

Abstract #159141

Use of a human breast cancer cell proliferation assay as an exposure assessment tool for total bioaccumulated xenoestrogens in channel catfish caught in Pittsburgh's three rivers.

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# **Introduction**

**Accumulation of xenoestrogenic compounds in waterways is a world-wide public health problem.**

**Fish are sensitive sentinels of waterway xenoestrogen contamination.**

# Effects of xenoestrogens on male fish

- Disruption of testicular development
- Elevation of serum vitellogenin levels
- Conversion to intersex phenotype

# Potential sources of xenoestrogens in Pittsburgh's three rivers

- Legacy waste from steel mill sites
- Untreated sewage
- Residential waste
- Agricultural runoff

# Routes of exposure to xenoestrogens in water

- **Recreational activities**
- **Subsistence fishing**
- **Municipal drinking water**

# Aim

**To assess the efficacy of a cell proliferation assay (CPA) to determine the presence of estrogenic substances in the flesh of channel catfish caught in Pittsburgh's three rivers**

# Methods

- **Fish caught**
- **Location of fish catch recorded**
- **Biometric parameters recorded**
- **Fish fillets extracted**
- **Extracts tested for estrogenicity via cell proliferation & estrogen receptor binding assays**

# Extraction Method

- 1g ( $\pm 5\%$ ) flesh, skin, and fat
- Extracted with  $\text{CHCl}_3/\text{CH}_3\text{OH}$  (9:1 v/v)
- Reduced to residue using  $\text{N}_2$  gas
- Stored at  $-20^\circ\text{C}$  under  $\text{N}_2$
- Solubilized in ethanol/glycerol (70:30 v/v)



# Cell proliferation assay (CPA)

## Cell lines used:

MCF-7: Estrogen receptor-positive

BT20: Estrogen receptor-negative

## Cell treatments:

Physiological estrogen (estradiol,  $10^{-9}$ M)

Fish extracts (varying doses)

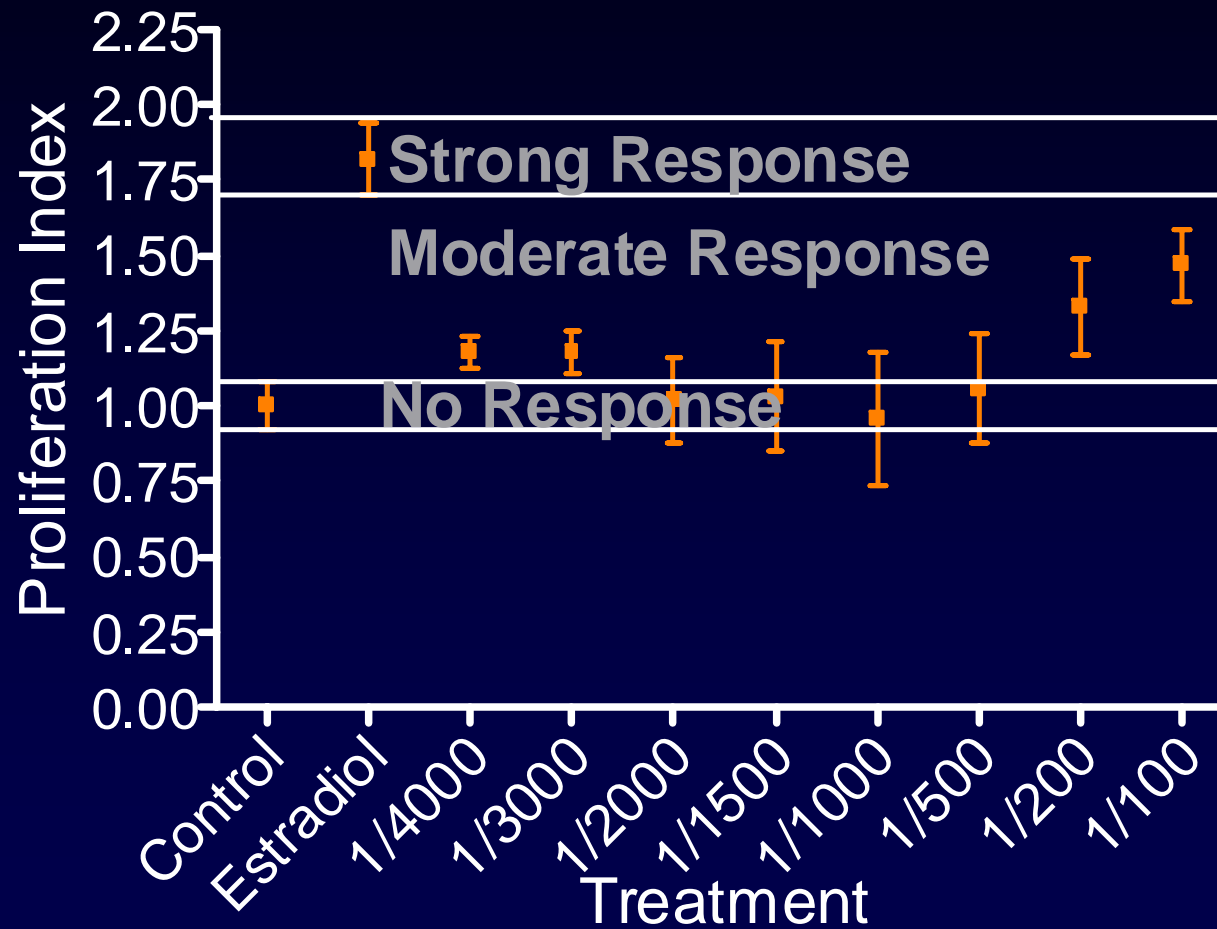
Medium only (negative control)

