TAML Catalysis and the Chesapeake Bay

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Meeting of the Chesapeake Bay Commission, Beacon Hotel, Washington DC, May 4, 2017

Definitions

- **Endocrine disruptor** (ED): a compound, either natural or synthetic, which, through environmental or inappropriate developmental exposures, alters the hormonal and homeostatic systems that enable the organism to communicate with and respond to its environment[1]—EDs can alter development to produce impaired creatures.

- **Intersex:** in fish correlates with sex characteristics (including genitals, gonads and protein characteristics) that do not fit typical binary notions of males or females—high percentages of intersex can be considered as indicative of exposure to endocrine disrupting compounds.

[1]Diamanti-Kandarakis, E.; Bourguignon, J.-P.; Giudice, L. C.; Hauser, R.; Prins, G. S.; Soto, A. M.; Zoeller, R. T.; Gore, A. C. *Endocr. Rev.* **2009**, *30*, 293-342.

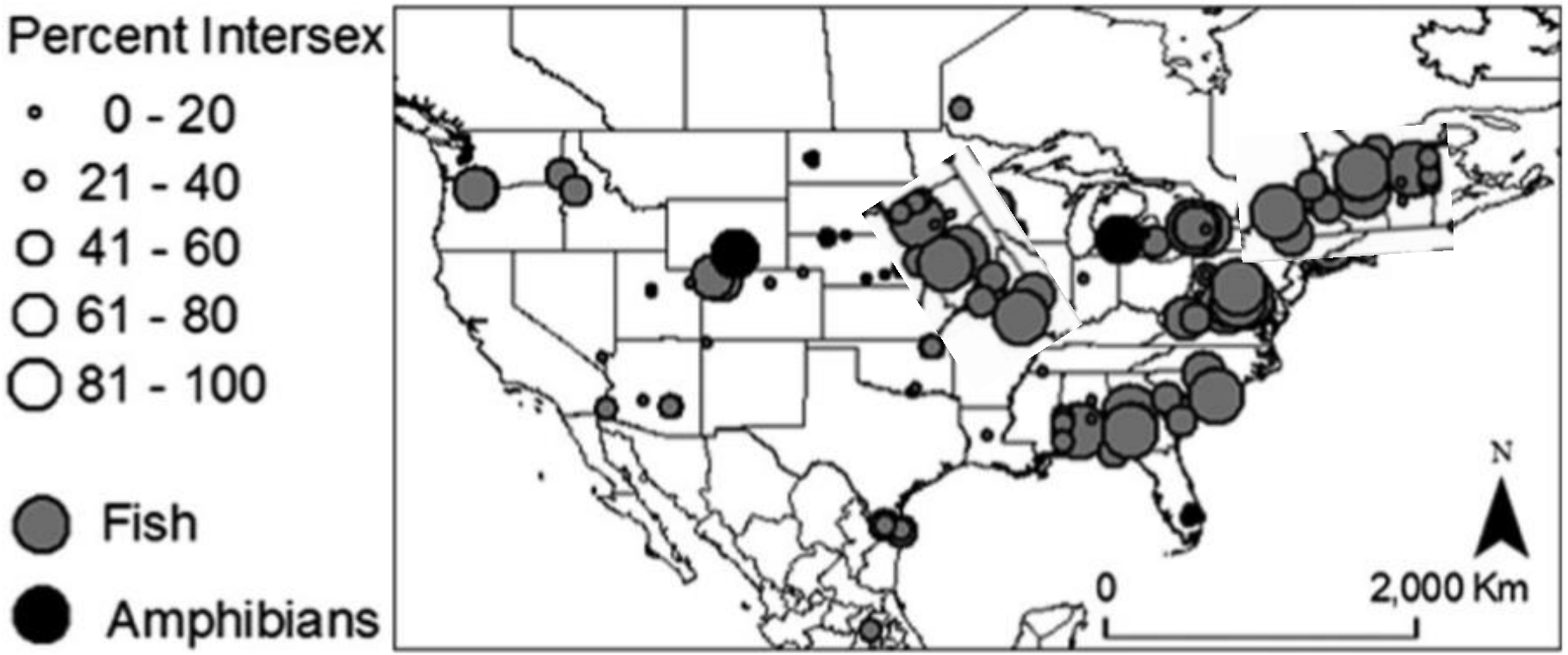
Indicators of Compromised CB Fish Health

