Characterization of the endocrine potencies of municipal effluents across Canada using \textit{in vitro} bioassays

Tabata Bagatim

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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

**Flame retardants**
- Organobromide compounds

**Life-Stock Operations**
- Hormones
- Pharmaceuticals

**Household Cleaning Products**
EDCs in MWWEs and challenges

- Incomplete understanding of the effluents from WWTPs contribution to the environment in Canadian surface waters.

- Complex mixture – problematic to identify the specific compounds that are responsible for biological effects in exposed organisms.

Figure 3 – Saskatoon WWTP
EDCs identification approaches

- Traditional targeted chemical analyses is not able to provide a complete and objective exposure assessment.

- Targeted *in vitro* bioassays can characterize the specific endocrine activity of complex mixtures, including unknowns chemicals.

- *In vitro* bioassays 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
  - Wild Fish Study (In Stream)
- 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 endocrine disrupting activities of MWWEs across Canada.
- Characterize the efficiency of WWTPs to remove EDCs.
- Evaluate different treatment levels of WWTPs (primary, secondary, etc.).
- Determine whether season (temperature) influences EDCs removal efficiency.
Methods

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

- Figure 5 - Particulate Filtration.
- Figure 6 - SPE – Capture of organic compounds.
- Figure 7 - SPE – Sample preparation for bioassay.

MDA (Anti) androgenicity

MVLM (Anti) estrogenicity

H295R Steroidogenesis Disruption
Cytotoxicity Test

City (10x concentrated)

- Control
- Influent
- Effluent

* Significant difference compared to Control
Dose Response - Saskatoon Spring

Androgenicity

Anti-Androgenicity

AEQ [ng DHT]

% Inhibition

Concentration

Influent

Effluent

Control

Influent

Effluent

Concentration

Control

0.1x

0.3x

1x

3x

10x

0.1x

0.3x

1x

3x

10x

0.1x

0.3x

1x

3x

10x

0.1x

0.3x

1x

3x

10x

0.1x

0.3x

1x

3x

10x

*
WWTPs across Canada - Spring 2014

Androgenicity

Anti-Androgenicity

City (1x concentrated)

AEQ [ng DHT]

% Inhibition

Control  Influent  Efluent

Control  Regina  Saskatoon  Guelph  Kitchener  Montreal  Quebec

City (1x concentrated)

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Androgenicity across WWTPs

Spring 2014

Summer 2014

AEQ [ng DHT]
Montreal Androgenicity - Summer 2014

• Montreal Effluent (1x) showed androgenic activity (12-14ng/L AEQ) in spring/summer.
WWTPs across Canada - Spring 2014

**Estrogenicity**

- Montreal Effluent (1x) = 1.78ng/L EEQ in Spring.
- Segner, 2003 - EE2>1.67ng/L ↓ egg number and fertilization success, delay in time to spawn.
Steroidogenesis Disruption across Canada - Summer 2014

Control Regina Saskatoon Guelph Kitchener Montreal Quebec

Fold-Change [SC=1]

City (1x concentrated)
Non-target screening for contaminants in WWTP Effluents

- Large number of known and unknown chemicals are present in effluents
- So far, identified approx. 400 different compounds, ranging from pharmaceuticals and personal care products to pesticides
## Chemical Analytical Data (ng/L)

<table>
<thead>
<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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<tr>
<td>Atrazine</td>
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Summary

- Most of the WWTPs had a high removal efficiency of androgenic activity.

- Selected effluents (e.g. Montreal) had significantly increased androgenic potencies.

- Removal efficiencies differed significantly among WWTPs.

- Further analysis need to be completed regarding efficiency of different treatment levels of WWTPs and determine whether population and temperature affects EDCs removal efficiency.
Conclusion

• *In vitro* assays supported *in vivo* findings, suggesting a 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.
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- Ashley Moate, Craig Baird, Shawn Beitel, Leanne Flahr, J.X. Sun, Bryanna Eisner
Questions?

**Fig8:** FHM field sampling in Saskatoon Upstream and Downstream
References

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10. Kidd et al., 2014 - Phil. Trans. R. Soc. B 369