Modeling the loading and fate of estrogens in wastewater treatment plants

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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, 17$\beta$-estradiol, and 17$\alpha$-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. Predicted average concentrations for EE\textsubscript{2} and E\textsubscript{2} in all the study sites exceed the threshold concentrations that could induce vitellogenin production by order of 13 and 2.3, respectively. The results demonstrate reasonable accuracy against previous measurements with $r^2$ values ranging from 0.79 to 0.99 and RMSE values ranging from 0.5 to 9.4 ng/l 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.

Subjects: Civil, Environmental and Geotechnical Engineering; Environmental Health; Pollution

Keywords: endocrine-disrupting compounds; estrogen; wastewater treatment plants; modeling
1. Introduction
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, O’Brien, & Woodruff, 2011). Recently, occurrence of estrogens in aquatic environments have received considerable interest as they may lead to infertility, developmental disorders, disorders of the nervous system, and improper functioning of the immune system in humans and wildlife even at extremely low concentrations (National Institute of Environmental Health Sciences, 2015). Estrogens, estrone (E1), 17\(\beta\) estradiol (E2), and estriol (E3) are synthesized and excreted by humans (de Mes, Zeeman, & Lettinga, 2005). 17\(\alpha\) estradiol (EE2) is a synthetic hormone which has molecular structure similar to that of E2, is the main estrogen used in oral contraceptives (OC), which is the most prescribed drug worldwide (Briciu, Kot-Wasik, & Namiesnik, 2009).

Concentrations as low as 0.5 ng/l of EE2 (Hansen et al., 1998; Purdom et al., 1994), 1 ng/l of E2 (Routledge, Parker, Odum, Ashby, & Sumpter, 1998), and 25 ng/L of E1 (Routledge et al., 1998) are reported to induce vitellogenin production in fish. In fact, recent studies have shown the presence of intersex fish downstream of wastewater treatment plants (WWTPs) at locations where estrogens are detected frequently and at higher concentrations among other endocrine disruptors (Evans, Jackson, Habibi, & Ikonomou, 2012). WWTPs are classified as a major point source for estrogens contaminants entering our waterways (Belfroid et al., 1999; Drewes, Heberer, Rauch, & Reddersen, 2003; Ternes, Kreckel, & Mueller, 1999). However, there are limited amounts of estrogen data collected since estrogens are currently unregulated pollutants (Umali, Pagsuyoin, & Parker, 2012). Furthermore, some cities in Canada are expanding and growing rapidly. 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 (Robinson, Valeo, Ryan, Chu, & Iwanyshyn, 2009).

Measuring estrogen compounds in wastewater is challenging, expensive, and intermittent due to: the extremely low concentrations (pg–ng/L in water) of occurrence; complexity of the wastewater matrix; and the laborious analysis protocols (Johnson & Williams, 2004; Umali et al., 2012). Typical analytical procedure includes extraction (liquid–liquid or solid phase extraction), purification, hydrolysis, derivatization, and evaporation used in tandem with chromatography and mass spectrometry (Briciu et al., 2009; Umali et al., 2012). However, it has been previously acknowledged that monitoring estrogen concentrations in treated effluent is a requirement for accurate risk assessments (Jones, Voulvoulis, & Lester, 2001). Given that humans excrete estrogen on a daily basis (Johnson & Williams, 2004), and the major point source for estrogen are WWTPs (Belfroid et al., 1999; Drewes et al., 2003; Ternes et al., 1999), it should be practical to estimate the concentrations of estrogen that enter and leave WWTPs. Previous research by Johnson and Williams (2004) and others (Atkinson et al., 2012; Umali et al., 2012) have estimated estrogen concentrations using demographic group excretion rates, census statistics, flow rates, and WWTP operational parameters. Modeling estrogen is required in order to: (i) allow estimation of estrogen in avoiding high costs and time associated with laboratory analysis; (ii) better formulate a citywide risk estimate for these contaminants of concern; (iii) predict risks posed by individual WWTPs due to the presence of steroid estrogens in effluent in a city or region; and (iv) developing management strategies that focus on the contaminant(s) of most concern. As such, the objective of this research is to model the wastewater concentrations of E1, E2, and EE2 in order to predict the range of concentrations that can be expected to occur at WWTP’s in Calgary and Edmonton, Alberta, and Brandon, Manitoba.

2. Study sites
In this study, estrogen release and its fate during wastewater treatment process was modeled in four selected WWTPs located within cities of Calgary and Edmonton, Alberta and in Brandon, Manitoba (Figure 1). All the WWTPs chosen to simulate the model employ tertiary technologies; they have available data, and are in Western Canadian cities that are in relative close proximity to each other (Table 1a).
Figure 1. Location of the sample sites of: (i) Calgary, Alberta; (ii) Edmonton, Alberta; and (iii) Brandon, Manitoba; used in this study.

