Intersex and Other Signs of Endocrine Disruption in Fishes Within the Chesapeake Watershed

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Chesapeake Bay Executive Order

Historically – primary concerns

- Nutrients, suspended sediment
- Legacy contaminants – whole body or fillet
  • Human consumption

EO

- Nutrients, suspended sediment
- Fish and wildlife health
- Contaminants of emerging concern
  • Endocrine disruption, intersex
“Toxic” Chemicals

- Ecosystem or Environmental Health
  - See serious biological effects when no one chemical is above “threshold benchmarks”

- Classic toxicology/current regulations
  - Benchmark criteria – one contaminant at a time and one or two, often fairly unsensitive species
    - Acute toxicity - death
    - Chronic toxicity – growth

- Endocrine disruption

- Immune system/disease resistance
Complexities of Contaminants in Wild Populations

- Many were produced to have a biological effect and so may affect nontarget organisms at very low levels.
- Endocrine/Immune systems - chemical communication and feedback mechanisms.
- Lack of classic dose response curve – hormesis.
- Multiple contaminant exposure routes - water, sediment, food (yolk sac).
- Short term exposure at sensitive life stages can have long term effects.
Biological Effects

- Fish kills and skin lesions
  - Centrarchids and suckers in the Potomac and Susquehanna drainages
  - Striped bass in the Bay and selected tributaries
- High prevalence of intersex and other signs of reproductive endocrine disruption
- Lack of recruitment of yellow perch in certain urbanized tributaries
- Skin and liver tumors in brown bullhead
Intersex in Normally Gonochorist Fishes

- Immature oocytes within testes
- Suggested as a marker of endocrine disruption
- Used as an indicator of exposure to estrogenic compounds
Induction of Testicular Oocytes Experimentally

- Estradiol
- Ethinyl estradiol – synthetic estrogen
- Nonylphenol
- 4-tert-pentylphenol and octylphenol
- DDT
- Bisphenol A
- Phytoestrogens/Isoflavones – equol, genistein
- Atrazine - amphibians
Experimental exposures of numerous fish species have shown that the most sensitive time period for induction of intersex is the first few weeks of life (sexual differentiation).

Exposure during that time can increase sensitivity later in life.

Exposure throughout life can increase incidence and severity.
Distribution, Prevalence, Severity
Intersex in the Potomac Watershed

Moderate (30%) to high (100%) prevalence
Low (< 0.2) to moderate (> 2.0) mean severity
Varied by season, site and species
Out-of-basin sites on Gauley, Tygart, Greenbrier had significantly lower prevalence and severity
Appeared to be associated with increased human population and increased agricultural landuse

Subsequent and Ongoing Studies to Address Intersex and CEC

- Wastewater treatment plants as sources
  - Collaborative study with FWS and MD DNR
  - Upstream and downstream sites on Conococheague Creek and Monocacy River


## Upstream/Downstream of WWTP

<table>
<thead>
<tr>
<th>Site</th>
<th>No. Males</th>
<th>Intersex Prevalence</th>
<th>Intersex Severity</th>
<th>% males w Vtg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susquehanna – U</td>
<td>14</td>
<td>93%</td>
<td>1.4</td>
<td>21%</td>
</tr>
<tr>
<td>Susquehanna – D</td>
<td>9</td>
<td>89%</td>
<td>1.7</td>
<td>11%</td>
</tr>
<tr>
<td>Swatara – U</td>
<td>4</td>
<td>75%</td>
<td>0.6</td>
<td>0%</td>
</tr>
<tr>
<td>Swatara – M</td>
<td>6</td>
<td>67%</td>
<td>1.0</td>
<td>50%</td>
</tr>
<tr>
<td>Swatara – D</td>
<td>6</td>
<td>100%</td>
<td>2.0</td>
<td>0%</td>
</tr>
<tr>
<td>Monocacy – U</td>
<td>11</td>
<td>82%</td>
<td>1.2</td>
<td>45%</td>
</tr>
<tr>
<td>Monocacy – D</td>
<td>7</td>
<td>100%</td>
<td>1.8</td>
<td>33%</td>
</tr>
<tr>
<td>Conococheague – U</td>
<td>10</td>
<td>100%</td>
<td>2.2</td>
<td>60%</td>
</tr>
<tr>
<td>Conococheague – D</td>
<td>10</td>
<td>90%</td>
<td>1.8</td>
<td>90%</td>
</tr>
</tbody>
</table>

Potomac Spawning Study

Objectives

- Comprehensive assessment of chemicals in water and sediment
- Evaluation of biological endpoints

Approach

- 7 sites, 6 in the Potomac and one reference (Gauley)
- Represent a range of TO and landuse
- Sampling during an important life stage
- Multiple sample types collected
## Intersex and Land-use

