Effects of Endocrine Disrupting Compounds on Fish - What Do We Know and What Don’t We Know:

Part A: Overview

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Aquatic Toxicology Laboratory 2006 Fall Research Retreat
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• What are Endocrine Disrupting Compounds (EDCs)?
• Effects Observed in Laboratory Studies.
• Lessons From Field Studies
• Relevance to MWRDGC
Endocrine Disrupting Compound - One Definition

“An Endocrine Disrupting Compound is an Anthropogenic Compound that may have an Adverse Effect Mediated Directly through the Endocrine System of Fish, Wildlife, or Humans.”
- Interact with hormone receptors.
- Little change in the past 500 million years.
- Hormones in fish and humans are remarkably similar.
Endocrine Disrupting Compound - Diversity

Pharmaceuticals & Personal Care Products (PPCPs)

Bath additives, shampoos, skin care products, hair sprays, oral hygiene, soaps, detergents

Fragrances

Preservatives

Disinfectants/Antiseptics

Sunscreen Agents

Daughton and Ternes
Alkylphenol Ethoxylates (APEs)

- Nonyl & Octyl Phenol Ethoxylate
- High Production Volume Chemical
  - ~ 391.5 million lbs/year NPEs and 77 million lbs/yr OPEs used in North America (U.S. & Canada) in 2003
- Uses - Detergents, wetting agents, dispersants, emulsifiers, solubilizers and foaming agents
- Industrial applications - Pulp and paper, textiles, coatings, agricultural pesticides, lube oils and fuels, metals and plastics
- Chief concern is NP based compounds.
  - OP is also toxic, and more potent ED but only = 10 – 15% of APEs used
A snapshot from effluents flowing into the Mississippi River:

**Natural Estrogens:**
- Estradiol: nd - 50 ng/L
- 17β-Estradiol: nd - 7.3 ng/L

**Synthetic Emerging Contaminants:**
- Ethynylestradiol (birth control): nd - 3 ng/L
- Total alkylphenols (detergents): > 50,000 ng/L
- Bisphenol A (plasticizer): 35.5 - 9,026 ng/L
- Carbamazepine (anti-epileptic): 823 - 1,360 ng/L
- Triclosan (anti-microbial): 82 - 318 ng/L
- Perfluorochemicals (non-stick Teflon): 3.6 - 28 ng/g in fish tissue

*nd = non-detectable; ng/L = parts per trillion*
Endocrine Disrupting Compound - Pharmaceuticals

Total SSRI concentration: > 5,000 ng/L!
Emerging contaminants enter the aquatic environment as mixtures:

1. What are the effects of individual compounds?
2. What are the effects of mixtures?
3. Can the sum of effects of individual compounds account for the effect of whole effluent?
The Ultimate Endpoint
**Reproductive Fitness** - ability to produce the greatest number of offspring.

Laboratory Studies - Concept

Population Size

- Food & Predation
- Sexual Competition
- Reproductive Fitness

Survival - Parent Generation

Reproductive Success - F1 Generation

F2 Generation
Laboratory Studies - Endpoints

- Tubercles & Dorsal Pad
- Female Ovary
- Intersex Condition
- Male Testis
- Brain Activity
- Vitellogenin - Egg Yolk Protein
- Liver
Expose mature male fathead minnows for 21 days to graded series of nonylphenol.

- Competitive spawning
- Secondary Sex Characters
- Vitellogenin
- Histology
Laboratory Studies - Male exposures

Measured NP Concentration (μg/L)

- 0.3
- 5
- 11
- 15

Vitellogenin Concentrations (mg/mL)

- day 1: p=0.38
- day 4: p=0.085
- day 7: p=0.013
- day 14: p=0.012
- day 35: p=0.25

Plasma VTG Concentration (mg/mL)

Vitellogenin Concentrations (mg/mL)
Laboratory Studies - Male exposures

-20% -10% +10% +20% no difference

50%

0.3 μg/L (47)  p<0.001
5 μg/L (57)  p<0.001
11 μg/L (58)  0.035
15 μg/L (74)  p<0.001

EPA Limit

Competitive Spawning

Schoenfuss et al. 2008. Aquatic Toxicology.
Exposure of newly hatched fathead minnows for 63 days to nonylphenol or alkylphenol mixture based on effluent.

- Record survival and rear in clean water to maturity.
- Allow exposed mature males to compete with control males for access to spawning opportunities.
Laboratory Studies - Larval exposures

Survival

Larval Survival in Nonylphenol

Larval Survival in Alkylphenol Mixture
Larval fish exposed to an alkylphenol mixture at half the concentration measured in a treaded wastewater effluent cannot compete with unexposed fish once they are adults.

