Characterization of the endocrine potencies of municipal effluents across Canada using *in vitro* bioassays

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Endocrine Disrupting Compounds

- There is increasing concern about chemicals with the potential to adversely affect the endocrine system of humans and wildlife.

- EDCs of primary toxicological concern:
  (anti)estrogenic, (anti)androgenic and steroidogenesis disruption properties

- Receptor mediated processes

- Non-receptor mediated processes

Figure 1 - EDCs mimicking endogenous hormones

http://www.precisionnutrition.com/all-about-environmental-toxin
MWWEs are considered to be the major source of EDCs in Canadian surface waters.

Figure 2 – Sources of EDCs (Adapted from Kirsten Moore et al. 2011)
Emergent Contaminants

- Plasticizers
  - Bisphenol A
  - Phthalates

- Pharmaceuticals
  - Birth control
  - Cimetidine

- Life-Stock Operations
  - Hormones
  - Pharmaceuticals

- Flame retardants
  - Organobromide compounds

- Household Cleaning Products
EDCs in MWWEs and challenges

- Our understanding of the contribution of effluents from WWTPs to the environment in Canadian surface waters is incomplete.

- MWWEs contain complex mixtures – problematic to identify the specific compounds responsible for biological effects in exposed organisms.

Figure 3 – Challenges of Complex Mixtures (www.ufz.de)
EDCs identification approaches

- Traditional targeted chemical analyses alone is not able to provide a complete and objective exposure assessment.
  - Does not provide a complete analysis of all biologically active contaminants in a sample.
  - Only searches for specific compounds.
- Targeted \textit{in vitro} bioassays can characterize the specific endocrine activity of complex mixtures, including unknown chemicals, therefore, have the potential to serve as predictors of potential hazards for wildlife.
AIME Overall Project

Assessment of Environmental Impacts of Municipal Effluents (AIME)

- **In Vivo** Studies with Native Species (FHM)
  - FHM Reproductive Assays
  - WP184
  - Wild Fish Study (In Stream)
  - WP168

- **In Vitro** Studies with Validated Bioassays

- Chemical Analytical Studies
Objectives

Determine whether MWWEs represent a significant source of EDCs to aquatic environments in Canada using an *in vitro* bioassay-directed analysis approach.

- Evaluate and quantify these compounds in MWWEs across Canada.

- Characterize WWTPs with different treatment levels (e.g. primary, secondary), population and temperature (season) removal efficiencies.
Methods

Figure 4 – Locations of wastewater treatment plants (WWTPs) in Quebec, Ontario and Saskatchewan, and the population (Pop) served.
Methods

**Figure 5** - Particulate Filtration.

**Figure 6** - SPE to Capture hormones, pharmaceuticals and other EDCs

**Figure 7** - SPE – Sample preparation for bioassay.

MDA (Anti) androgenicity

MVLN (Anti) estrogenicity

H295R Steroidogenesis Disruption
Cytotoxicity Test

Androgen Receptor Cell Line (Summer)

![Bar graph showing relative difference for different cities: Saskatoon, Regina, Guelph, Kitchener, Montreal, Quebec. The bars represent SC=1 and indicate a significant difference (*) between influent and effluent samples. The x-axis represents city names, and the y-axis represents the relative difference.](image)
Dose Response

Androgenicity – Montreal Influent (Summer)

Relative Difference [SC=1]

Concentration

0.0 0.1x 0.3x 1x 3x 10x

0.0 5.0 10.0 15.0

*
WWTPs across Canada - Spring 2014

Androgenicity

- Saskatoon
- Regina
- Guelph
- Kitchener
- Quebec
- Montreal

Relative Difference [SC=1]

City (1x Concentrated)

Anti-Androgenicity

- Saskatoon
- Regina
- Guelph
- Kitchener
- Quebec
- Montreal

Relative Difference [PC=1]

City (1x Concentrated)
Montreal Androgenicity - Summer 2014

Graph showing relative difference in concentration across different concentrations and dates.
Identification of endocrine-disrupting effects in aquatic vertebrates and invertebrates: report from the European IDEA project

H Segnera, K Carrollb, M Fenskea, C.R Janssenb, G Maackb, D Pascoeb, C Schäfersd, G.F Vandenberghec, M Wattek, A Wenzeld

Table 1
Summary of Effect Concentrations of Zebrafish, Danio rerio, Exposed to EE2 and BPA