<table>
<thead>
<tr>
<th>Landuse Characteristics</th>
<th>Intersex Prevalence</th>
<th>Intersex Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r²</td>
<td>p</td>
</tr>
<tr>
<td></td>
<td>r²</td>
<td>p</td>
</tr>
<tr>
<td>Human population</td>
<td>0.39</td>
<td>0.10</td>
</tr>
<tr>
<td># WWTP</td>
<td>0.22</td>
<td>0.24</td>
</tr>
<tr>
<td>WWTP flow</td>
<td>0.32</td>
<td>0.15</td>
</tr>
<tr>
<td>Percent agriculture</td>
<td>0.63</td>
<td>0.02</td>
</tr>
<tr>
<td># Animal feeding operations</td>
<td>0.28</td>
<td>0.17</td>
</tr>
<tr>
<td>Total animal numbers</td>
<td>0.27</td>
<td>0.18</td>
</tr>
<tr>
<td>Animal density</td>
<td>0.49</td>
<td>0.05</td>
</tr>
<tr>
<td>Poultry Houses</td>
<td>0.27</td>
<td>0.18</td>
</tr>
</tbody>
</table>

$R = 0.986, \ p<0.001$
Intersex and Chemical Correlations

<table>
<thead>
<tr>
<th>Chemical Contaminants</th>
<th>Intersex Prevalence</th>
<th>Intersex Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r^2$</td>
<td>$P$</td>
</tr>
<tr>
<td>Atrazine</td>
<td>0.93</td>
<td>0.003</td>
</tr>
<tr>
<td>Deethylatrazine</td>
<td>0.78</td>
<td>0.039</td>
</tr>
<tr>
<td>Acetochlor</td>
<td>0.65</td>
<td>0.116</td>
</tr>
<tr>
<td>Metolachlor</td>
<td>0.87</td>
<td>0.011</td>
</tr>
</tbody>
</table>

## Agricultural Pesticides

### Fall (Spring)

<table>
<thead>
<tr>
<th>Chemical Estimated ng/L</th>
<th>Con Up</th>
<th>Con Down</th>
<th>Mon Up</th>
<th>Mon Down</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metolachlor</td>
<td>0.73 (7.5)</td>
<td>1.1 (9.0)</td>
<td>12.0</td>
<td>10.8 (97)</td>
</tr>
<tr>
<td>Atrazine</td>
<td>47 (380)</td>
<td>110 (430)</td>
<td>92</td>
<td>2 (2100)</td>
</tr>
<tr>
<td>Prometon</td>
<td>1.1 (1.2)</td>
<td>3.2 (1.4)</td>
<td>2.1</td>
<td>1.4 (1.8)</td>
</tr>
</tbody>
</table>

2.5 ppb recently shown to induce complete feminization and chemical castration in frogs - Hayes et al. (March 2010)

Earlier work found 0.1 ppb induced intersex in frogs
Fish Kill Issues
Related to Intersex and Other Reproductive Findings?

- Estrogens and estrogenic chemicals (estrogen mimics) are most often associated with intersex and vitellogenin production in male fishes.
- Increasing evidence that estrogenic chemicals and other endocrine-disrupting substances modulate the immune response and disease resistance.
- Also other chemicals such as arsenic, atrazine, PCBs act as immunosuppressors by other mechanisms.
Overall Findings/Conclusions

- Sensitive species, stressed populations close to a threshold between healthy and sick (dead)
- Variety of stressors leading to immunosuppression
- Numerous pathogens contributing to the skin lesions and eventual death – no consistent findings
  - Bacteria – *Aeromonas hydrophila, A. salmonicida, Flavobacterium columnare*
  - Virus – largemouth bass virus
  - Numerous parasites
- Prevalence/severity of intersex appears to be related to fish lesions/kills

Populations Effects

- Increased mortality due to opportunistic infectious and parasitic disease
  - Smallmouth bass
  - Roach (Europe)

- Reduced reproductive success
  - Experimental lake work (Kidd et al. 2007)
  - Smallmouth bass – decreased sperm/motility
  - Roach (Harris et al. 2011)
Yellow Perch Study

- Lack of recruitment of yellow perch in certain urban Chesapeake tributaries
- Relationship with percent impervious surface
- Five tributaries varying percent of urban landuse and impervious surface
  - South and Severn rivers – high % urban landuse and impervious surface
  - Mattawoman Creek – moderate and increasing
  - Allen’s Fresh and Choptank - low
Yellow Perch

Histological findings

- Lack of final oocyte maturation,
- Abnormal zona pellucida and
- Leydig cell proliferation/Leydig cell tumors

Both responses are regulated by dopamine
- Raises question of exposure to dopamine agonists
Acknowledgements

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  MD Department of Natural Resources
  University of Tennessee, Center for Environmental Biotechnology
  Virginia Tech
  PA Fish and Boat Commission
  Potomac/ Shenandoah River Keeper
  Friends of the Shenandoah