- **Intersex in largemouth (LMB) and smallmouth bass (SMB) and other signs of endocrine disruption.**
- Increased incidences of infectious disease and parasite infestations contributing to increased mortality in several species of fish.
- Reduced reproductive success and recruitment of yellow perch in tributaries in certain highly urbanized drainage basins.
- Tumors in bottom dwelling fish.
- 80% loss of adult Shenandoah River smallmouth bass and redbreast sunfish during 2004 and 2005 fish kills.

2012 Toxic Contaminants in the Chesapeake Bay and its Watershed: Extent and Severity of Occurrence and Potential Biological Effects; USEPA Chesapeake Bay Program Office: Annapolis, MD, 2012; p 175.
Blazer, V. S.; Iwanowicz, L. R.; Iwanowicz, D. D.; Smith, D. R.; Young, J. A.; Hedrick, J. D.; Foster, S. W.; Reeser, S. J. *J. Aquat. Anim. Health* **2007**, *19*, 242-253.
Ripley, J.; Iwanowicz, L.; Blazer, V.; Foran, C. *Environ. Toxicol. Chem.* **2008**, *27*, 1756-1767.

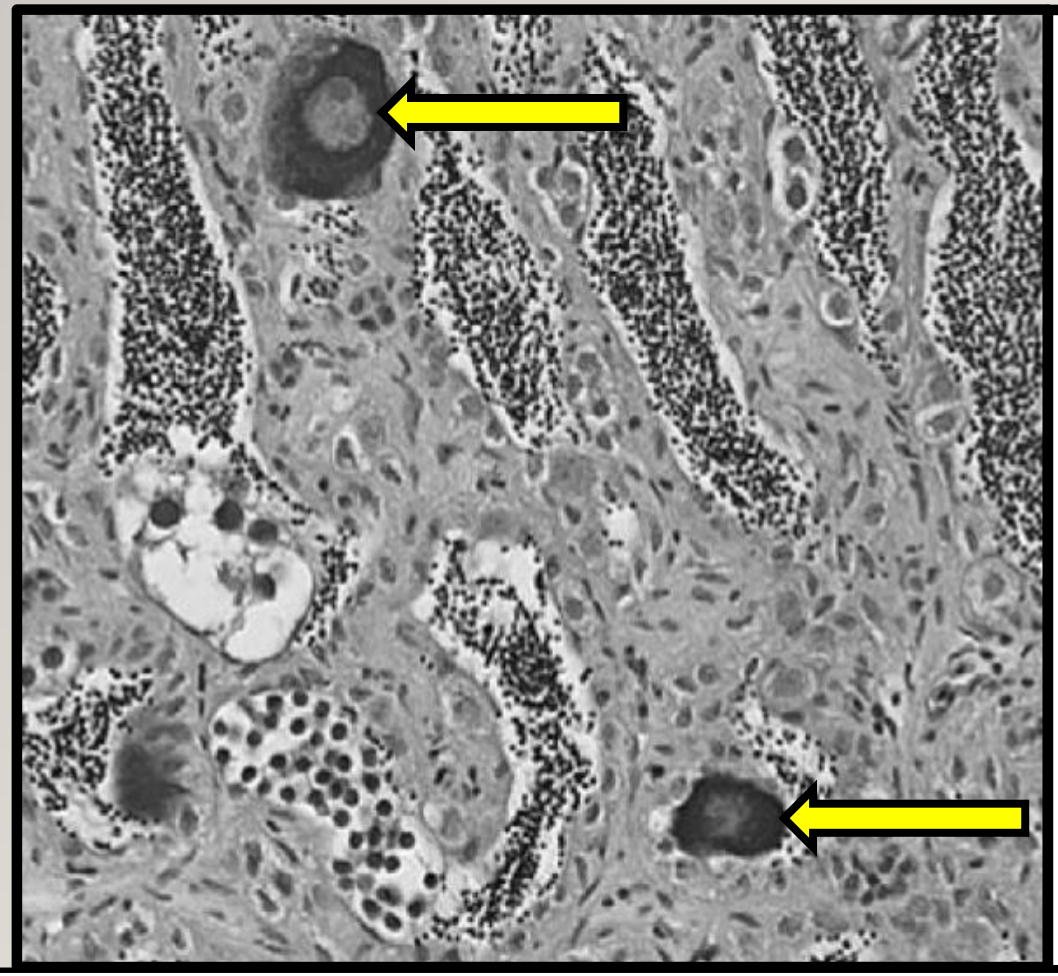
Literature Reports of Gonadal Intersex (2015)[1]