Table 1a. Process characteristics of the wastewater treatment plants (WWTPs)

| WWTP     | Plant class | Primary treatment | Secondary treatment | N. P. C.<sup>d</sup> removal | Disinfection<sup>e</sup> | Lagoon |
|----------|-------------|-------------------|---------------------|-------------------------------|--------------------------|--------|
| Fish creek<sup>a</sup> | Tertiary     | Yes               | Activated sludge   | P.C.                          | UV (High)                | Yes    |
| Bonnybrook<sup>a</sup> | Tertiary     | Yes               | Activated sludge   | Yes                           | UV (High)                | Yes    |
| Brandon<sup>b</sup>     | Tertiary     | Yes               | Activated sludge   | When needed                   | UV (High)                | Yes    |
| Goldbar<sup>c</sup>     | Tertiary     | Yes               | Activated sludge   | Yes                           | UV (High)                | Yes    |

<sup>a</sup>Obtained from Calgary through personal communication.
<sup>b</sup>Cicek et al. (2007).
<sup>c</sup>Fernandez et al. (2008).
<sup>d</sup>N = nitrogen, P = phosphorus, C = carbon.
<sup>e</sup>UV = ultraviolet.
The Calgary wastewater flow data and historical population served by each WWTP was provided by the City of Calgary. The Brandon Manitoba WWTP parameters were obtained from Cicek, Londry, Oleszkiewicz, Wong, and Lee (2007), while the Edmonton WWTP parameters were obtained from Fernandez, Buchanan, and Ikonomou (2008) (Table 1b). The estrogen data used for comparison and validation to the simulated model were obtained from WWTPs located in Alberta and Manitoba (Figure 1). As limited data were available 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 WWTP, Edmonton. The estrogen data collected to calculate the influent-to-effluent removal rates were obtained from various sources, mainly in Canada (Table 2); the estrogen removal matrix was calculated using a combination of estrogen data collected in Brandon, Manitoba (Cicek et al., 2007) and Wiesbaden, Germany (Andersen, Siegrist, Halling-sørensen, & Ternes, 2003) due to worldwide limited information. Data showing an influent-to-effluent concentration increase within WWTPs were not included in the model due to reported limitations in analytical methods preventing the detection of conjugated estrogen compounds commonly present in raw influent (Fernandez, Ikonomou, & Buchanan, 2007; Lee, Peart, & Svoboda, 2005).

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

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**Table 1b. Operational parameters of the wastewater treatment plants WWTPs**

| WWTP   | Population served | Average daily flow rate (m³/d) | Wastewater temperature | Wastewater source | SRT* (days) | HRT (hrs) |
|--------|-------------------|-------------------------------|------------------------|-------------------|-------------|-----------|
| Fish creek | 168,951           | 66,240                        | N/A                    | Domestic          | N/A         | 10        |
| Bonnybrook | 794,227           | 384,870                       | N/A                    | Domestic          | N/A         | 23        |
| Brandon | 43,020            | 16,523                        | 10–12                  | Domestic          | 1.2         | 6         |
| Goldbar | 750,000           | 250,000                       | 15.2–21.6              | Domestic          | 4–8         | 6–7       |

*Parameters are averages for the following years: Edmonton 2006; BB 2003, FC 2003; Brandon 2003.
*Obtained from Calgary through personal communication.
*Cicek et al. (2007).
*dFernandez et al. (2008).
*eSRT = suspended retention time.
*fHRT = hydraulic retention time.

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The Calgary wastewater flow data and historical population served by each WWTP was provided by the City of Calgary. The Brandon Manitoba WWTP parameters were obtained from Cicek, Londry, Oleszkiewicz, Wong, and Lee (2007), while the Edmonton WWTP parameters were obtained from Fernandez, Buchanan, and Ikonomou (2008) (Table 1b). The estrogen data used for comparison and validation to the simulated model were obtained from WWTPs located in Alberta and Manitoba (Figure 1). As limited data were available 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 WWTP, Edmonton. The estrogen data collected to calculate the influent-to-effluent removal rates were obtained from various sources, mainly in Canada (Table 2); the estrogen removal matrix was calculated using a combination of estrogen data collected in Brandon, Manitoba (Cicek et al., 2007) and Wiesbaden, Germany (Andersen, Siegrist, Halling-sørensen, & Ternes, 2003) due to worldwide limited information. Data showing an influent-to-effluent concentration increase within WWTPs were not included in the model due to reported limitations in analytical methods preventing the detection of conjugated estrogen compounds commonly present in raw influent (Fernandez, Ikonomou, & Buchanan, 2007; Lee, Peart, & Svoboda, 2005).