Cell number assessed: *CellTiter96*

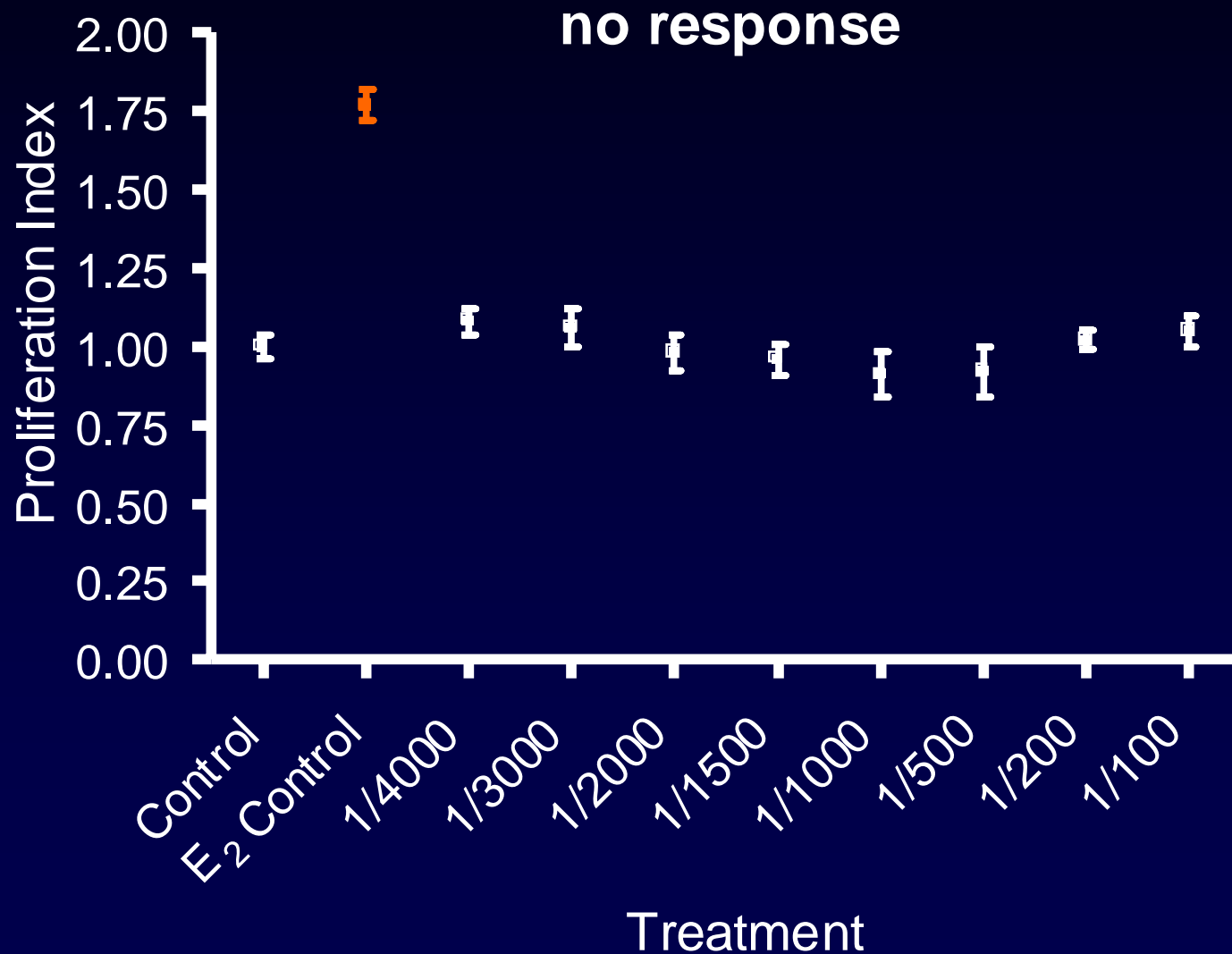
## Proliferation index (PI)