- **Adding MO River data[2]**
- **Adding NE US National Wildlife Refuge Waters fish data[3]**



[1]Abdel-moneim, A.; Coulter, D. P.; Mahapatra, C. T.; Sepúlveda, M. S. *J. Appl. Toxicol.* **2015**, *35*, 1228-1240.
[2]Papoulias, D., Intersex Sturgeon on the Missouri River? The Effects of Emerging Contaminants on a Big River. October 28, 2014.
[3]Iwanowicz, L. R.; Blazer, V. S.; Pinkney, A. E.; Guy, C. P.; Major, A. M.; Munney, K.; Mierzykowski, S.; Lingenfelser, S.; Secord, A.; Patnode, K.; Kubiak, T. J.; Stern, C.; Hahn, C. M.; Iwanowicz, D. D.; Walsh, H. L.; Sperry, A. *Ecotoxicol. Environ. Saf.* **2016**, *124*, 50-59.

Testicular Oocytes (TOs)



- TO: presence of immature oocytes in the testes.
- Baseline TO prevalence in small mouth bass (SMB) is thought to be 14–22%.
- TO found in SMB of the South Branch of the Potomac (**Spring 2004**: **69–100% Prevalence**) and the Shenandoah (**Spring 2005**: **80–100%**)
- **Thought to be indicative of early-life exposure to estrogens**[1]

[1]Blazer, V. S.; Iwanowicz, L. R.; Iwanowicz, D. D.; Smith, D. R.; Young, J. A.; Hedrick, J. D.; Foster, S. W.; Reeser, S. J. *J. Aquat. Anim. Health* **2007**, *19*, 242-253.
Iwanowicz, L. R.; Blazer, V. S.; Guy, C. P.; Pinkney, A. E.; Mullican, J. E.; Alvarez, D. A. *Environ. Toxicol. Chem.* **2009**, *28*, 1072-1083.

Endocrine Disruption in Male Bass of the Conococheague (C), Monocacy (M), and Blue Plains

Collected Sept 6–14, 2005	Vitellogenin (VTG)	
Site	Samples with VTG / %	Plasma VTG / mg mL^{-1}
UC	60 A	0.117 ± 0.051 A
DC	90 A	0.050 ± 0.036 B
UM	45	0.059 ± 0.010 A
DM	33	0.143 ± 0.085 A
BP	85	0.577 ± 0.165

- **Vitellogenin (VTG) is a protein that is an egg yolk precursor**
- **VTG synthesis regulated by an estrogen receptor**
- **VTG is not produced by males under normal conditions**
- **Normal, male LMB plasma concentrations are usually <0.05 mg mL^{-1}**
- **The presence of elevated serum VTG is a marker for recent or prolonged estrogen exposure**

Iwanowicz, L. R.; Blazer, V. S.; Guy, C. P.; Pinkney, A. E.; Mullican, J. E.; Alvarez, D. A. *Environ. Toxicol. Chem.* **2009**, *28*, 1072-1083.