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**Table 2. Data sources and calculated Canadian WWTP mean estrogen removal percentage**

| Reference                          | E1(n) | E2(n) | EE2(n) |
|-----------------------------------|-------|-------|--------|
| Baronti et al. (2000)             |       |       | 30     |
| Cicek et al. (2007)               | 4     | 4     |        |
| Lee, Oleszkiewicz, and Cicek (2013)| 11   | 11    | 6      |
| Lee et al. (2005)                 | 8     | 8     |        |
| Pauwels, Noppe, De Brabander, and Verstraete (2006) | 6     | 3     |        |
| Servos et al. (2005)              | 9     | 9     |        |
| Mean removal (%)                  | 85 (±7.9) | 89 (±7.2) | 80.5 (±6.7) |

*Reference provides estrogen sample averages only, the raw data related to this reference were obtained from N. Cicek, University of Manitoba through personal communication.
*n raw data sample size used.
*Calculated 95% confidence limits.
*EE2 data supplemented from a WWTP in Italy due to limited data within Canada; ND = no data.
measurements within the Brandon facility and also data from the Calgary facilities. Though estrogens are not detected in Calgary WWTPs during their wastewater analysis, it is assumed to be present but below the applicable detection limits. These concentrations below the detection limits were assumed to be zero and used to validate the calibrated model.

The data composed of a combination of grab samples and 24-h composite samples taken in 2003, for the Brandon and Calgary facilities; 2006 for the Edmonton facility, and 2012 again for the Calgary facilities (Figure 2). Eight influent and effluent samples were obtained from the Brandon facility, 4 effluent samples from each Calgary facilities, and 29 influent samples were used from the Edmonton WWTP.

The estrogen data collected from the WWTPs in Calgary and Brandon are compound-specific, while the estrogen data collected from the Edmonton WWTP in 2006 measures the total estrogenic activity or recombinant yeast assay (RYA) activity measured $E_2$-eq data for the initial influent. Although, the RYA-measured $E_2$-eq data correlate to the total estrogenic activity, $E_1$ and $E_2$ have been identified as the major contributors to RYA (Fernandez et al., 2008); however, since RYA measures the total estrogenic activity and lacks compound specificity, it was compared to the total estrogen concentration from the model or $E_1 + E_2 + EE_2$. The detailed estrogen data collection methodology and analysis for the Brandon, Calgary, and Edmonton facilities are fully described in Cicek et al. (2007), Chen et al. (2006), and Fernandez et al. (2008), respectively.

3. Methods of data analysis
A schematic diagram illustrating the methods employed in this study is shown in Figure 3. 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) influent and effluent model simulations and comparisons, and (3) calculated removal matrix and sensitivity analysis. Brief descriptions of the three major components are provided in the following paragraphs.

3.1. Demographic profile parameters
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 pills (BCP), 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; however, the group sizes have been revised based on local data (Table 3). In addition, the male and female parameter definitions were revised to exclude prepubescent individuals, i.e. 13 years of age and under, due to extremely low estrogen excretion concentrations (Jorgensen, Keiding, & Skakkebaek, 1991). The parameter revisions should improve model accuracy over previous simulations completed by Johnson and Williams (2004), where excretion rates were assumed to be constant over the entire female population and did not exclude prepubescent individuals.
3.2. Biological transformation rate of estrogens during transit in sewers

Researchers have reported that E2 can readily biodegrade to E1 during sewer transit due to the presence of native biofilms in sewers that have metabolic capabilities associated with biodegradation (D’Ascenzo et al., 2003; de Mes, 2007; Johnson & Williams, 2004; Ternes et al., 1999). In contrast, EE2 degradation occurs at much slower rates and in some cases no degradation occurs due to the presence of the ethinyl group that’s responsible for delaying enzyme expression, substrate–receptor binding, and metabolism (de Mes, 2007; Racz & Goel, 2009). In fact, previous observations in activated sludge microcosms have shown no transformation of E1 & EE2 during sewer transit (Johnson & Williams, 2004). Therefore, only the transformation of E2 to E1 will be considered for this study.

