

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

- **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 N_2 gas
- Stored at -20°C under N_2
- Solubilized in ethanol/glycerol (3:1 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)

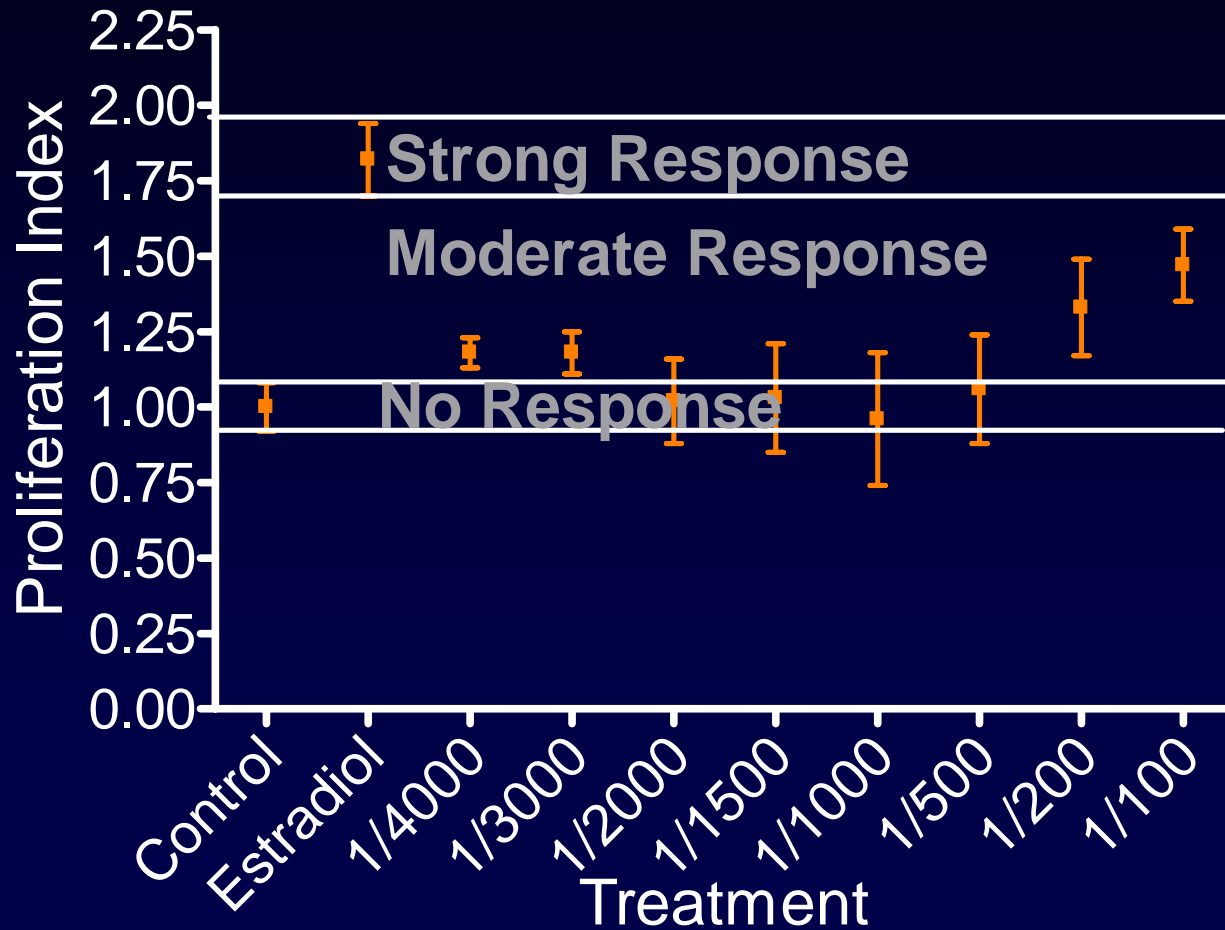
Medium only (negative control)