Compounds Associated With Intersex

Compound	Compartment	Effect	Likely Sources
Estrone (E1)	**Water, Sediment**	**TO, VTG**	**WWTP Effluent; Poultry, Bovine, and Swine Manures; Aquaculture/Hatchery Effluent**
17β-Estradiol (E2)	Water, Sediment	VTG	WWTP Effluent, Sewage Discharges, Agricultural Runoff
Atrazine	Water	TO	Agricultural Runoff
β-Sitosterol	Sediment	TO, VTG	Plant Sterols, may derive mostly from non-human feces
β-Stigmastanol	Sediment	TO	
Trans-nonachlor	Sediment	TO	Chlordane insecticide, banned for all uses in 1988

Blazer, V. S.; Iwanowicz, D. D.; Walsh, H. L.; Sperry, A. J.; Iwanowicz, L. R.; Alvarez, D. A.; Brightbill, R. A.; Smith, G.; Foreman, W. T.; Manning, R. *Environ. Monit. Assess.* **2014**, *186*, 6471-6491.
Kolpin, D. W.; Blazer, V. S.; Gray, J. L.; Focazio, M. J.; Young, J. A.; Alvarez, D. A.; Iwanowicz, L. R.; Foreman, W. T.; Furlong, E. T.; Speiran, G. K.; Zaugg, S. D.; Hubbard, L. E.; Meyer, M. T.; Sandstrom, M. W.; Barber, L. B. *Sci. Total Environ.* **2013**, *443*, 700-716.