The estimated sewer biological transformation rate of E2 to E1 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 difference 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 Johnson and Williams (2004) model will form the basis used to determine the total amount of estrogen arriving at the WWTP:

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

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

For \( E2 \):

\[ T_{E2} = \frac{R_T \sum_{i=1}^{n} [P_{G,HU_1,f_1}]}{Q} \]  

(3)

For \( E1 \):

\[ T_{E1} = \frac{\sum_{i=1}^{n} [P_{G,HU_1,f_1}]}{Q} + E_c \]  

(4)

For \( EE2 \):

\[ T_{EE2} = \frac{\sum_{i=1}^{n} [P_{G,HU_1,f_1}]}{Q} \]  

(5)

where \( R_T \) represents the percentage of \( E2 \) removed in the sewer system, \( P_{G,HU_1} \) is the number of people within the demographic group of the WWTP served population, \( U_1 \) (μg/d) is the estrogen excretion rate in urine per individual of a particular demographic profile, \( F_1 \) (μg/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 preliminary simulation. \( E_c \) for Equation (4) will be the amount of degraded \( E2 \) (i.e. 27%). The excretion value rates used account for the free,
glucuronide, and sulfate forms of the compound (Johnson & 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)

### 3.3. Model effluent simulation

The following equation describes the removal efficiency calculation based on the raw measured influent and effluent data as referenced in Table 2:

$$R_E = \frac{(E_{\text{infl}} - E_{\text{Effl}})}{E_{\text{infl}}} \times 100$$  \hspace{1cm} (7)

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

The mean percent estrogen influent-to-effluent removal values were calculated with 95% confidence limits using the $R_E$ results calculated from Equation (7) above. 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 (Table 2). The following equation describes how the 95% confidence limits were calculated using the mean percent estrogen influent-to-effluent removal values:

$$CL_{95} = X \pm \left[ \text{Margin of error} \right]$$  \hspace{1cm} (8)

$$CL_{95} = X \pm \left\{ 1.96 \left[ \text{SD} / (\sqrt{n}) \right] \right\}$$  \hspace{1cm} (9)

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.

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

$$F_{EE\text{Effl}} = E_{\text{infl}} - (E_{\text{infl}} \times R_E)$$  \hspace{1cm} (10)

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

### 3.4. Calculated removal matrix and sensitivity analysis

The WWTP concentrations estimated to be found after each individual treatment process was calculated using data from the Brandon and Wiesbaden WWTPs. Since some components of the Brandon and Wiesbaden treatment process differs from Bonnybrook and Fish Creek, only the data obtained from relevant process units was 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 does not employ UV disinfection. As a result, UV disinfection data from the Brandon facility were 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 were used verses the vortex grit removal process found in Brandon. The percent difference was calculated using Equation (1) as noted above.
The sensitivity index (SI), developed by Hoffman and Gardner (1983), accounted for the possible values when determining parameter sensitivity:

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

where \(\max(P_i)\) and \(\min(P_i)\) are maximum and minimum output values, respectively, from the range of input values used. The SI for each demographic profile and associated excretion rate variables were completed within the upper and lower range of the given value (see in Table 4).

The model was validated based on the following criteria: (i) the 95% confidence intervals for the estrogen influent and effluent predicted by the model for a given WWTP should cover 90% of all daily mean estrogen measurements—a similar approach used by Ram and Gillett (1993); (ii) WWTP’s with limited estrogen data was validated using supplemental data from similar WWTP’s where 90% of all daily mean estrogen measurements lie within the 95% confidence intervals; and (iii) a regression analysis between measurements and predictions.

### 4. Results and discussion

Figure 4 shows a comparison between the modeled WWTP influent and effluent verses measurements. The model produced good results as all of the \(E1\) and \(E2\) influent and effluent mean measurements per day are within the upper and lower confidence limits of the model. Discrepancies between the predicted and measured concentrations for \(EE2\) may be due to underestimating the number of people taking OC and deviations from the average daily flow (Johnson, Belfroid, & Di Corcia, 2000). Only through extensive calibration, does the model allow for all of the mean measurements of \(EE2\) to fall within the upper and lower limits. Model calibration was achieved by adjusting the upper and lower limits within the Brandon WWTP model to a point that included all of the measurements for half of the Brandon data-set while running the model against the remaining samples. In this case, the \(EE2\) influent upper limits increased by factor of 6.4 and the lower limits decreased by a factor of 1.4; for \(EE2\) effluent, the upper and lower removal percentage decreased to 62% and 0%, respectively. The calibration model for \(EE2\) was also partially validated using effluent data from

### Table 4. Minimum and maximum sensitivity parameter input values

| Sensitivity index parameter | Per capita mean excretion estrogen (μg/day) | Group size (%)<sup>b</sup> |
|-----------------------------|-------------------------------------------|---------------------------|
|                             | Min (EI/E2) | Max (EI/E2) | Min | Max |
| Males                       | 1.4/1.3     | 2.9/2.4     | 83.3 | 84.6 |
| Menstruating females        | 7.5/1.7     | 15.4/4.6    | 56.3 | 59.3 |
| Menopausal females          | 0.0/0.0     | 5.7/3.5     | 23.0 | 27.0 |
| Women on HRT<sup>c</sup>    | 24.0/51.5   | 33.0/61.5   | 3.2  | 7.6  |
| Pregnant Women<sup>d</sup>  | 432.0/340.0 | 668.0/445.0 | 2.3  | 3.1  |
| Women on BCP<sup>e</sup>    | 9.6         | 11.3        | 17.0 | 28.0 |

<sup>a</sup> The upper and lower values were are derived by Johnson and Williams (2004) from two standard errors to give 95% confidence limits.