Cell number assessed: *CellTiter96*

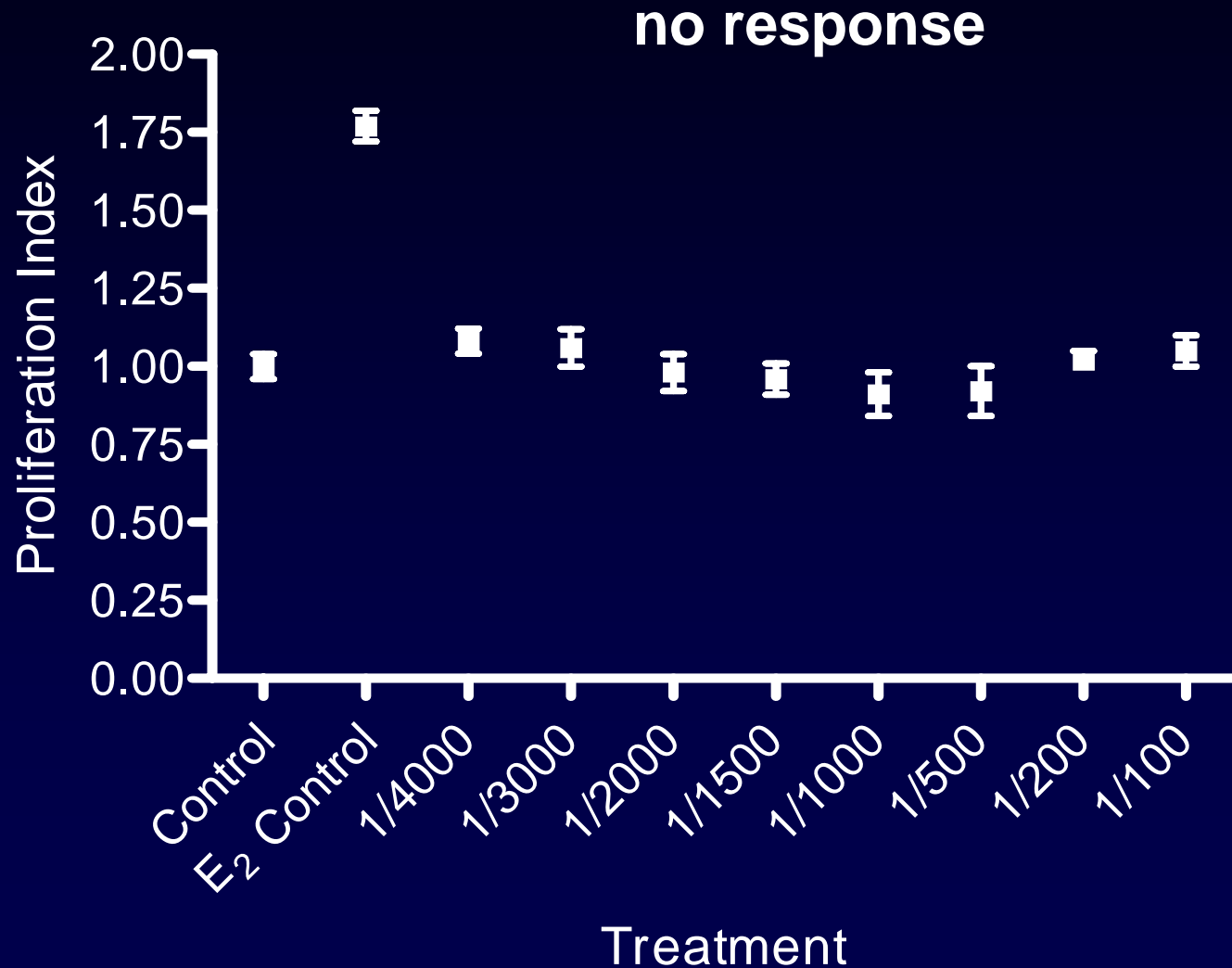
Proliferation index (PI)

