assist researchers in developing comprehensive models.
References


City of Calgary (2010) Environmental Performance Plan


Input/output balance of estrogenic active compounds in a major municipal sewage plant in Germany. *Chemosphere*. 40: 1131-1142


Tetreault, GR, Bennett CJ, Shires K, Knight B, Servos MR, McMaster ME (2011) Intersex and reproductive impairment of wild fish exposed to multiple municipal wastewater discharges. Aquatic Toxicology 104: 278-290


### Appendix A: Fish Creek and Bonnybrook WWTPs Data

Table IV Calgary Wastewater Treatment Plants

<table>
<thead>
<tr>
<th>Year</th>
<th>Parameter</th>
<th>Bonnybrook Plant</th>
<th>Fish Creek Plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002</td>
<td>Total Population Served</td>
<td>748,634</td>
<td>192,718</td>
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<tr>
<td></td>
<td>Total Wastewater Flow Treated</td>
<td>135,167,530 m³</td>
<td>25,998,585 m³</td>
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<td></td>
<td>Average Daily Flow Treated</td>
<td>370,322 m³/d</td>
<td>71,229 m³/d</td>
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<tr>
<td></td>
<td>Wastewater Flow Per Capita</td>
<td>495 L/Person/d</td>
<td>370 L/person/d</td>
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<td>2003</td>
<td>Total Population Served</td>
<td>794,227</td>
<td>168,951</td>
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<td>Total Wastewater Flow Treated</td>
<td>140,477,550 m³</td>
<td>24,243,300 m³</td>
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<tr>
<td></td>
<td>Average Daily Flow Treated</td>
<td>384,870 m³/d</td>
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<td></td>
<td>Wastewater Flow Per Capita</td>
<td>485 L/person/d</td>
<td>392 L/person/d</td>
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<tr>
<td>2004</td>
<td>Total Population Served</td>
<td>804,156</td>
<td>176,605</td>
</tr>
<tr>
<td></td>
<td>Total Wastewater Flow Treated</td>
<td>134,041,250 m³</td>
<td>24,703,648 m³</td>
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<tr>
<td></td>
<td>Average Daily Flow Treated</td>
<td>366,233 m³/d</td>
<td>67,496 m³/d</td>
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<tr>
<td></td>
<td>Wastewater Flow Per Capita</td>
<td>455 L/person/d</td>
<td>382 L/person/d</td>
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<tr>
<td>2005</td>
<td>Total Population Served</td>
<td>815,854</td>
<td>190,503</td>
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<td></td>
<td>Total Wastewater Flow Treated</td>
<td>148,546,680 m³</td>
<td>27,432,944 m³</td>
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<tr>
<td></td>
<td>Average Daily Flow Treated</td>
<td>406,977 m³/d</td>
<td>75,159 m³/d</td>
</tr>
<tr>
<td></td>
<td>Wastewater Flow Per Capita</td>
<td>499 L/person/d</td>
<td>395 L/person/d</td>
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<tr>
<td>2006</td>
<td>Total Population Served</td>
<td>844,767</td>
<td>197,431</td>
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<td>Total Wastewater Flow Treated</td>
<td>142,394,970 m³</td>
<td>25,977,165 m³</td>
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<td></td>
<td>Average Daily Flow Treated</td>
<td>390,123 m³/d</td>
<td>71,170 m³/d</td>
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<tr>
<td></td>
<td>Wastewater Flow Per Capita</td>
<td>462 L/person/d</td>
<td>360 L/person/d</td>
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<tr>
<td>2007</td>
<td>Total Population Served</td>
<td>880,643</td>
<td>199,867</td>
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<tr>
<td></td>
<td>Total Wastewater Flow Treated</td>
<td>145,397,750 m³</td>
<td>26,549,928 m³</td>
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<td>Average Daily Flow Treated</td>
<td>398,350 m³/d</td>
<td>72,740 m³/d</td>
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<td></td>
<td>Wastewater Flow Per Capita</td>
<td>452 L/person/d</td>
<td>364 L/person/d</td>
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<tr>
<td>2008</td>
<td>Total Population Served</td>
<td>891,100</td>
<td>215,331</td>
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<td></td>
<td>Total Wastewater Flow Treated</td>
<td>138,455,735 m³</td>
<td>27,590,961 m³</td>
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<td></td>
<td>Average Daily Flow Treated</td>
<td>378,294 m³/d</td>
<td>75,385 m³/d</td>
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<td>Wastewater Flow Per Capita</td>
<td>425 L/capita/d</td>
<td>350 L/capita/d</td>
</tr>
<tr>
<td>2009</td>
<td>Total Population Served</td>
<td>859,395</td>
<td>95,192 *</td>
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<tr>
<td></td>
<td>Total Wastewater Flow Treated</td>
<td>129,023,270 m³</td>
<td>11,634,998 m³</td>
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<tr>
<td></td>
<td>Average Daily Flow Treated</td>
<td>353,488 m³/d</td>
<td>31,877</td>
</tr>
<tr>
<td></td>
<td>Wastewater Flow Per Capita</td>
<td>411 L/Person/d</td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>Total Population Served</td>
<td>838,761</td>
<td>96,453 *</td>
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<td>Total Wastewater Flow Treated</td>
<td>126,130,500 m³</td>
<td>11,668,203 m³</td>
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<td></td>
<td>Average Daily Flow Treated</td>
<td>345,563 m³/d</td>
<td>31,968 m³/d</td>
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<tr>
<td>Year</td>
<td>Total Population Served</td>
<td>Total Wastewater Flow Treated</td>
<td>Average Daily Flow Treated</td>
</tr>
<tr>
<td>------</td>
<td>------------------------</td>
<td>------------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>2011</td>
<td>848,450</td>
<td>133,231,400 m³</td>
<td>365,018 m³/d</td>
</tr>
<tr>
<td>2012</td>
<td>880,058</td>
<td>133,315,440 m³</td>
<td>364,250 m³/d</td>
</tr>
</tbody>
</table>