<sup>b</sup> Group size percent is derived from the total male population and total female population. The min and max values are derived using the demographic data for the City of Calgary from 2001 to 2011 in association with the age classes associated with each Sensitivity Index Parameter.

<sup>c</sup> The min and max % of women on HRT is derived from Canadian Institute for Health Information (2008).

<sup>d</sup> The min and max % of pregnant women is derived from Government of Alberta (2011).

<sup>e</sup> The min and max % of pregnant women on HRT is derived from Johnson and Williams (2004), Fisher et al. (1999).

<sup>f</sup> The min and max treated flow rate is derived from the City of Calgary data.
the Calgary facilities. For the Goldbar facility, 79% of the mean daily influent estrogen simulated values lie within the 95% confidence intervals for the model. 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 $E_2$-eq estrogen measurements are known to account for. Overall, the model shows agreement with the measurements, and the model results are also consistent with concentrations reported in the literature (Baronti et al., 2000; Fernandez et al., 2007; Lee et al., 2005; Lishman et al., 2006; Servos et al., 2005).

As mentioned previously, concentrations to induce vitellogenin production in fish have been reported as low as 0.5 ng/l of $EE_2$ (Hansen et al., 1998; Purdom et al., 1994), 1 ng/l of $E_2$ (Routledge et al., 1998), and 25 ng/L of $E_1$ (Routledge et al., 1998). The model concentrations for $E_2$ and $E_2$ averaged in all the study sites exceed the threshold concentrations by order of 13 and 2.3, respectively. These predicted concentrations suggest potential risk to fish may exist as vitellogenin production in fish could be induced. 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 could dramatically increase or decrease concentrations and need to be taken into consideration to accurately assess risk.

Figure 5 shows the relationship between measured $E_2$-eq. data and total estrogen modeled values for the Edmonton Goldbar facility; Figure 6 shows the relationship between compound specific data for $E_1$, $E_2$, and $EE_2$. The model produced good results with influent and effluent $r^2$ values ranging from 0.79 to 0.99 and RMSE values ranging from 0.5 to 9.4. However, modeling exercises in the past has been confounded by conflicting results. In one modeling study using data from five Italian and three Dutch WWTPs, Johnson et al. (2000) produced reasonable influent predictions for $E_1$ ($r^2$ value of 0.50) and $E_2$ ($r^2$ value of 0.47), while $EE_2$ ($r^2$ value of 0.149) proved to be less accurate. In another study, Johnson and Williams (2004) modeled estrogen for six Roman and one German WWTP, and produced reasonably good results for predicting influent concentrations with $r^2$ values ranging from 0.70 ($p < 0.001$) for $E_1$, 0.66 ($p < 0.001$) for $E_2$, and 0.53 ($p = 0.06$) for $EE_2$. In addition, effluent predictions for $E_2$ and $EE_2$ fell within the ranges of observed values, while $E_1$ proved difficult to predict. Umali et al. (2012) modeled $E_2$ influent loading for WWTPs in Bethlehem and Allentown, and produced good results that varied 1.2–2.5% from observed values for Bethlehem and Allentown, respectively. In contrast, Atkinson et al. (2012) found that measured $E_1$ and $E_2$ concentrations in wastewater influent for WWTPs in Ottawa and Cornwall were higher than predicted estimates—by a factor of 2 for $E_1$. 

Figure 4. Comparison between the modeled and actual concentration of $E_1$, $E_2$, $EE_2$, and total estrogen for both influent (a) and effluent (b) regimes at various WWTP sites. Note that actual measurements at Bonnybrook site for: (i) $E_1$, $E_2$, and $EE_2$ for influent; and (ii) $EE_2$ for effluent, were unavailable for this study. Error bars represent the standard deviation from the mean.
As discussed previously, deviations between the model and measurements for the Goldbar facility 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 $E_2$-eq estrogen measurements are known to account for. Also, reported limitations in analytical methods are known to prevent the detection of conjugated estrogen compounds commonly present in raw influent (Fernandez et al., 2007; Lee et al., 2005), whereas the model accounts for unconjugated estrogen compounds. Deviations between the model and measurements for the Brandon facility could be explained by sampling times during the day which may capture peak estrogen loading, deviations in flow data, and grab samples that do not represent daily averages. Note that limited available data for the Calgary facilities prevented the completion of a regression analysis, and suggested that additional data would be required to better corroborate the Calgary models.
4.1. Calculated removal matrix and sensitivity analysis

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 (Table 5 and Figure 7). 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. It is 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 estrogen (Andersen et al., 2003). The reductions also suggest that de-nitrification and aerobic biological degradation appear to play a role in reducing the amount of $E_1/E_2$ and $EE_2$, respectively. The reduced concentrations may also suggest slow sorption kinetics and no equilibrium between the absorbed 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.