$$PI = \frac{\text{Absorbance of treated well}}{\text{Mean of negative control wells (n=10)}}$$

# Estrogen response profile (idealized)

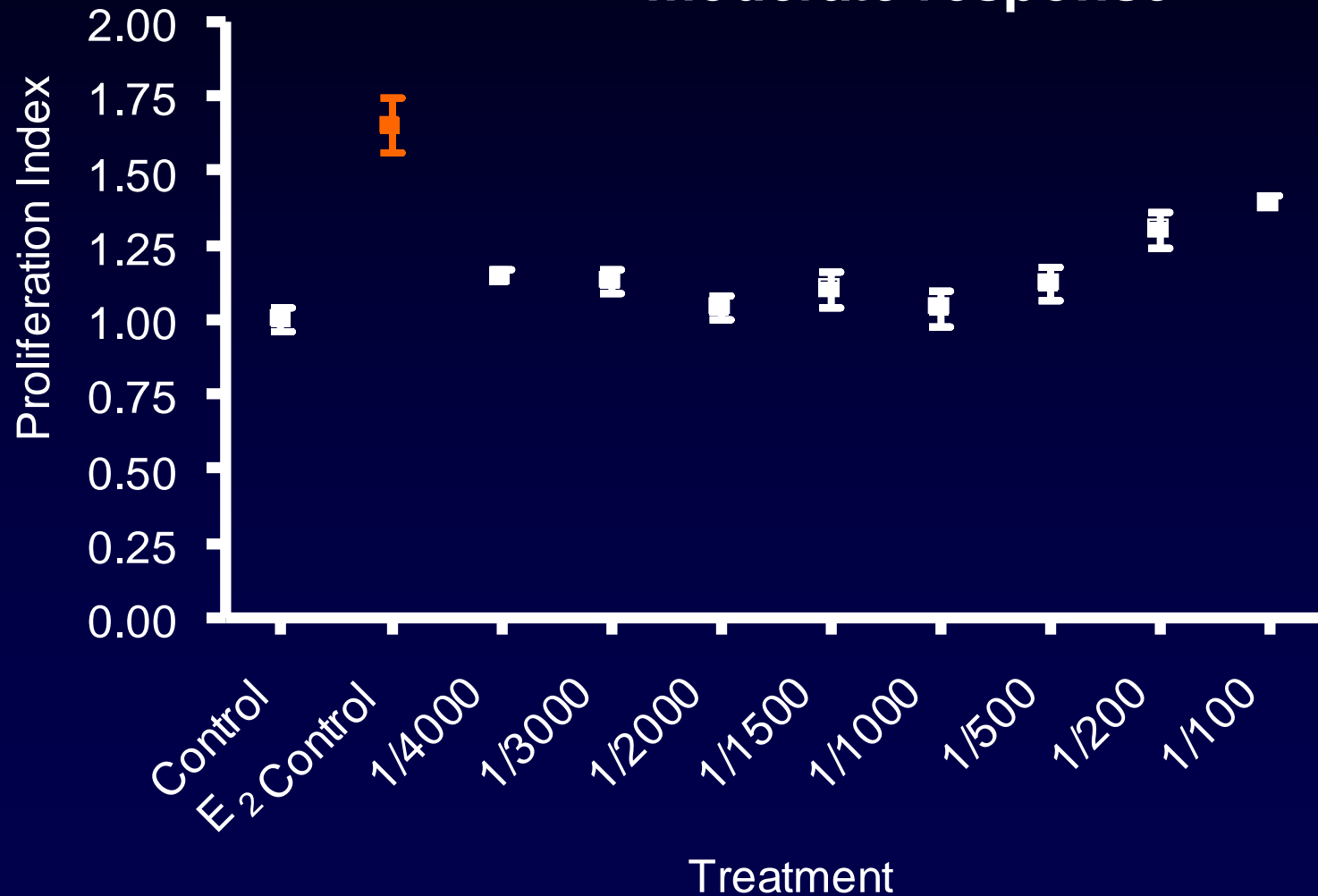