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

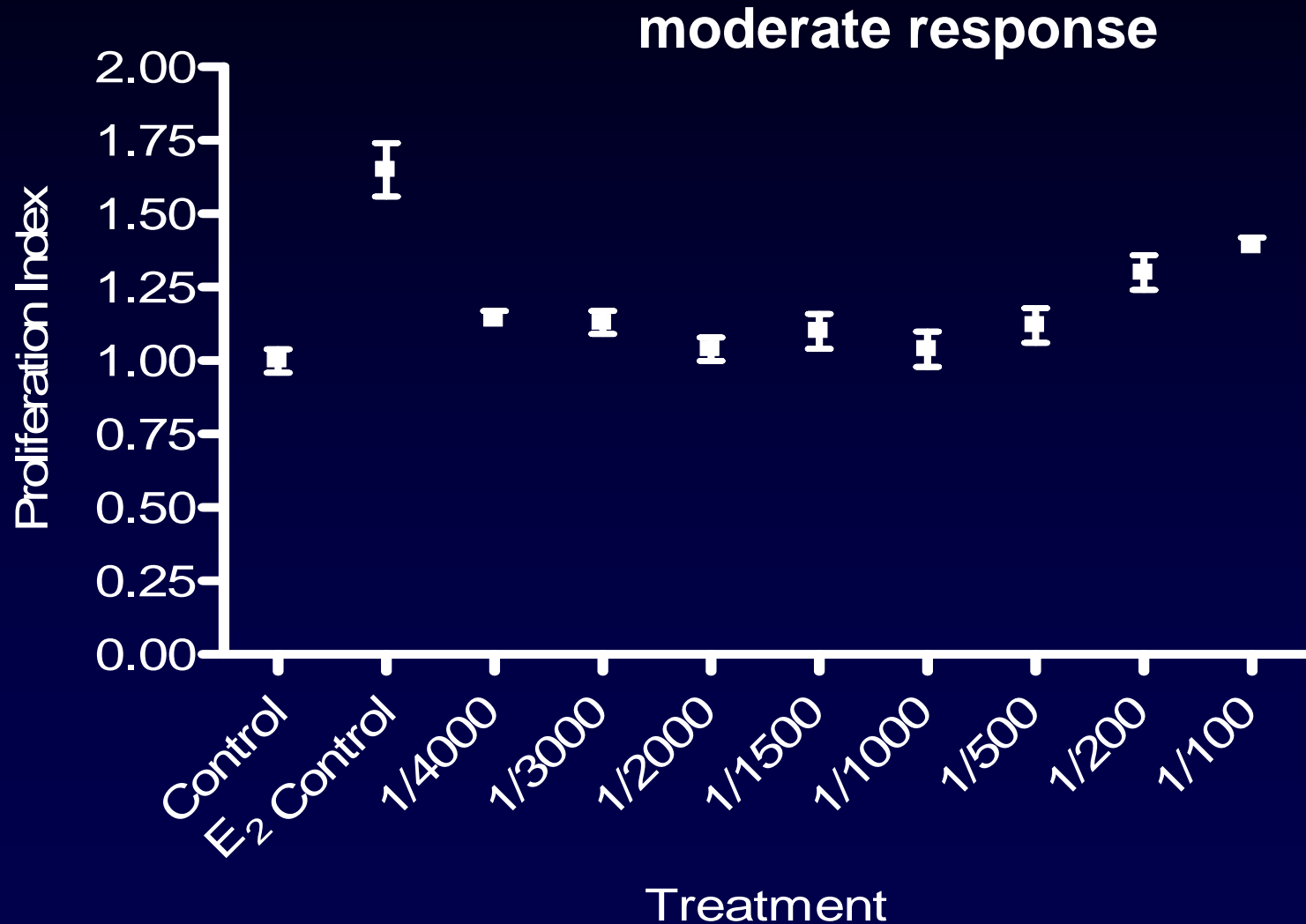
Estrogen response profile (idealized)



Estrogen response profile (channel catfish 138)

