Impact of EDCs on Trout in an Effluent Dominated Stream

2009 GWPC Water/Energy Sustainability Symposium
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Presentation Summary

- SBWRD Background information
- Introduction to endocrine disrupting compounds (EDCs), pharmaceuticals and personal care products (PPCPs)
- Implications for aquatic life
- SBWRD’s research efforts
What are Endocrine Disrupting Compounds (EDCs)?

- Natural and Synthetic Hormones
- Pharmaceuticals and Personal Care Products
- Pesticides
- Detergents
- Industrial Compounds
SBWRD is Concerned About EDCs Discharged to East Canyon Creek
SBWRD’s Reason for Concern

Brown Trout
(Salmo trutta)

Bonneville Cutthroat
(Oncorhynchus clarki)

Upstream reach of East Canyon Creek in late summer
Detection of Trace Level EDCs Possible by Advances in Analytical Methods

Liquid Chromatography/Mass Spectrometer
Public Perception of EDCs in Water is a Challenge for Water Professionals
Natural and Synthetic Hormones Thought to be the EDCs of Greatest Concern

<table>
<thead>
<tr>
<th>Microconstituent</th>
<th>MRL¹ (ng/L)</th>
<th>Type/Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>1.0</td>
<td>Pain Relief</td>
</tr>
<tr>
<td>Caffeine</td>
<td>3.0</td>
<td>Stimulant</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>5.0</td>
<td>Anti-Epileptic</td>
</tr>
<tr>
<td>Cotinine</td>
<td>1.0</td>
<td>Stimulant</td>
</tr>
<tr>
<td>Diazepam</td>
<td>1.0</td>
<td>Anti-Anxiety</td>
</tr>
<tr>
<td>Estrone</td>
<td>1.0</td>
<td>Natural Hormone</td>
</tr>
<tr>
<td>Estradiol</td>
<td>1.0</td>
<td>Natural Hormone</td>
</tr>
<tr>
<td>Ethinyl Estradiol- 17αβ</td>
<td>1.0</td>
<td>Synthetic Hormone</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>1.0</td>
<td>Anti-Depressant</td>
</tr>
<tr>
<td>Progesterone</td>
<td>1.0</td>
<td>Natural Hormone</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>1.0</td>
<td>Antibiotic</td>
</tr>
<tr>
<td>Testosterone</td>
<td>1.0</td>
<td>Natural Hormone</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>1.0</td>
<td>Antibiotic</td>
</tr>
<tr>
<td>Triclosan</td>
<td>5.0</td>
<td>Anti-Microbial</td>
</tr>
</tbody>
</table>

¹Method Reporting Limit
Sources of Estrogens

- Mature woman
  - 4.8 μg estriol
  - 3.5 μg estradiol
  - 8.0 μg estrone

- Post Menopause Woman
  - 7 μg/day (all 3)

- Men
  - 7 μg/day (all 3)

- Pregnant Woman
  - 6,000 μg estriol
  - 600 μg estrone
  - 259 μg estradiol

- Synthetic Hormones
  - 26% of ethyl estradiol in birth control pills is excreted

- Hormone & estrogen replacement therapy
  - 65% estradiol excreted
  - 15% estrone excreted
Timeline of Research Efforts

JUNE 2007
- Influent & Effluent Grab Samples
- GAC Benchscale Test

OCT 2007
- Brown Trout Investigation

NOV 2007
- Treatment Technology Evaluation

MAR 2008
- Sex Ratio Investigation

NOV 2008
- Sentinel Study
EDCs Detected at Low Concentrations in ECWRF Effluent

JUNE 2007
SBWRD Collects Influent & Effluent Grab Samples
# Historical EDC/PPCP Detections