*Data obtained from the City of Calgary*
Appendix B: Effluent Data collected for Fish Creek and Bonnybrook WWTPs.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Concentration in WWTPs (µg/L) ( ^a )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BB 15/01/2003</td>
</tr>
<tr>
<td>EE2</td>
<td>&lt;0.0039</td>
</tr>
<tr>
<td>E2</td>
<td>0.0015</td>
</tr>
<tr>
<td>E1</td>
<td>0.0026</td>
</tr>
</tbody>
</table>

\( ^a \) Data obtained from the City of Calgary
Appendix C: Sampling Analysis

Brandon WWTP Sampling Analysis

The Brandon WWTP sampling analysis is fully described in detail by Cicek et al. (2007). Cicek et al. (2007) stated that, “Eight different sample types were collected from the WWTP at Brandon, in either grab or 24-hour-composite fashion. The composite samples were collected over a single 24-hour period between 11:00 a.m. on May 20, 2003, to 11:00 a.m. on May 21, 2003. Tygon tubing (9.5-mm [0.375-in.] diameter) was used, leading to the autosampler, to limit estrogen losses. The potential losses of estrogens resulting from the use of Tygon tubing were not quantified, but the water spent a very short time in the tubing and most of the collection time in the glass bottle to prevent sorption. The grab samples were collected in glass bottles on a single day (4 samples of 4 L each). The plant is centered around two non-nitrifying aerobic sequencing batch reactors (SBRs), with a total HRT of approximately 6 hours and SRT of less than 1.2 days. All containers used during sampling and storage throughout this study were glass and were acid-washed and rinsed with 50% methanol to prevent adsorption of estrogens (unless otherwise stated). In addition, methanol (20 mL, high-performance liquid chromatography [HPLC]-grade) was added to the 4-L collection bottles to help reduce the loss of estrogens onto glass surfaces. Four composite samplers were used simultaneously during sampling around the SBR and UV disinfection units. A collection rate of 333 mL/h over a 24-hour period was used to obtain a total sample volume of 8 L. Water in each carboy was dispensed into smaller (4-L) bottles and transported in coolers packed with ice to the University of Manitoba, Winnipeg,
Manitoba, Canada.”

Cicek et al. (2007) further stated that, “each of the eight WWTP samples was analyzed as at least four replicate subsamples. Each 1-L subsample was sequentially filtered through 2.5- (μm) then 0.7-μm glass fiber (GF/C) filters. The filtrate was refrigerated for less than 24 hours before solid-phase extraction (SPE) was performed. The SPE cartridges (LC-18, 6 mL, 0.5 g, Supelco, Sigma Aldrich Canada, Ontario) were preconditioned with 5 mL acetone and then 10 mL Milli-Q water. Samples were filtered at, < 20 min/L, then estrogens were eluted with acetone (4 x 3 mL). All extracts were stored frozen (-20°C) for as little time as possible between treatment steps. As a prelude to the testing of the WWTP, the procedures were tested by spiking estrogens (from a stock solution containing E1, E2, and EE2 at 100 ng/mL in acetone) directly to laboratory-grade water to final concentrations of 1, 10, or 100 ng/L for each estrogen. Recoveries from water were 62, 56, and 50% for E1, E2, and EE2 for the water and 64, 29, and 21% for the filters. The relatively lower recoveries for the glass fiber filters were of concern, limiting the interpretation of the results to comparative assessments of estrogen levels between samples. Wastewater samples were likewise amended with estrogens just before filtration, and recoveries through the entire procedure with the eight different samples were 77 +/- 58, 76 +/- 34, and 141 +/- 30% for E1, E2, and EE2, respectively. Overall, recoveries from the entire procedure averaged 82%.”

