Biodegradation of CECs in Streams
USGS Toxics Program CEC Research

Sources & Pathways

Environmental Occurrence

Transport & Fate

Receptors & Impacts

Methods Development
CEC = umbrella term
CEC Ecological Impacts

- Endocrine Disruption
- Contaminant Toxicity
- Antibiotic Resistance
- Pathogens
- etc.

TRUE STORY: THE ESTROGEN, STEROIDS, PROZAC AND OTHER DRUGS WE TAKE... ARE NOT BEING FILTERED OUT OF OUR WASTE BY SEWAGE TREATMENT PLANTS OR SEPTIC SYSTEMS

AND THEY'RE TURNING UP IN INCREASING CONCENTRATIONS IN RIVERS AND LAKES, ...AND IN THE FISH THAT INHABIT THEM.

THAT WAS DELICIOUS TROUT! SUDDENLY, I FEEL LESS DEPRESSED ABOUT MY ERECTILE DYSFUNCTION

*11/14/03 CNN.COM
Managing EDC Impacts

I. No environmental release.
Summary - National Recon Studies

- Streams (1999-2000)
- Ground Water (2000)
- Drinking Water Sources (2001)
- Streambed Sediment (2002)

(Kolpin, et al., 2002, Barnes et al., 2008; Focazio et al., 2008)

Furlong et al.
Managing EDC Impacts

I. No environmental release.
II. Environmentally benign.
EDCs Effects on Aquatic Life

• Biochemical:
  – Vitellogenin (egg yolk protein) production in male fish

• Organism:
  – Intersex (male and female tissue) fish

• Population:
  – “Feminization” of fish populations
  – Population crashes
EDC Impacts – Biochemical
e.g. male vitellogenin Induction

- Vitellogenin (egg yolk protein):
  - Induced in males – 14 days
  - 10-25% effluent
  - Log scale

*Effluent Elevates Plasma Vitellogenin*

- Bar graph showing elevated plasma vitellogenin with different effluent concentrations.

*Pimephales promelas (fathead minnow)*
EDC Impacts – Organism
e.g. intersex

- Gonads removed
- Embedded & sectioned
- Examined microscopically
  - male intersex = oocytes in testes
EDC Impacts – Population
e.g. Feminization

(Vajda et al. ES&T. 2008)
Kidd et al. PNAS 2007;104:8897-8901:

- ELA.
- *Pimephales promelas* (fathead minnow).
- 5-6 ng/L EE2 (2001-2003).
- Population crash within 1-3 years.
Managing EDC Impacts

I. No environmental release.
II. Environmentally benign.
III. Impacts subject to responsible management:
   I. Improved treatment prior to release.
   II. Assimilative capacity of receptor.
As a contaminant is transported through the environment, it is subject to a variety of processes; some of which have the potential to attenuate its environmental toxicity.

Mass destruction preferred over media shift or dilution:

• Biodegradation
• ...
Potential for EDC Biodegradation

• Endocrine Hormones:
  • Natural:
    • 17β-Estradiol (E2)
    • Estrone (E1)
    • Testosterone
  • Synthetic:
    • 17α-Ethinylestradiol (EE2)

• Endocrine Disrupting Compounds:
  • Biochemical Mimics:
    • Phenol Group
    • Extended Carbon Backbone
  • APE Metabolites
    • e.g. 4-nonylphenol
### Potential for EDC Biodegradation:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>17β-Estradiol</th>
<th>4-NP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative Estrogenicity*</td>
<td>1000</td>
<td>1</td>
</tr>
<tr>
<td>Concentration$_{\text{effluent}}$</td>
<td>10 ng/L</td>
<td>to 343 µg/L</td>
</tr>
<tr>
<td>Concentration$_{\text{receptor}}$</td>
<td>10 ng/L</td>
<td>to 644 µg/L</td>
</tr>
<tr>
<td>Concentration$_{\text{sediment}}$</td>
<td>10 µg/kg</td>
<td>to 14 mg/kg</td>
</tr>
<tr>
<td>Detection$_{\text{WWTP-Streams}}$</td>
<td>&lt;20 %</td>
<td>majority</td>
</tr>
</tbody>
</table>

*Depends on study and NP isomer
Potential for EDC Biodegradation: $^{14}$C-Radiotracer Experiment

17β-Estradiol

4-\(n\)-Nonylphenol

$^{14}$C-EDC

- Ring labeled.
- $^{14}$C-mineralization indicates ring destruction.
- $^{14}$C-mineralization indicates loss of estrogenicity.
**14C-Radiotracer Experiment**

**Radiotracer approach:**
- [14C] EDC.
- GC with TCD and Radiometric Detection.
- 14CO2, 14CH4.
- Sediment & water microcosms.
Triple Watershed Study

Fourmile Creek (IA)
- Drainage Area = 410 km²
- Gradient = 1 m/km
- Population ~ 27,000
- Fine Grain Sand
- WWTP Discharge = 15-80 % DSF