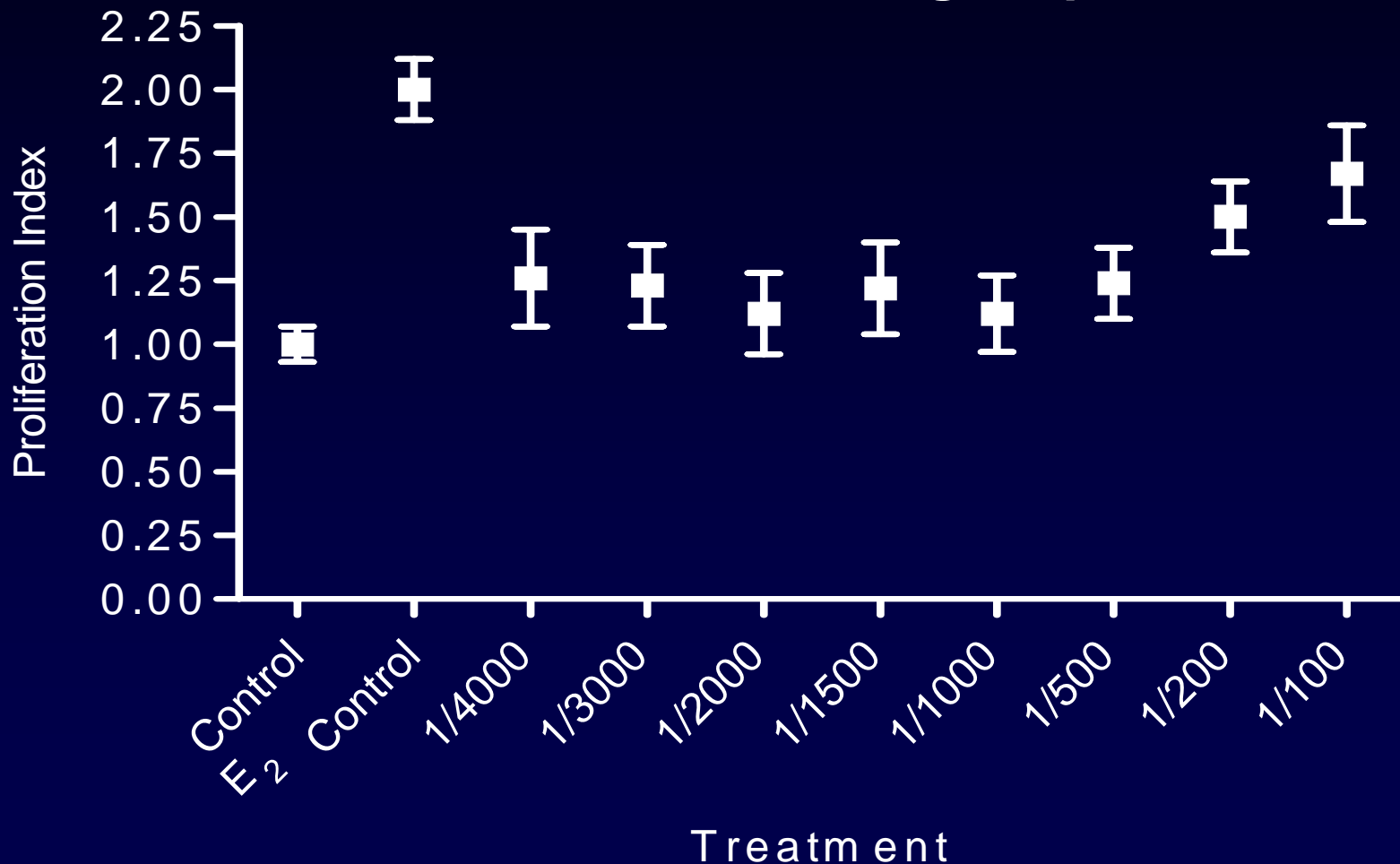


Estrogen response profile (channel catfish 100)