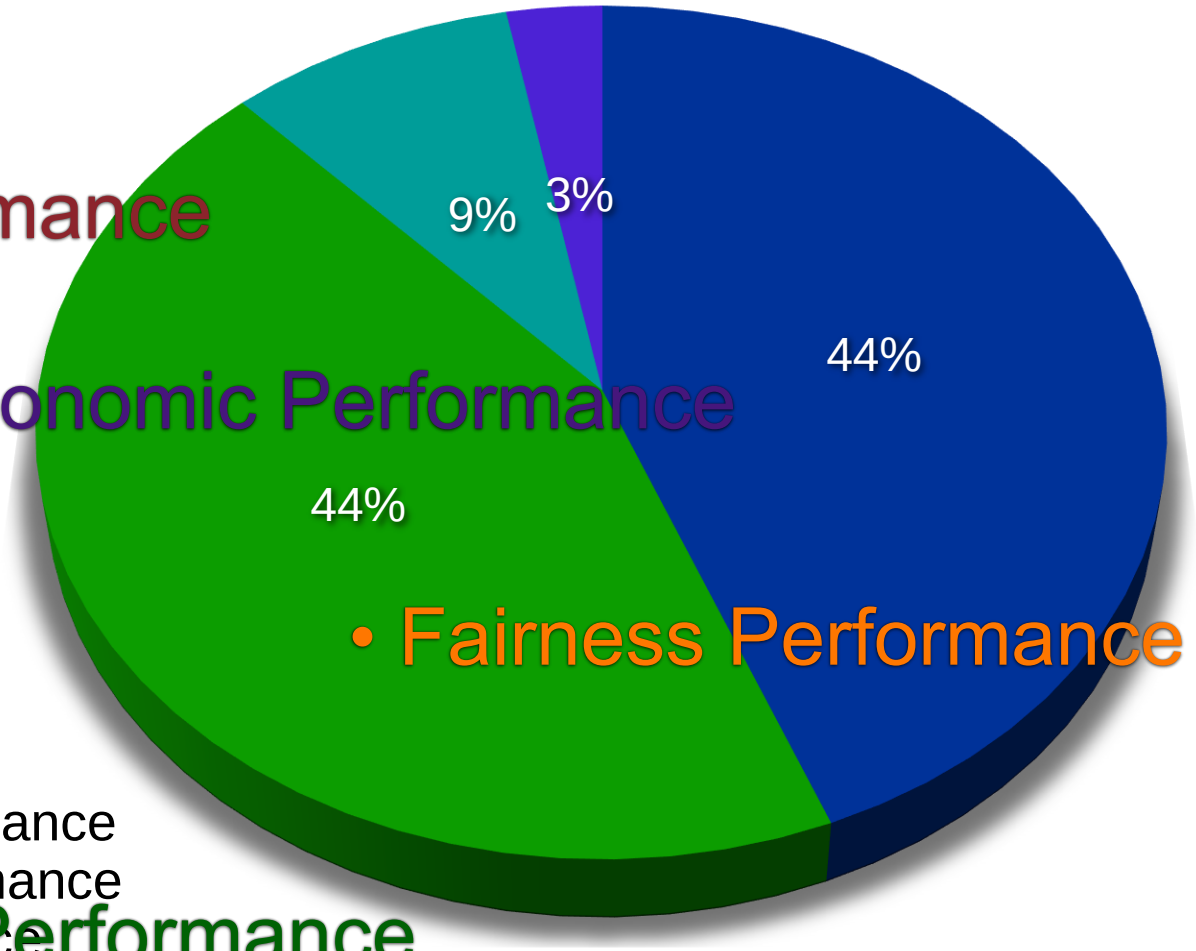
Conclusions

- Male fish in the CB watershed are impaired by early-life and adult exposure to estrogens
- Agricultural sources, animal feeding operations, and poultry houses appear to be the main sources driving TO and abnormal plasma VTG concentrations in CB, male SMB
- WWTP discharges and pulp mills are also contributors.
- Each location on each waterway represents a different combination of sources and MPs.
- **A simple-to-use, cost-effective, potent treatment process that can be adapted for all these various challenges would be a major advance in water purification.**
- **Such a technology could bring the great economic benefit of healthier, more abundant aquatic life with major positive impacts on the CB state economies.**

What Performances are critical for sustainable water treatment technologies?

Guestimates of average performance priorities embedded in most existing non-pharma chemical technologies

- Technical Performance
- Economic Performance
- Health Performance
- Fairness Performance
- Environmental Performance