Bistodeau et al. 2006. Aquatic Toxicology.
Experimental Analysis

- **Treatment**
  - 21 Day Exposure
- **Control**
  - Subsample
- **Pre SSC**
- **4 Day Competitive Challenge**
  - Post SSC
  - VTG
  - GSI
  - HSI
  - Histology
## Nominal Exposure Concentrations

<table>
<thead>
<tr>
<th>(ng/L)</th>
<th>Low</th>
<th>Med</th>
<th>High</th>
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</thead>
<tbody>
<tr>
<td>Estrone (E1)</td>
<td>5 (0.5)</td>
<td>50 (5)</td>
<td>100 (10)</td>
</tr>
<tr>
<td>Estradiol (E2)</td>
<td>1</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>Ethynylestradiol (EE2)</td>
<td>0.1 (1)</td>
<td>2.5 (25)</td>
<td>10 (100)</td>
</tr>
<tr>
<td>Mixture</td>
<td>E1 10</td>
<td>N/A</td>
<td>E1 30</td>
</tr>
<tr>
<td></td>
<td>E2 1</td>
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<td>E2 3</td>
</tr>
<tr>
<td></td>
<td>EE2 0.1</td>
<td></td>
<td>EE2 0.3</td>
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</tbody>
</table>

(3) \(=\) Estradiol Equivalencies

(9)
Comparing estradiol equivalencies, VTG analysis demonstrate E1-H, E2-M, Mix-H all had similar VTG induction indicating that the addition of estrogens (E1, E2, EE2) has an additive effect.
% Intersex In Males

Comparing estradiol equivalencies, data indicates a synergistic effect.
Field Studies - Questions

Emerging Contaminants have multi-tier effects on model organisms exposed in the laboratory.

1. Are these effects observable in wild fish?
2. Are these effects localized or wide-spread?
3. Are all fish affected equally?

2006 Mississippi River Survey
Field Studies - Mississippi River Survey

- Does repeated influx of contaminants result in cumulative increases in chemical load in the Mississippi River and concurrent increases in endocrine disruption?
- Does endocrine disruption correlate with the degree of dietary specialization?

![Smallmouth Bass](image1.png)

![Redhorse](image2.png)

![Carp](image3.png)

Map of the Mississippi River showing key locations: Bemidji, Grand Rapids, Brainerd, Saint Cloud, Twin Cities, Red Wing, Winona.
Field Studies - Mississippi River Survey

<table>
<thead>
<tr>
<th>Site</th>
<th>Sediment</th>
<th>Water</th>
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<tbody>
<tr>
<td>Bemidji</td>
<td>30</td>
<td>8</td>
</tr>
<tr>
<td>Grand Rapids</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>Brainerd</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>St. Cloud</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>Anoka</td>
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<td>2</td>
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<td>St. Paul WWTP</td>
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<td>4</td>
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<tr>
<td>Hastings</td>
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<td>2</td>
</tr>
<tr>
<td>Red Wing</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Lake City</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>La Crescent</td>
<td>20</td>
<td>1</td>
</tr>
</tbody>
</table>

*Most commonly detected compounds: Atrazin, Cholesterol, Alkylphenols, DEET*
Mean vitellogenin concentrations (μg/mL) in male fish in the Mississippi River (5 highest concentrations for each species)

2. Location & Dilution & Sampling

Field Studies - Mississippi River Survey
Does repeated influx of contaminants result in cumulative increases in chemical load in the Mississippi River and concurrent increases in endocrine disruption?

- “Hotspots” rather then continuum on Upper River, continuous endocrine disruption on Lower River, noticeable effects of tributary dilution.

Does endocrine disruption correlate with degree of dietary specialization?

- Carp and redhorse had less induction of vitellogenin despite being close to substrate with higher contaminant load.
Land use appears to be an important factor in buffering the effects of EDCs!
2007 Tributary Study
Field Studies - Tributary study

Common shiner (*Luxilus cornutus*)

Fathead Minnow (*Pimephales promelas*)

Creek Chub (*Semotilus atromaculatus*)

Smallmouth Bass (*Micropterus dolomieu*)

Redhorse (*Moxostoma*)

Common carp (*Cyprinus carpio*)

White Sucker (*Catostomus commersonii*)
Fish response varies dramatically by species.
Endocrine Disruption is a widespread problem in the developed world. Any assessment of adverse effects requires a broad understanding of the biology of the aquatic ecosystem and of the chemistry of the EDCs. The “Tragedy of the Commons” scenario truly applies to EDCs, therefore education is key.
Thank You!