# Estrogen response profile (channel catfish 138)



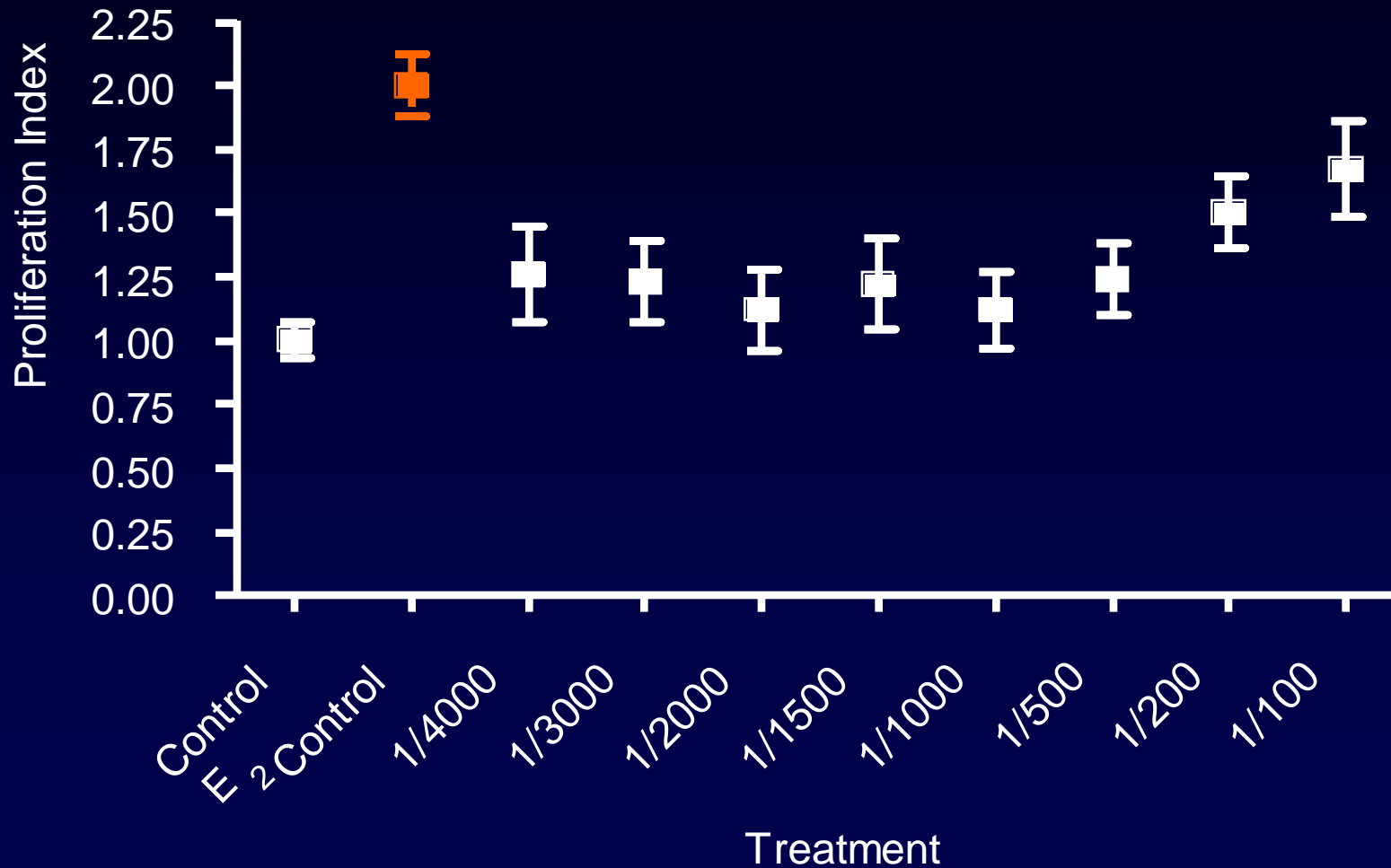
# Estrogen response profile (channel catfish 100)