Devastation of Sports Fishing Industry

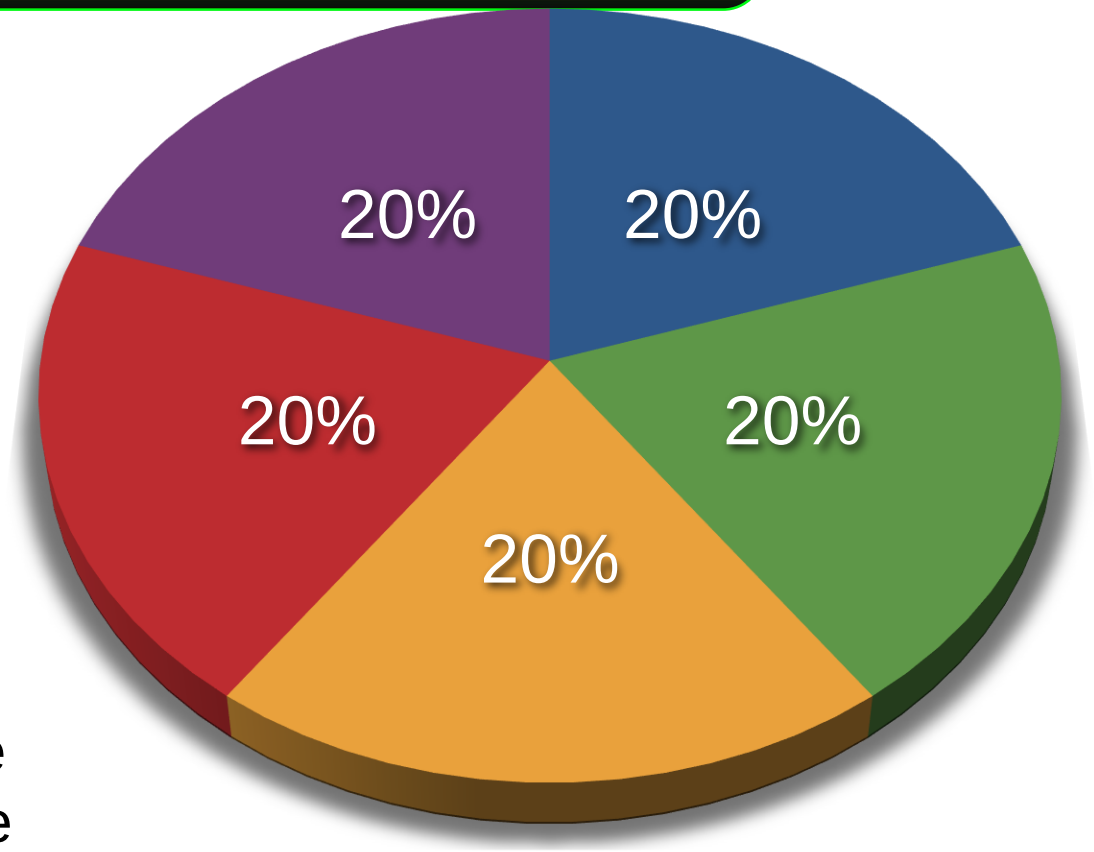
- Middletown smallmouth guide Bob Clouser has for more than a decade sounded the alarm that Susquehanna smallmouth in the Harrisburg reaches were disappearing.
- He watched his guiding business shrink from 75 smallmouth taken per day to 5 or less. Increasingly, he had to inform his customers from as far away as Cleveland, Ohio: "Don't come. It's not worth fishing."

http://www.flyfisherman.com/news/susquehannas-lost-smallmouth-fishery/#ixzz4eq935qAi

Performances: what does sustainability need?

CMU's IGS catalyst technologies have been developed to achieve this picture.

Fundamental challenge:
"How can we parameterize the health, environmental and fairness performances of chemical products and processes?"



GREENSCIENCE

Two great challenges presented by EDs to green scientists:

Tiered Protocol for Endocrine Disruption (TiPED) produced:

Schug T., Abagyan R.,Blumberg B., Collins T., Crew, D., DeFur P., Dickerson S., Edwards T., Gore] A., Guillette L., Hayes T., Heindel J,, Moores A., Patisaul H. Tal, T., Thayer K., Vandenberg L., Warner J., Watson C., vom Saal F., Zoeller T., O'Brien K., Myers JP., *Green Chem.*, **2013**,15, 181-198