Boulder Creek (CO)
- Drainage Area = 1160 km²
- Gradient = 4 m/km
- Population ~ 174,000
- Coarse Sand & Gravel
- WWTP Discharge = 20-50 % DSF

South Platte River (CO)
- Drainage Area = 10000 km²
- Gradient = 2 m/km
- Population ~ 1,700,000
- Coarse Sand and Gravel
- WWTP Discharge > 90 % DSF
Sediment Red-Ox Conditions

“Oxic”

Water-Column Dissolved Oxygen:
- YSI 556 multiparameter meter.
- Four points, mid-depth.
- Saturated.
  - Mean = 8.8 mg/L
- No upstream-downstream change.

Sediment Dissolved Oxygen:
- Chemets colorimetric.
- Four points, 10 cm depth.
- 0.4-4.0 mg/L.
  - Mean 1.3 mg/L
- No upstream-downstream change.
Potential for EDC Biodegradation

- **Endocrine Hormones:**
  - **Natural:**
    - 17β-Estradiol (E2)
    - Estrone (E1)
    - Testosterone
  - **Synthetic:**
    - 17α-Ethinylestradiol (EE2)

- **Endocrine Disrupting Compounds:**
  - **Biochemical Mimics:**
    - Phenol Group
    - Extended Carbon Backbone
  - **APE Metabolites**
    - e.g. 4-nonylphenol

- **Potential for EDC Biodegradation**
Potential for 4-NP Biodegradation: Oxic Sediment

- Sediment upstream of WWTP outfall.
- Efficient mineralization under oxic conditions.
  - 15-20% in 48 h.
  - Complete in 32 d.

Bradley et al. ET&C. 2008
Potential for EDC Biodegradation

- **Endocrine Hormones:**
  - **Natural:**
    - $17\beta$-Estradiol (E2)
    - Estrone (E1)
    - Testosterone
  - **Synthetic:**
    - $17\alpha$-Ethinylestradiol (EE2)

- **Endocrine Disrupting Compounds:**
  - **Biochemical Mimics:**
    - Phenol Group
    - Extended Carbon Backbone
  - **APE Metabolites**
    - e.g. 4-nonylphenol

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**17\beta\text{-estradiol}**

**4-n-alkylphenol**

$n = 2$ (ethyl), 4 (butyl), 6 (hexyl), 8 (octyl), and 9 (nonyl)
Potential for Steroid Hormone Biodegradation: 
$^{14}$C-Radiotracer Experiment

[4-$^{14}$C] model hormones

- “A” ring labeled.
- $^{14}$C-mineralization indicates ring destruction.
- $^{14}$C-mineralization indicates loss of estrogenicity.
Potential for 17β-Estradiol Biodegradation: Oxic Sediment?

- Upstream sediment:
  - 30-40% in 32 d.
- Outfall sediment:
  - 40-90% in 32 d.

• Potential for 17β-Estradiol biodegradation in oxic sediments.

Bradley et al. ES&T. 2009
Potential for 17β-Estradiol Biodegradation: Oxic Water?

- 154 days.
- FC - no biodegradation.
- Biodegradation at outfall in BC & SPR.

- Biodegradation efficiency stimulated by effluent:
  - Adapted microbial population?
  - …

Bradley et al. ES&T. 2009
Potential for Estrone/Testosterone Biodegradation
Oxic Sediment?

Bradley et al. ES&T. 2009

[Graphs showing the mineralization of Estrone and Testosterone in Boulder Creek, South Platte River, and Fourmile Creek over a period of 35 days. The graphs illustrate the percentage of theoretical mineralization over time, with error bars indicating variability.]
Potential for EDC Biodegradation

- **Endocrine Hormones:**
  - **Natural:**
    - 17-β-Estradiol (E2)
    - Estrone (E1)
    - Testosterone
  - **Synthetic:**
    - 17α-Ethinylestradiol (EE2)

- **Endocrine Disrupting Compounds:**
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\[ n = 2 \text{ (ethyl)}, 4 \text{ (butyl)}, 6 \text{ (hexyl)}, 8 \text{ (octyl), and 9 (nonyl)} \]
Potential for Steroid Hormone Biodegradation: 
$^{14}\text{C}$-Radiotracer Experiment

$^{14}\text{C}$-mineralization indicates ring destruction.
$^{14}\text{C}$-mineralization indicates loss of estrogenicity.

[4-$^{14}\text{C}$] model hormones:
- “A” ring labeled.
- $^{14}\text{C}$-mineralization indicates ring destruction.
- $^{14}\text{C}$-mineralization indicates loss of estrogenicity.
Potential for $17\alpha$-Ethinylestradiol Biodegradation?