moderate response



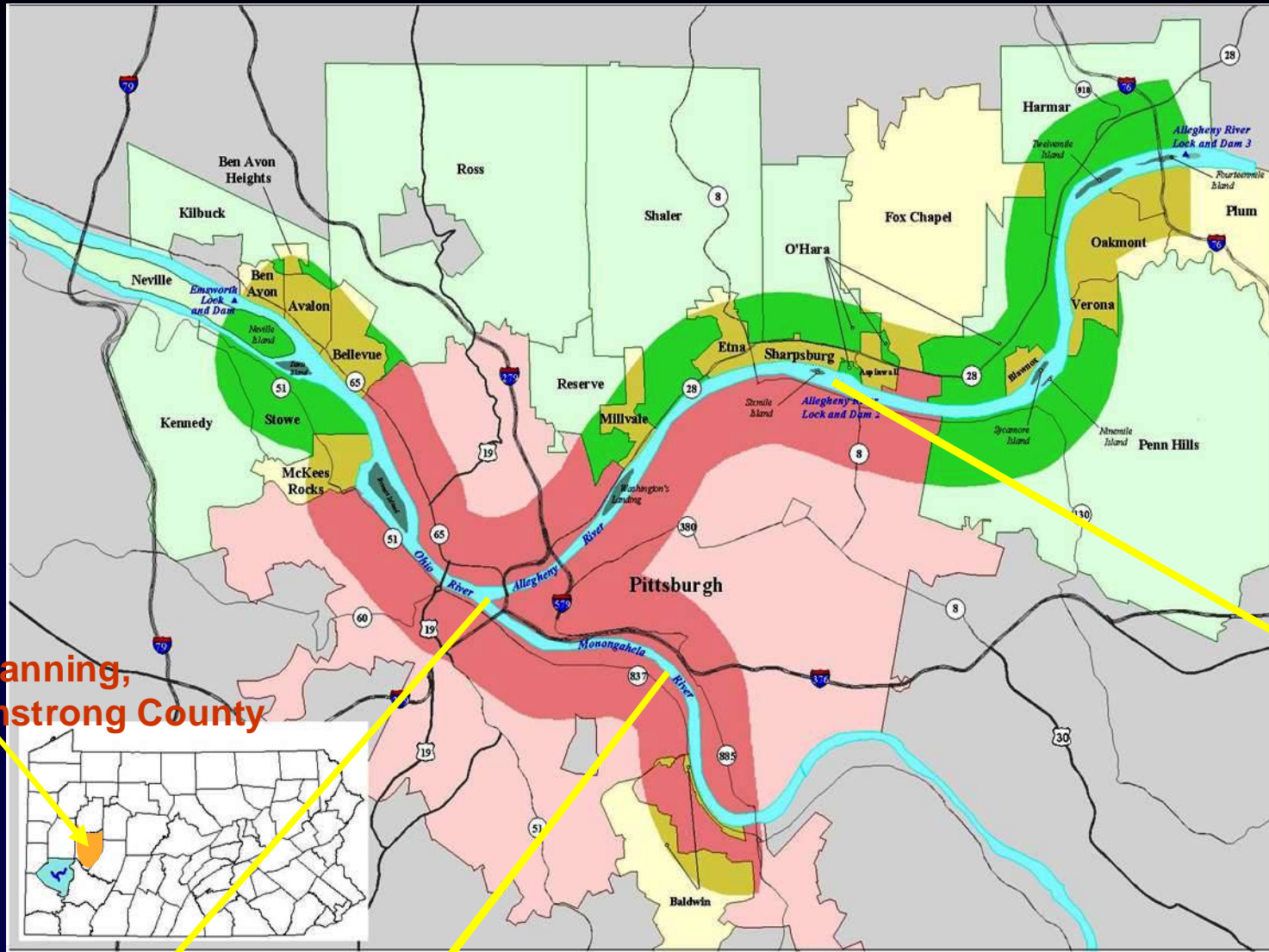
# Estrogen response profile (channel catfish 47)

strong response



# CPA Results

- **N=21**
- **1 extract exhibited a strong response**
- **13 extracts displayed a moderate response**
- **7 extracts produced no response**



**Kittanning,  
Armstrong County**

**Highland Park**

**Point State Park**

**Monongahela River**

▲ Dams  
 ■ Allegheny County  
 ■ Islands  
 ■ City of Pittsburgh  
 ■ Boroughs  
 ■ Townships

Darker shades represent areas contained within the corridor.

0 1 Miles  
 Scale 1:90,000  
 Accuracy: 5 ft.

# Results

**In general, extracts that are stimulators of cell proliferation also compete for ER binding (data not presented)**



# Conclusions

**These results support the hypothesis that bioaccumulated xenoestrogens are highest in channel catfish caught nearest to legacy-contaminated locations and areas of untreated sewage overflows.**

# Acknowledgements

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Bassmasters

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