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Samples</th>
<th>Detection Frequency</th>
<th>AVG (ng/L)</th>
<th>MRL (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-Screen Bioassay</td>
<td>18</td>
<td>100%</td>
<td>0.69</td>
<td>0.03</td>
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<tr>
<td>Cotinine</td>
<td>15</td>
<td>100%</td>
<td>29</td>
<td>1.0</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>24</td>
<td>100%</td>
<td>846</td>
<td>1.0</td>
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<tr>
<td>Gemfibrozil</td>
<td>19</td>
<td>95%</td>
<td>85</td>
<td>1.0</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>22</td>
<td>95%</td>
<td>73</td>
<td>1.0</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>34</td>
<td>94%</td>
<td>81</td>
<td>5.0</td>
</tr>
<tr>
<td>Triclosan</td>
<td>20</td>
<td>90%</td>
<td>36</td>
<td>5.0</td>
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<tr>
<td>Ibuprofen</td>
<td>19</td>
<td>89%</td>
<td>29</td>
<td>1.0</td>
</tr>
<tr>
<td>Caffeine</td>
<td>24</td>
<td>88%</td>
<td>23</td>
<td>3.0</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>24</td>
<td>88%</td>
<td>50</td>
<td>1.0</td>
</tr>
<tr>
<td>Diazepam</td>
<td>17</td>
<td>47%</td>
<td>87</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Ethinyl Estradiol – 17α</strong></td>
<td>34</td>
<td>29%</td>
<td>5.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Estrone</td>
<td>34</td>
<td>26%</td>
<td>47</td>
<td>1.0</td>
</tr>
<tr>
<td>Estradiol</td>
<td>34</td>
<td>18%</td>
<td>1.6</td>
<td>1.0</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>24</td>
<td>13%</td>
<td>2.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Iopromide</td>
<td>20</td>
<td>n/a</td>
<td>12&lt;sup&gt;1&lt;/sup&gt;</td>
<td>5.0</td>
</tr>
<tr>
<td><strong>Testosterone</strong></td>
<td>28</td>
<td>n/a</td>
<td>1.2&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.0</td>
</tr>
<tr>
<td>DEET</td>
<td>1</td>
<td>n/a</td>
<td>437&lt;sup&gt;1&lt;/sup&gt;</td>
<td>25</td>
</tr>
<tr>
<td>TDCPP</td>
<td>1</td>
<td>n/a</td>
<td>222&lt;sup&gt;1&lt;/sup&gt;</td>
<td>25</td>
</tr>
<tr>
<td>Tris (2-chloroethyl) phosphate</td>
<td>1</td>
<td>n/a</td>
<td>166&lt;sup&gt;1&lt;/sup&gt;</td>
<td>25</td>
</tr>
</tbody>
</table>

<sup>1</sup>Result shown is a single detection (not an average)
Current Literature Describes Potential Effects of EDC Exposure for Fish

Estrogenicity (feminization)
  - Vitellogenin induction in male fish
  - Intersex fish
  - Skewed sex ratios
  - Population collapse

Bioaccumulation

Chronic Toxicity

(Nash et al, 2004)
Vitellogenin (Vtg) is a Biomarker of Estrogen Exposure in Male Fish

Egg yolk protein

Vtg not normally found in male fish

Vtg detected after exposures as low as 1.0 ng/L (ppt)

No known short-term health consequences
Connection Between Vtg Induction and More Severe Effects Not Yet Understood

FEMALES: MALES

Testis

Undetermined gonadal (ovary-type) tissue
Estrogen Activity Measured by E-Screen Bioassay

Breast cancer cell line with growth response to estrogen

Negative Control  Positive Control

Reported as estradiol equivalents (MRL = 0.030 ppt)
Valuable Lessons Learned Along the Way

OCT 2007
GAC Benchscale Test

NOV 2007
Brown Trout Investigation
Ozone Oxidation Recommended as Treatment Technology of Choice

GAC Filtration

Ozone/Peroxide

UV/Peroxide

MAR 2008

Treatment Technology Testing

NF/RO not considered due to cost and concentrate disposal constraints
EDC Removal Using Existing Infrastructure: GMF to GAC