**Boulder Creek:**
- Significant mineralization of EE2 in sediments.
  - Upstream
  - Downstream
- No EE2 mineralization in water or epilithon treatments.
Relative Biodegradation?
Boulder Creek

Boulder Creek:
- Significant mineralization of EE2 in sediments.
  - Upstream
  - Downstream
- No EE2 mineralization in water or epilithon treatments.
- Rate of mineralization of EE2 substantially lower than for E2 or NP:
  - EE2 ~ 15-25% in 70 days
  - E2 & NP ~ 20-30% in 7 days

Writer et al. ES&T In Press
EDC Assimilative Capacity in Streams

- **EDCs:**
  - Endocrine Hormones.
    - Natural
    - Synthetic
  - Endocrine Mimics.

- Potential for EDC biodegradation in streams affected by:
  - Matrix (sediment > epilithon > water).
  - Contaminant (e.g. E2, NP > EE2).
  - Red-Ox conditions (oxic > anoxic).
  - etc.

Exploitable EDC Assimilative Capacity
Managing EDC Impacts

I. No environmental release.

II. Environmentally benign.

III. Impacts subject to responsible management:
   I. Improved treatment prior to release.
   II. Assimilative capacity of receptor.
   III. Discontinued use.
Managing EDC Impacts

- Improve Treatment (Occurrence)
- Identify Impact Thresholds
- Assess Assimilative Capacity
- Manage EDC Risk
Managing EDC Impacts

Impending regulation?
- “Aquatic Life Criteria”
- “Drinking Water Standards”
Managing EDC Impacts

Impending regulation?
- “Aquatic Life Criteria”
- “Drinking Water Standards”

USEPA Candidate Contaminant List 3:
- 104 chemicals.
- 14 PPCPs.
- 9 hormones:
  - 17-β-Estradiol (E2)
  - Estrone (E1)
  - 17α-Ethinylestradiol (EE2)

<table>
<thead>
<tr>
<th>Substance Name</th>
<th>PPCP</th>
<th>CASRN</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>17alpha-estradiol</td>
<td>P</td>
<td>57-91-0</td>
<td>It is an estrogenic hormone and is used in pharmaceuticals.</td>
</tr>
<tr>
<td>equilenin</td>
<td>P</td>
<td>517-09-9</td>
<td>It is an estrogenic hormone and is used in pharmaceuticals.</td>
</tr>
<tr>
<td>equilin</td>
<td>P</td>
<td>474-86-2</td>
<td>It is an estrogenic hormone and is used in pharmaceuticals.</td>
</tr>
<tr>
<td>Erithromycin</td>
<td>P</td>
<td>114-07-8</td>
<td>It is used in pharmaceutical formulations as an antibiotic.</td>
</tr>
<tr>
<td>Estradiol (17-beta estradiol)</td>
<td>P</td>
<td>50-28-2</td>
<td>It is an estrogenic hormone and is used in pharmaceuticals.</td>
</tr>
<tr>
<td>estriol</td>
<td>P</td>
<td>50-27-1</td>
<td>It is an estrogenic hormone and is used in veterinary pharmaceuticals.</td>
</tr>
<tr>
<td>estrone</td>
<td>P</td>
<td>53-16-7</td>
<td>It is an estrogenic hormone and is used in veterinary and human pharmaceuticals.</td>
</tr>
<tr>
<td>Ethinyl Estradiol (17-alpha ethynyl estradiol)</td>
<td>P</td>
<td>57-63-6</td>
<td>It is an estrogenic hormone and is used in veterinary and human pharmaceuticals.</td>
</tr>
<tr>
<td>Mestranol</td>
<td>P</td>
<td>72-33-3</td>
<td>It is an estrogenic hormone and is used in veterinary and human pharmaceuticals.</td>
</tr>
<tr>
<td>Norethindrone (19-Norethisterone)</td>
<td>P</td>
<td>68-22-4</td>
<td>It is a progestational hormone used in pharmaceuticals.</td>
</tr>
<tr>
<td>Quinoline</td>
<td>P</td>
<td>91-22-5</td>
<td>It is used in the production of other substances, and as a pharmaceutical (anti-malarial) and as a flavoring agent.</td>
</tr>
<tr>
<td>2-Methoxyethanol</td>
<td>PCP</td>
<td>109-86-4</td>
<td>It is used in consumer products, such as synthetic cosmetics, perfumes, fragrances, hair preparations, and skin lotions.</td>
</tr>
<tr>
<td>2-Propan-1-ol</td>
<td>PCP</td>
<td>107-18-6</td>
<td>It is used in the production of other substances, and in the manufacture of flavorings and perfumes.</td>
</tr>
<tr>
<td>Butylated hydroxyanisole</td>
<td>PCP</td>
<td>25013-16-5</td>
<td>It is used as a food additive (antioxidant).</td>
</tr>
</tbody>
</table>
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The Toxics Program:
[toxics.usgs.gov]

Emerging Water Quality Issues:
[toxics.usgs.gov/regional/emc.html]