<table>
<thead>
<tr>
<th>Species</th>
<th>Response criterion</th>
<th>Exposure period</th>
<th>Effect conc EE2</th>
<th>Effect Conc BPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zebrafish,</td>
<td>Vitellogenin</td>
<td></td>
<td>Significant induction ≥ 1.67 ng/L</td>
<td>Significant induction ≥ 375 μg/L</td>
</tr>
<tr>
<td>Danio rerio</td>
<td></td>
<td></td>
<td>Changes ≥ 3 ng/L</td>
<td>Changes ≥ 375 μg/L</td>
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<tr>
<td></td>
<td>Gonad histology</td>
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<td>No effect</td>
<td>No effect</td>
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<tr>
<td></td>
<td>Survival</td>
<td></td>
<td>Significant reduction ≥ 1.67 ng/L</td>
<td>Significant reduction ≥ 1500 μg/L</td>
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<td></td>
<td>Juvenile growth</td>
<td></td>
<td>Significant delay ≥ 1.67 ng/L</td>
<td>Significant delay ≥ 1500 μg/L</td>
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<tr>
<td></td>
<td>Time to spawn</td>
<td></td>
<td>Altered at ≥ 1.67 ng/L</td>
<td>Altered at 1500 μg/L</td>
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<tr>
<td></td>
<td>Mating behavior</td>
<td></td>
<td>Significant reduction ≥ 1.67 ng/L</td>
<td>Significant reduction at 1500 μg/L</td>
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<tr>
<td></td>
<td>Egg number per female</td>
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<td>No effect</td>
<td>No effect</td>
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<tr>
<td></td>
<td>Fertilization success</td>
<td></td>
<td></td>
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<td></td>
<td>Hatching rate of offspring</td>
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* Significant
## Chemical Analytical Data (ng/L)

<table>
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<tr>
<th>Endpoint</th>
<th>Season</th>
<th>Regina</th>
<th>Saskatoon</th>
<th>Guelph</th>
<th>Kitchener</th>
<th>Quebec</th>
<th>Montreal</th>
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<tbody>
<tr>
<td>Atrazine</td>
<td>spring</td>
<td>&lt;MDL</td>
<td>0.06</td>
<td>0.33</td>
<td>0.61</td>
<td>0.23</td>
<td>10.11</td>
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<tr>
<td></td>
<td>summer</td>
<td>0.08</td>
<td>0.16</td>
<td>0.21</td>
<td>N/A</td>
<td>&lt;MDL</td>
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<td>Carbamazepine</td>
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<td>1.24</td>
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<td>16.55</td>
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<td>Clofibrate</td>
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<td>37.80</td>
<td>39.82</td>
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<td>30.93</td>
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<td>DEET</td>
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<td>113.70</td>
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<td>&lt;MDL</td>
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<tr>
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<td>0.11</td>
<td>&lt;MDL</td>
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<td>&lt;MDL</td>
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<td>spring</td>
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<td>&lt;MDL</td>
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<td>1637.31</td>
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<tr>
<td></td>
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<td>&lt;MDL</td>
<td>&lt;MDL</td>
<td>&lt;MDL</td>
<td>N/A</td>
<td>320.99</td>
<td>&lt;MDL</td>
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<tr>
<td>Naproxen</td>
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<td>&lt;MDL</td>
<td>&lt;MDL</td>
<td>&lt;MDL</td>
<td>&lt;MDL</td>
<td>18.73</td>
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<tr>
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<td>summer</td>
<td>&lt;MDL</td>
<td>&lt;MDL</td>
<td>&lt;MDL</td>
<td>N/A</td>
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<td>Triclosan</td>
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<td></td>
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<td>8.74</td>
<td>2.27</td>
<td>N/A</td>
<td>106.08</td>
<td>13.93</td>
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</tbody>
</table>
Summary

- Most of the WWTPs had a high removal efficiency of androgenic activity.
- Selected effluents (e.g. Montreal and Quebec) had significantly increased androgenic potencies.
- Regina and Guelph showed significant increase in estrogenicity.
- Anti-androgenicity and anti-estrogenicity seem to represent a greater concern at most of the TPs.
- Removal efficiencies differed significantly among WWTPs.
- Further analysis need to be completed regarding efficiency of different treatment levels, and determine whether population and temperature affects EDCs removal efficiency.
Conclusion

• *In vitro* assays supported *in vivo* findings, suggesting that *in vitro* assays represent a relevant and cost-effective tool for predicting EDCs in aquatic environments.

• Targeted chemical analysis did not show a presence of chemicals with estrogenic properties, aligning with *in vitro* assays findings.

Figure 8. Regina WWTP outflow
Acknowledgement

- Saskatoon Wastewater Treatment Plant
- City of Regina Wastewater Treatment Plant
- Department of Biological Science, University of Lethbridge, Lethbridge, AB, Canada;
- Biology Department, University of Waterloo, Waterloo, ON
- INRS-ETE et UQTR, Quebec, QC, Environment Canada
- Ashley Moate, Craig Baird, Shawn Beitel, Leanne Flahr, Dr. J.X. Sun, Dr. Hui Peng and Bryanna Eisner.
Questions?

Figure 9: FHM field sampling in Saskatoon Upstream and Downstream

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References

8. Kime and Nash, 1999 – Aquaculture 177: 345-352
10. Kidd et al., 2014 - Phil. Trans. R. Soc. B 369
Steroidogenesis Disruption across Canada

(Summer-2014)

Relative Difference [SC = 1]

City (1x Concentrated)

Saskatoon  Regina  Guelph  Kitchener  Montreal  Quebec

SC - Influent  Effluent

* Indicates significant difference.