Existing Granular Media Filter (Parkson Dynasand)  GAC Contactor
Efforts to Identify Impacts of Effluent on Downstream Trout

NOV 2008
Sex Ratio Investigation

DEC 2008
Sentinel Study
Study Objectives

- Determine if EDC concentrations are high enough to induce vitellogenesis by holding sentinel fish in the effluent
- Determine if downstream fish populations were being feminized by conducting a field investigation of sex ratios
- Evaluate fish tissue for bioaccumulation of EDCs
Sentinel Study Methods

Holding pen at fish hatchery

Holding pen in effluent aeration basin
Sentinel Study Methods

Blood Sample (0.5mL) Collected From Each Fish
Sentinel Study Methods

Vtg Analysis Requires Blood Plasma
**Vtg Levels Increased in Fish Exposed to ECWRF Effluent**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ECWRF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>3 weeks</td>
</tr>
<tr>
<td>Vitellogenin (ug/ml)</td>
<td>0.343 ± 0.09</td>
<td>0.110 ± 0.03</td>
</tr>
<tr>
<td>Total length (mm)</td>
<td>255.2 ± 3.6</td>
<td>265.6 ± 3.9</td>
</tr>
<tr>
<td>Total weight (g)</td>
<td>188.3 ± 8.0</td>
<td>207.9 ± 9.8</td>
</tr>
<tr>
<td>GSI</td>
<td>0.21 ± 0.07</td>
<td></td>
</tr>
</tbody>
</table>
Objectives of Brown Trout Sex Ratio Investigation

Compare Females vs Males

Sample Size Goal (n=70)

Expected 50:50 Ratio

Blood Samples (Vtg)

Tissue Samples (Bioaccumulation)
Sex Ratio Investigation Methods

Electrofishing  Transport  Holding Pen
Sex Ratio Investigation Methods

Gender Determination: Palpitation or Necropsy
Sex Ratio Investigation Methods
Summary of Fish Collected For the Sex Ratio Investigation

71 Fish Captured

43 Released (Sexually Mature)

28 Harvested (Gender by Necropsy)

5 Additional Fish Sampled Upstream (Negative Control)
Sex Ratio Investigation Results

Altered Sex Ratio Not Seen

38 Female (54%) to 33 Male (46%)

Vtg Not Detected in Male Fish

4 to 1 Dilution of Effluent in Stream

Fish Tissue Results – Still Pending
Conclusions

- EDCs detected at low concentrations (ppt) in ECWRF
- Effluent EDCs concentrations high enough to induce vitellogenesis in male trout
- ECWRF effluent does not appear to have altered the sex ratio of the brown trout
Observations

- Additional research needed to understand which of the compounds are responsible for vitellogenin induction in male fish
- Bioassays and biomarkers are cost effective indicators of estrogenicity potential
- EDC’s can be removed using advanced treatment technologies
- Water professionals need to be prepared to address EDC concerns with customers
Questions?

For more info visit: http://www.sbwrdd.com
Concentrations of Estrogens that begin to affect Male Fish

**Inducement of vitellogenin production**
- $\approx 5 \text{ ng/L} \ 17\beta$-estradiol
- estriol is 30 times less potent than above
- $\approx 3.2 \text{ ng/L} \ for \ estrone$
- $\geq 1 \text{ ng/L} \ for \ 17\alpha$-ethinylestradiol

**Inducement of intersex**
- $\approx 10 \text{ ng/L} \ for \ estrone, \ or \ 17\beta$-estradiol
- estriol is 100 times less potent than above
- $\approx 4 \text{ ng/L} \ for \ 17\alpha$-ethinylestradiol
- estrogenic substances are additive
Fish Tracking Results for Negative Control Test Group

Hatchery Group (Negative Control):

50 Fish
- 7 Mortalities
- 4 Lost Tags
- 39 Pre & Post Exposure
Fish Tracking Results for Effluent Exposure Group

ECWRF Effluent Group:
50 Fish
10 Mortalities
9 Lost Tags
31 Pre & Post Exposure