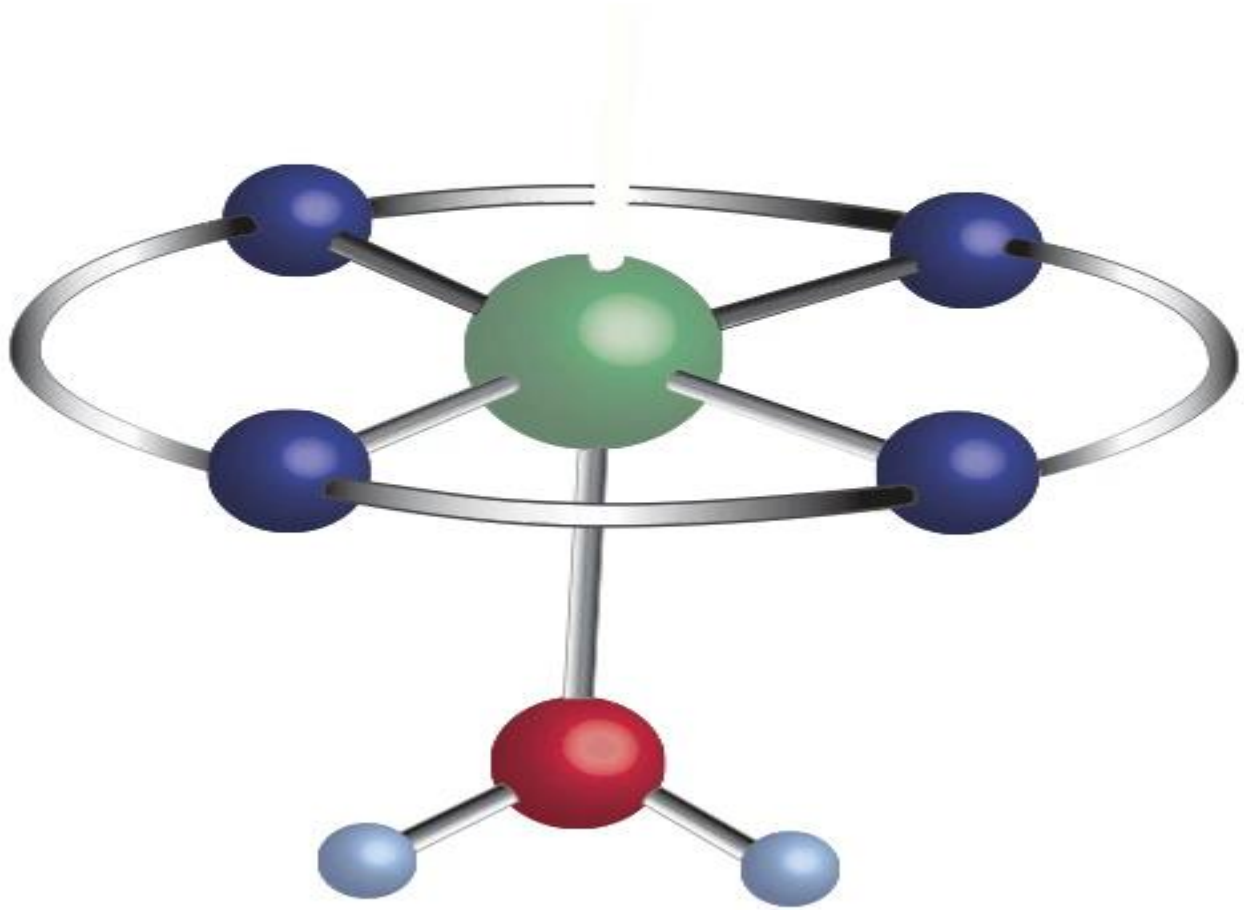
Many EDs are integral to the way our city-dominated civilization has been constructed—"damned if you do, damned if you don't" challenges. Effective product stewardship can be a powerful tool in these cases.

Schug T., Johnson, A,. Birnbaum, L., Colborn T., Guillette L., Crews D, Collins T., Soto A., vom Saal F., McLachlan, Sonnenschein C., Heindel J. Minireview: Endocrine Disruptors: Past Lessons and Future Directions, Mol. Endocrinol. **2016,** *30*: 833–847

TiPED Assays Applied to TAML catalysts

- Cellular assays clear for androgen, estrogen and thyroid endpoints
- Zebrafish development assays clear for catalysts undergoing development
- Mouse uterotrophic assays and general organ toxicity clear for NewTAML
- Fish studies of TAML/H_2O_2 de-estrogenized (EE2) water show greatly reduced vitellogenin expression.

How TAMLs were designed and developed as small-molecule oxidation enzyme mimics.