### Table 5. The relative removal percent difference for each pre- and post-treatment unit process

| Estrogen | Primary clarifier$^a$ | Activated sludge bioreactor$^a$ | Secondary clarifier$^a$ | UV disinfection$^a$ |
|----------|-----------------------|-------------------------------|------------------------|-------------------|
| $E_1$    | No data               | 91 (89–92)                   | 5 (0–10)               | 48 (0–84)         |
| $E_2$    | 30 (25–35)$^c$        | 76 (69–82)                   | 17 (0–33)              | 25 (0–81)         |
| $E_1 + E_2$ | No data             | 89 (87–91)                   | 9 (0–17)               | 47 (0–82)         |
| $EE_2$   | 38 (31–44)           | 51 (34–67)                   | 4                      | 18 (0–68)         |

$^a n = 2$ Andersen et al. (2003).  
$^b n = 8$ Cicek et al. (2007).  
$^c$Brackets indicate the range.

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**Figure 7. Example of modeled 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.
Finally, a sensitivity analysis was completed by calculating the estrogen influent loading concentration for $E_1$, $E_2$, and $EE_2$ while varying each individual variable to its upper and lower range (Table 4); these calculations represent the maximum and minimum output values max ($P_i$) and min ($P_i$) used in the SI equation (Equation (10)) to determine the output % difference. The results for $E_1$ 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 $E_2$ 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 > HRT excretion rates > menstruating females > menopausal females > males; and the results for $EE_2$ can be expressed as females taking BCP > daily treated flow of wastewater > $EE_2$ excretion rate (Figure 8). The results of $E_1$ and $E_2$ 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 $EE_2$.

5. Conclusions
In this paper, a simple model was employed to determine whether an accurate range of estrogen can be predicted to arrive and leave a WWTP in Western Canada. This study employed (i) estrogen excretion rates and revised parameter ratios (ii), model simulations using a calculated sewer biological transformation rate and influent-to-effluent removal rates, and (iii) a calculated removal matrix and sensitivity analysis. The analysis revealed reasonably good results for predicting $E_1$ and $E_2$ values and calibration was required to produce good results for $EE_2$. Rate of excretion of $E_1$ during pregnancy, population of pregnant females and population taking OC are found to be the most sensitive parameters that influence the model predictions. Improvement in model predicted $E_1$, $E_2$, and $EE_2$ concentrations suggest that the mathematical model presented here could be used to assist with formulating a citywide risk estimate for these contaminants of concern.
Acknowledgments
The authors would like to thank the City of Calgary and Dr. Cicek for providing WWTP and estrogen data pertaining to Brandon site.

Funding
The authors received no direct funding for this research.

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Citation information
Cite this article as: Modeling the loading and fate of estrogens in wastewater treatment plants, Michael Fleming, Gopal Achari & Quazi K. Hassan, Cogent Environmental Science (2016), 2: 1222690.