Cicek et al. (2007) also stated, “The GF/C filters were combined and extracted with acetone by accelerated solvent extraction (ASE). An ASE 400 instrument (Dionex, Sunnyvale, California) was used to extract filters or sludges from 11-mL cells (filled with Ottawa Sand, Fisher Scientific, Hampton, New Hampshire) with acetone (HPLC-grade, Fisher Scientific) at approximately 140 bar (2000 psi) and 100°C (1 cycle, 5 minutes heat, 5 minutes static, 60% flush,
90 seconds purge). In a test of the extraction procedure, filters were directly spiked with 0, 1, 10, or 100 ng each of E1, E2, and EE2; extracted; and analyzed. Recoveries were similar for all three estrogens, averaging 79%. Sludge Samples (2 and 4). Sludge samples could not be filtered directly, so they were first centrifuged (10 000 x g, 15 minutes) in acid-washed and methanol-rinsed polypropylene centrifuge bottles. The supernatant was decanted and pooled in graduated cylinders, then filtered and analyzed, as described for the liquid samples. The pellets were transferred to glass vials, frozen, and lyophilized. The wet and dry weights were recorded, and the dried sludge was extracted with acetone by ASE, as described above. Some sludge samples were spiked with estrogens (100 to 1000 ng E1, E2, EE2) before centrifuging, to test recovery.

Sample Processing. Acetone extracts (from SPE or ASE extractions) were concentrated under a stream of nitrogen gas (N2) at 37°C. Each extract (100 to 200 μL) was applied to a new silica gel column (1 g silica gel [baked at 150°C for 8 hours, then deactivated with 15 μL water] suspended in 5 mL hexanes:acetone [65:35] in a pipet with a glass wool plug). Estrogens were eluted with 5 mL hexanes:acetone, and the eluant was concentrated under nitrogen gas at 37°C. To remove particulates (including silica gel) the sample (300 to 500 μL) was filtered through a 0.2-um polytetrafluoroethylene filter into a glass vial with a Teflon-lined cap. Samples were derivatized with 100 μL N-Methyl-N-(trimethylsilyl) trifluoro-acetamide (Sigma Aldrich Canada) and 10 μL pyridine for 2 hours at 65°C, then dried under nitrogen gas and resuspended in 100- to 500-μL hexanes. Blanks and standards (100 ng each of E1, E2, and EE2) were prepared with each set of samples derivatized. Samples were analyzed within 10 days of derivatization. Analysis. The trimethyl-silyl-derivatives of E1, E2, and EE2 were analyzed by GC-MS-MS. A Varian 3800 gas chromatographer (Varian Inc., Lexington, Massachusetts) with a Saturn 2000 mass spectrometer (Varian Inc.) was used with a DB-5ms column (30 m x 0.25 mm x 0.25 lm) with a 1 m x 0.53
mm precolumn. Samples of 2 to 4 μL were injected in splitless mode at 80°C, and the injector was heated to 250°C at 200°C/min. The oven temperature program was 80°C for 1.5 minutes, increased to 180°C at 50°C/min, then increased to 300°C at 20°C/min and held for 5 minutes. The mass spectrometer had a transfer line at 250°C, electron ionization ion source of 70 eV, and ion trap temperature of 200°C. The MS-MS was performed for E1 using a precursor ion of 342 (m/z) and quantified using the daughter ions 244, 245, and 257. The MS-MS was performed for E2 using a precursor ion of 416 and quantifying using the daughter ions 285 and 326. The MS-MS for EE2 used a precursor ion of 425 and quantifying using the daughter ions 193, 231, and 407. Estrogens were quantified by comparison of peak areas to five-point standard calibration curves generated daily using standards of 10 to 200 ng E2 and EE2 and confirmed with check standards and blanks.”