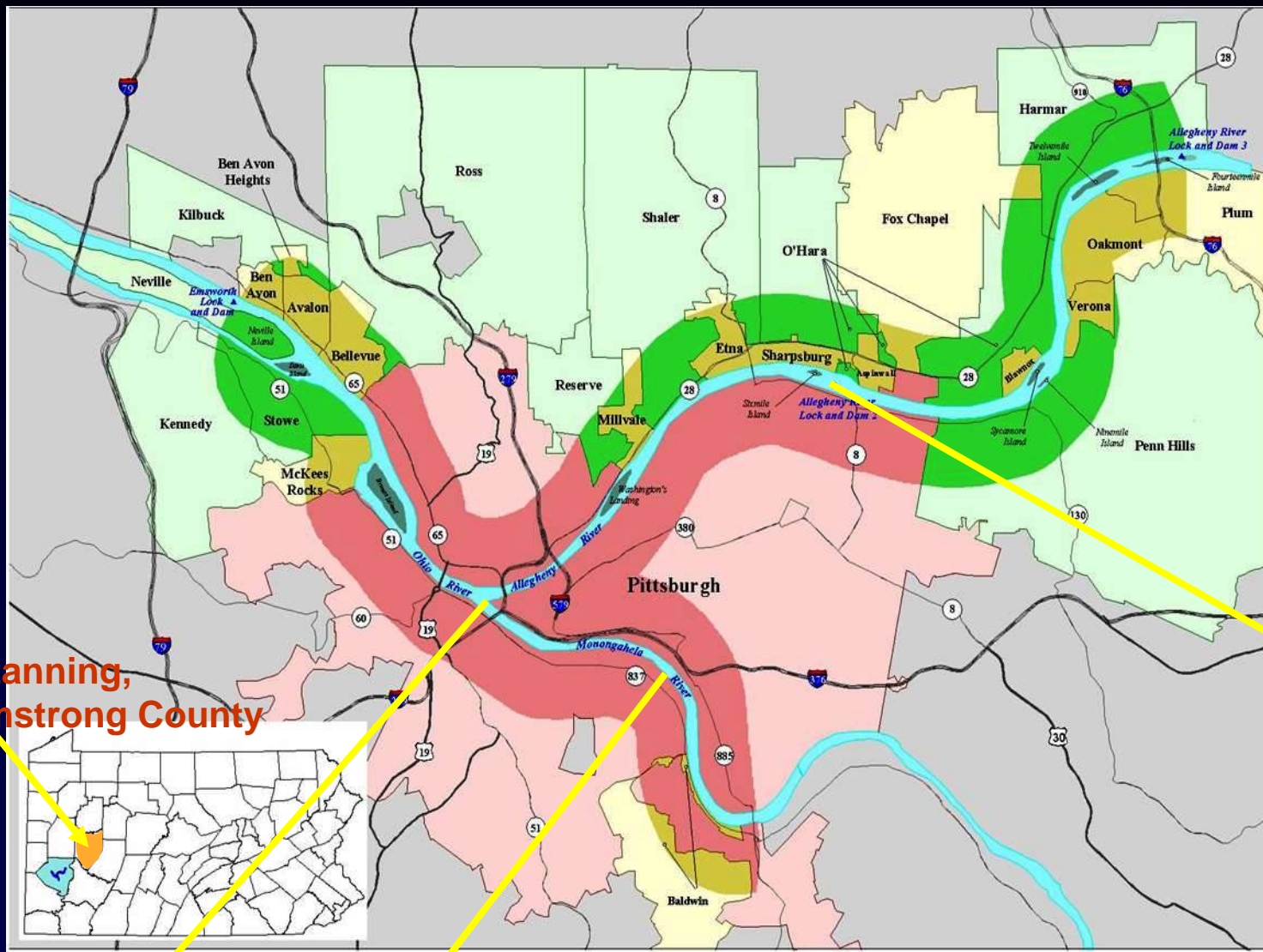
Estrogen response profile (channel catfish 47)

strong response

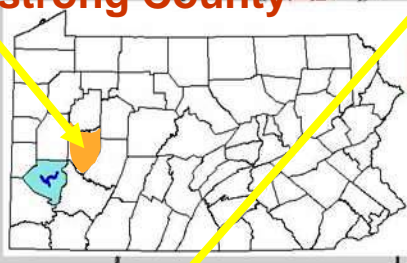


CPA Results

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



**Kittanning,
Armstrong County**



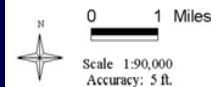
Highland Park

Point State Park

Monongahela River

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

Darker shades represent areas contained within the corridor.



Results

The strongest proliferative responses were from catfish caught near the steel works on the Monongahela River and the Point.

The weakest proliferative responses were from extracts from catfish caught upstream on the Allegheny River

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.

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