References
Al-Sahab, B., Ardern, C. I., Hamadheh, M. J., & Tamim, H. (2010). Age at menarche in Canada: Result from the national longitudinal survey of children & youth. BMC Public Health, 10, 1–8. doi:10.1186/1471-2458-10-736
Andersen, H., Siegrist, H., Holling-sørensen, B., & Ternes, T. A. (2003). Fate of estrogens in a municipal sewage treatment plant. Environmental Science & Technology, 37, 4021–4026. doi:10.1021/es021692o
Atkinson, S. K., Marliatt, V. L., Kinpe, L. E., Lean, D. R. S., Trudeau, V. L., & Blais, J. M. (2012). The occurrence of steroidal estrogens in south-eastern Ontario wastewater treatment plants. Science of The Total Environment, 430, 119–125. doi:10.1016/j.scitotenv.2012.04.069
Baronti, C., Curini, R., D’Ascenzo, G., Di Corcia, A., Gentili, A., & Sampieri, R. (2008). Monitoring natural and synthetic estrogens at activated sludge sewage treatment plants and in a receiving river water. Environmental Science & Technology, 34, 5059–5066. doi:10.1021/es001359q
Belfroid, A. C., Van der Horst, A., Vethaak, A. D., Schäfer, A. J., Rijs, G. B. J., Wegener, J., & Cofino, W. (1999). Analysis and occurrence of estrogenic hormones and their glucuronides in surface water and waste water in The Netherlands. Science of The Total Environment, 225, 101–108. doi:10.1016/S0048-9697(98)00316-2
Briciu, R. D., Kot-Wasik, A., & Narnoisnik, J. (2009). Analytical challenges and recent advances in the determination of estrogens in water environments. Journal of Chromatographic Science, 47, 127–139. doi:10.1093/ chromatoc/47.2.127
Belfroid, A. C., Van der Horst, A., Vethaak, A. D., Schäfer, A. J., Rijs, G. B. J., Wegener, J., & Cofino, W. (1999). Analysis and occurrence of estrogenic hormones and their glucuronides in surface water and waste water in The Netherlands. Science of The Total Environment, 225, 101–108. doi:10.1016/S0048-9697(98)00316-2
Briciu, R. D., Kot-Wasik, A., & Narnoisnik, J. (2009). Analytical challenges and recent advances in the determination of estrogens in water environments. Journal of Chromatographic Science, 47, 127–139. doi:10.1093/ chromatoc/47.2.127
Canadian Institute for Health Information. (2015). Fertility in Alberta. Retrieved September 19, 2015 from http://www.finance.alberta.ca/aboutalberta/demographic_highlight_old.html
Fleming, G., Won, D., & Lee, Y. (2007). Removal of selected natural and synthetic estrogenic compounds in a canadian full-scale municipal wastewater treatment plant. Water Environment Research, 79, 795–800. doi:10.2175/106143007x117544
D’Ascenzo, G. D., Di Corcia, A., Gentili, A., Mancini, R., Mastropasqua, R., Nazzari, M., & Sampieri, R. (2003). Fate of natural estrogen conjugates in municipal sewage transport and treatment facilities. The Science of The Total Environment, 302, 199–209. doi:10.1016/S0048-9697(02)00342-X
de Mes, T. (2007). Fate of estrogens in biological treatment of concentrated black water (Unpublished doctoral dissertation). Wageningen, The Netherlands: Wageningen University. de Mes, T., Zeeman, G., & Leitinga, G. (2005). Occurrence and Fate of Estrone, 17β-estradiol and 17α-ethynylestradiol in STPs for Domestic Wastewater. Reviews in environmental science and biotechnology, 4, 275–311. doi:10.1007/s11157-005-3216-x
Drewes, J. E., Heuberer, T., Rauch, T., & Reddersen, K. (2003). Fate of pharmaceuticals during ground water recharge. Ground Water Monitoring & Remediation, 23, 64–72. doi:10.1111/j.1745-6592.2003.tb06084.x
Evans, J. S., Jackson, L. J., Hoibi, H. R., & Ikonomou, M. G. (2012). Feminization of longnose dace (Rhinichthys cataractae) in the Oldman River, Alberta, (Canada) provides evidence of widespread endocrine disruption in an agricultural basin. Scientifica, 2012, 1–11. doi:10.6064/2012/521931
Fernandez, M. P., Buchanan, I. D., & Ikonomou, M. G. (2008). Seasonal variability of the reduction in estrogenic activity at a municipal WWTP. Water Research, 42, 3075–3081. doi:10.1016/j.watres.2008.02.022
Fernandez, M. P., Ikonomou, M. G., & Buchanan, I. L. (2007). An assessment of estrogenic organic contaminants in Canadian wastewaters. Science of The Total Environment, 373, 250–269. doi:10.1016/j.scitotenv.2006.11.018
Fisher, W. A., Boroditsky, R., & Bridges, M. L. (1999). The 1998 contraception study. Canadian Journal of Human Sexuality, 8, 161–227. Retrieved from http://eric.ed.gov/?id=EJ607403
Government of Alberta. (2011). Fertility in Alberta. Retrieved Retrieved September 19, 2015 from http://www.finance.alberta.ca/aboutalberta/demographic_highlight_old.html
Hansen, P. D., Dizer, H., Hock, B., Marx, A., Sherry, J., McMaster, M., & Blaise, C. (1998). Vitellogenin—a biomarker for endocrine disruptors. TAC Trends in Analytical Chemistry, 17, 448–451. doi:10.1016/S0165-9936(98)00020-X
Hoffman, F. O., & Gardner, R. H. (1983). Evaluation of uncertainties in environmental radiological assessment models. In J. E. Till & H. R. Meyer (Eds.), Radiological Assessment (pp. 1–55). Washington, DC: U.S. Nuclear Regulatory Commission.
Johnson, A. C., Belfroid, A., & Di Corcia, A. (2000). Estimating steroid oestrogen inputs into activated sludge treatment works and observations on their removal from the effluent. Science of The Total Environment, 256, 163–173. doi:10.1016/S0048-9697(00)00481-2
Johnson, A. C. & Williams, R. J. (2004). A model to estimate influent and effluent concentrations of estradiol, estrone, and ethinylestradiol at sewage treatment works. Environmental Science & Technology, 38, 3649–3658. doi:10.1021/es035342u
Jones, O. A. H., Voultouilfs, N., & Lester, J. N. (2001). Human pharmaceuticals in the aquatic environment a review. Environmental Technology, 22, 1383–1394. doi:10.1080/095933301.2001.11908073
Jorgensen, M., Keding, N., & Skokkebak, N. E. (1991). Estimation of spermarche from longitudinal spermatic data. Biometrics, 47, 177–193.
Kato, I., Toniole, P., Akhmedakhavan, A., Koenig, K. L., Shore, R., & Zeleniuch-Jacquotte, A. (1998). Prospective study of factors influencing the onset of natural menopause. Journal of Clinical Epidemiology, 51, 1271–1276. doi:10.1016/S0196-0644(98)00119-X

