Investigating the presence of and effects from Endocrine Disrupting Compounds (EDCs) within the mainstem Ohio River

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Ohio River Facts

- 981 miles long
- 305,000 sq. mile drainage area
- 600+ permitted discharges
- 180 municipal wastewater treatment plants
- 50 CSO communities
  - 1400 CSO outlets
  - 15% of the nation’s CSOs
Study Focus

- Can we detect EDCs in the water column?
- Can we demonstrate linkages between the occurrence of EDCs and adverse biological effects among indigenous fish species?
Study Design

- Two potential study locations chosen.
  - ALCOSAN - PA
    - 200+ MGD WWTP
      - Chosen because of size
      - No chemistry, looked at fish only
  - Wheeling - WV
    - 15 MGD WWTP
      - Earlier EPA screening study indicated low level estrogenic activity
      - Main focus of study
Study Design

- **Grab Samples – Effluent**
  - Chemistry
  - In-lab **Vitellogenin** (Vg) gene analysis
    - Vg is an egg yolk precursor protein expressed only in female fish and is normally dormant in male fish.

- **Deployments**
  - Vg - Caged (♂) Fathead Minnows (*Pimephales promelas*)
  - POCIS/SPMD

- **Indigenous Fish**
  - Vg
  - Histopathology
Site Set-up

29 lbs buoy to support deployments

- 2 Passive samplers per buoy 7 day time point
- 3 Minnow cages 1, 4, and 7 d (N = 10/cage)

Anchor
Site Set-up

WWTP Outfall

Flow

Upstream Reference.
- POCIS/SPMD
  - 7, 28 and 56 days
  - Fish deployment
    - 1, 4 and 7 days

Downstream.
- POCIS/SPMD
  - 7, 28 and 56 days
  - Fish deployments
    - 1, 4 and 7 days
Detecting EDCs in the Water Column

Wheeling Reference – July
POCIS

![Graph showing concentrations of different EDCs in the water column.](image-url)
Detecting EDCs in the Water

Wheeling - August

Graph showing the concentrations of various hormones in water samples taken in August, including:
- Dihydrotestosterone
- 17-b-estradiol (E2)
- Estrone (E1)
- Testosterone
- Androstenedione
- 17-a-ethynylestradiol (EE2)
- Estriol (E3)
- Progesterone
Detecting EDCs in the Water Column

- **Round 1**
  - 5 hormones at upstream reference site
    - Vandalism!
    - No outfall data

- **Round 2**
  - 2 hormones found
    - Reference and outfall locations
    - Outfall levels only slightly higher

- Results were highly variable and largely insignificant
Linking EDCs to Adverse Biological Effects

- Vitellogenin gene expression
- Histopathology
Grab Sample Exposure

![Graph showing normalized quantity for different treatments]

- Initial
- Control
- Time 0
- DMSO
- 5 ng/L EE2
- Wheeling

The graph displays the normalized quantity for each treatment condition.
July Deployments

Normalized Quantity

Ref day 1  Ref day 4  Ref day 7  Outfall day 1

trap A  Outfall day 1

trap B
August Deployments

Error bars are standard error
Vtg Results Summary

- Laboratory assay using effluent grab sample did not cause gene expression.
- Caged fish results
  - Higher gene expression rates in one of the downstream samples
  - Overall results too variable
Linking EDCs to Adverse Biological Effects

- Vitellogenin gene expression
- Histopathology
Linking EDCs to Adverse Biological Effects

- Fish collected using boat-mounted electrofishing.
- 355 individuals collected (244 submitted)
  - Round 1
    - 35 males (27 with anomalies)
  - Round 2
    - 142 males (108 with anomalies)
    - 67 females (56 with anomalies)
- Livers and gonads removed
Histopath Results

- No intersex found!!
- Other indications that may be due to exposure to EDCs.
  - Ripe males found at the wrong time of the year
  - Increase in spermatagonia
  - Many types of degenerative conditions noted
- Timing Issue
  - Increase in hybrids due to breakdown in timing isolation mechanism.
Problems Encountered

- $$ - Research isn’t cheap
- Sampling platform/logistics
- Vandalism
- Gender identification - difficult
- Gonad identification - difficult
- ‘Noisy’ results
  - EDCs are much more than PPCPs
  - Complex mixtures
Future Considerations

- Determine which species are most sensitive.
- Complex mixtures
  - Interactions between EDCs (mode of action)
  - Interactions with the environment (WQ)
  - Rank stressors/effects
- Develop fish health indicator
  - Incorporated into IBI process
    - or -
  - Stand alone
- Lots more to do
Questions?