Edmonton WWTP Sampling Analysis

The Edmonton WWTP sampling analysis is fully described in detail by Fernandez et al. (2007). Twenty-four-hour composite samples were collected twice a week from September to December 2006. Fernandez et al. (2007) stated that, “Each sample was filter sterilized using 0.2 um Puradiscs cartridge filters and stored in sterile glass vials at 20°C for collective RYA analysis. RYA was carried out in 96-well plates as described in Fernandez et al. (2007b). Samples were run in three serial dilutions of 1:4, 1:12, and 1:24, along with a positive control (1000 ng/L) for toxicity at the 1:12 dilution level. This was the optimal layout to analyze these samples for estrogenic activity, as both true dose response and toxicity effects could be evaluated in every sample. Concentration in E2-eq (ng/L wastewater) was then calculated from a simultaneously run 12-point calibration curve (n = 4; R²<0.998) of E2 from 10⁻⁴ to 10⁻¹⁰ g/L (see
Fernandez et al., 2007b for more details).”

Fernandez et al. (2007) further stated that, “Procedural blank samples were prepared from distilled water run identically to the wastewater samples, such that the blank values could be subtracted from the samples to obtain a final value. E2-eq were calculated from the 1:12 dilution unless this dilution did not show a response, in which case the 1:4 dilution value was used. For samples that responded greatly, the 1:24 value was used only if the 1:12 was out of range. To aid in the interpretation of the RYA results found in this work, four randomly selected 10mL aliquots (previously analyzed for RYA activity) were thawed and combined for each warm and cold temperature time periods. The 30–40mL composite was SPE extracted with 500mg Oasis HLB cartridges, worked-up and analyzed for free and conjugated estrogens via LC–MS/MS. The free estrogens measured were E2, E1, E3, 16a-hydroxyestrone, EE2, 19-norethindrone, norgestrel, diethylstilbesterol, progesterone, equilenin, equilin, a-zearalanol, 17b-estradiol-3-benzoate, and testosterone. The conjugated estrogens measured were estrone-3-sulfate (E1-3S), estrone-3-glucuronide (E1-3G), estradiol-3-sulfate (E2-3S), estradiol-3-glucuronide (E2-3G), estriol-3-sulfate (E3-3S), estiol-3-glucuronide (E3-3G), estriol-16-glucuronide (E3-16G), ethinylestradiol-3-sulfate (EE2-3S), and ethinylestradiol-3-glucuronide (EE2-3G). The sample workup conditions, the surrogate internal standards used for quantification, the LC–MS/MS instrumental analysis conditions, the calibration condition used for quantification, the method detection limits, and all the other quality assurance, quality control criteria used for analyte identification and quantification are described in detail in Fernandez et al. (2008).”
Calgary WWTP Sampling Analysis

The Calgary WWTP sampling analysis is fully described in detail by Chen et al. (2006). Chen et al. (2006) stated that, “Volumes of 12-L water samples or 4 L of WWTP final effluent were extracted for the analysis of EDCs. All samples were extracted at EnviroTest Laboratories by liquid-liquid extraction with chloroform, which was repeated twice. The organic phases were combined and filtered through glass frit funnels before concentrated to a final volume of 10 mL. The extracts were sent to the Institute of Ocean Sciences, where they were split quantitatively into three equal aliquots and analyzed for nonylphenol (NP), nonylphenol ethoxylayes (NPnEOs), phthalates and steroids. The first aliquot was spiked with a representative suite of surrogate standards, processed through a number of cleanup steps and analyzed for NP and NPnEOs according to the LC-MS analytical methodology described by Shang et al. (1999). The second aliquot was also spiked with a group of surrogate standards, processed through a number of cleanup steps and analyzed for phthalates according to the GC-MS method described by Lin et al. (2003). To the third aliquot, surrogate standards including 180 ng of Ring-13C6-NP and 500 ng of DnOP-d4 (Cambridge Isotope Laboratories Inc., Mass.) were added. The extracts were then filtered, concentrated, derivatized and analyzed for steroids by GC-MS according to the method developed by Ikonomou et al.

All samples collected for the EDCs analyses were stored in pre-cleaned glass bottles. Two types of blank samples were analyzed in the laboratory. One blank sample was prepared and extracted by EnviroTest Laboratories. The other type of blank sample was prepared by the laboratory at the Institute of Ocean Sciences. All the results were blank-corrected. Surrogate standards were added prior to sample extraction in order to monitor extraction efficiency. The analytical results were corrected, based on the recovery of the surrogate standards. Even though
EnviroTest Laboratories was involved in the extraction of samples in this study, this laboratory followed the protocols developed by Shang et al. (1999), Lin et al. (2003) and Ikonomou et al. (submitted for publication) for sample extraction. The instrumental analyses were performed at the Institute of Ocean Sciences.”