Lee, H.-B., Peart, T. E., & Svoboda, M. L. (2009). Determination of endocrine-disrupting phenols, acidic pharmaceuticals, and personal-care products in sewage by solid-phase extraction and gas chromatography-mass spectrometry. Journal of Chromatography A, 1094, 122–129. doi:10.1016/j.chroma.2005.07.070

Lee, Y. M., Oleksiwicz, J. A., & Cicik, N. (2013). Fate of pharmaceuticals and endocrine disrupting compounds (EDC) in municipal wastewater treatment facilities: Methodology for assessment of environmental levels (pp. 157–167). In Proceeding of 3rd International Conference on Pharmaceuticals and Endocrine Disrupting Chemicals in Water, National Ground Water Association, Minneapolis, Minnesota, USA.

Lishman, L., Smyth, S. A., Sarafin, K., Kleywegt, S., Toito, J., Peart, T., … Seto, P. (2006). Occurrence and reductions of pharmaceuticals and personal care products and estrogens by municipal wastewater treatment plants in Ontario, Canada. Science of The Total Environment, 367, 544–558. doi:10.1016/j.scitotenv.2006.03.021

Matsui, S., Takigami, H., Matsuda, T., Taniguchi, N., Adachi, J., Kawami, H., & Shimizu, Y. (2000). Estrogen and estrogen mimics in water and the role of sewage treatment. Water Science & Technology, 42, 173–179. Retrieved from http://wst.iwaponline.com/content/42/12/173.article-info

Matsui, S., Takigami, H., Matsuda, T., Taniguchi, N., Adachi, J., Kawami, H., & Shimizu, Y. (2000). Estrogen and estrogen mimics in water and the role of sewage treatment. Water Science & Technology, 42, 173–179. Retrieved from http://wst.iwaponline.com/content/42/12/173.article-info

Peart, T., … Seto, P. (2006). Occurrence and reductions of pharmaceuticals and personal care products and estrogens by municipal wastewater treatment plants in Ontario, Canada. Science of The Total Environment, 367, 544–558. doi:10.1016/j.scitotenv.2006.03.021

Racz, L. & Goel, R. K. (2009). Fate and removal of estrogens in municipal wastewater. Journal of Environmental Monitoring, 12, 58–70. doi:10.1039/B917298J

Ram, R. N., & Gillette, J. W. (1993). An aquatic/terrestrial foodweb model for polychlorinated biphenyls (PCBs). In W. G. Landis, J. S. Hughes, & M. A. Lewis (Eds.), Environmental Toxicology and Risk Assessment (pp. 192–212). Philadelphia, PA: American Society for Testing and Materials. http://dx.doi.org/10.1520/STP1179-EB

Robinson, K. L., Valeo, C., Ryan, M. C., Chu, A., & Ivanyshyn, M. (2009). Modelling aquatic vegetation and dissolved oxygen after a flood event in the Bow River, Alberta, Canada. Canadian Journal of Civil Engineering, 36, 492–503. doi:10.1139/L08-126

Routeledge, E. J., Parker, J., Odum, J., Ashby, J., & Sumpter, J. P. (1998). Estrogenicity of drinking water? Environmental Science & Technology, 32, 933–936. doi:10.1021/es1014482

Umali, H. M., Pagsuyoin, S. A., & Parker, W. J. (2012). Estimation of influent concentrations of estrogens and select prescription drugs in wastewater treatment plants (pp. 103–106). In Proceedings of the 2012 IEEE Systems and Information Design Symposium, Charlottesville, Virginia, USA.

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