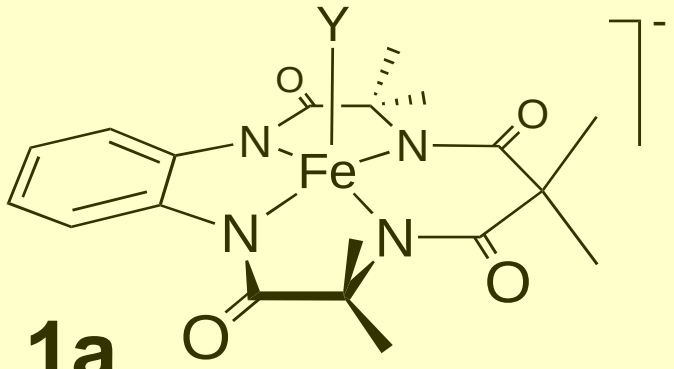


THE INSTITUTE FOR GREEN SCIENCE
CARNEGIE MELLON UNIVERSITY

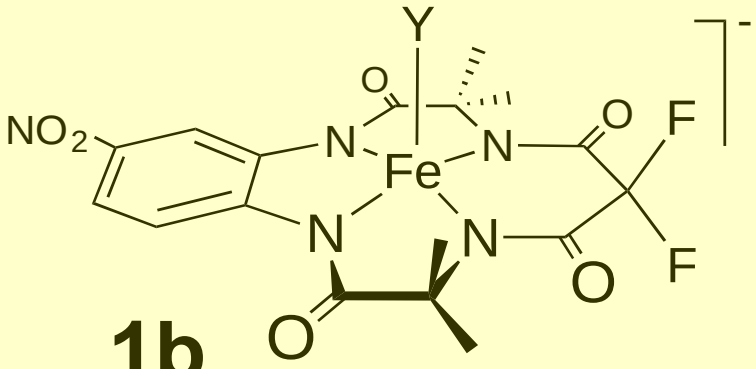
What is the football?

Iron *T*etra*A*mido *M*acrocylic *L*igand Complexes (TAML Activators)

‘OldTAML’

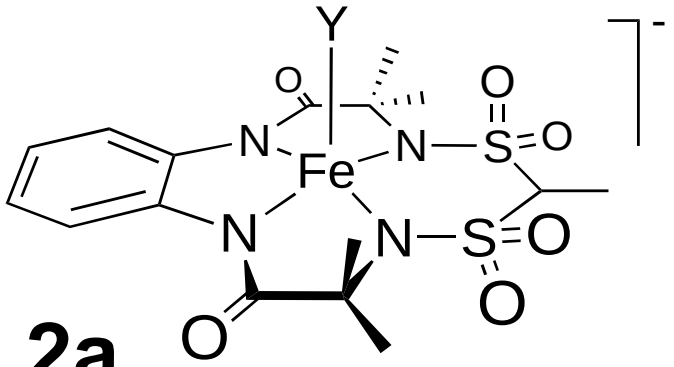


1a

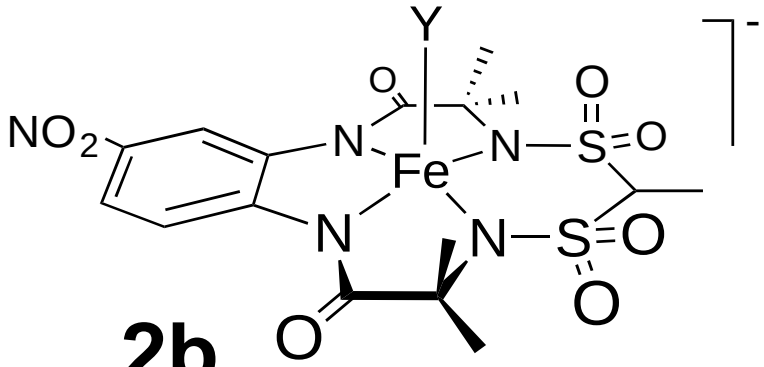


1b

‘NewTAML’

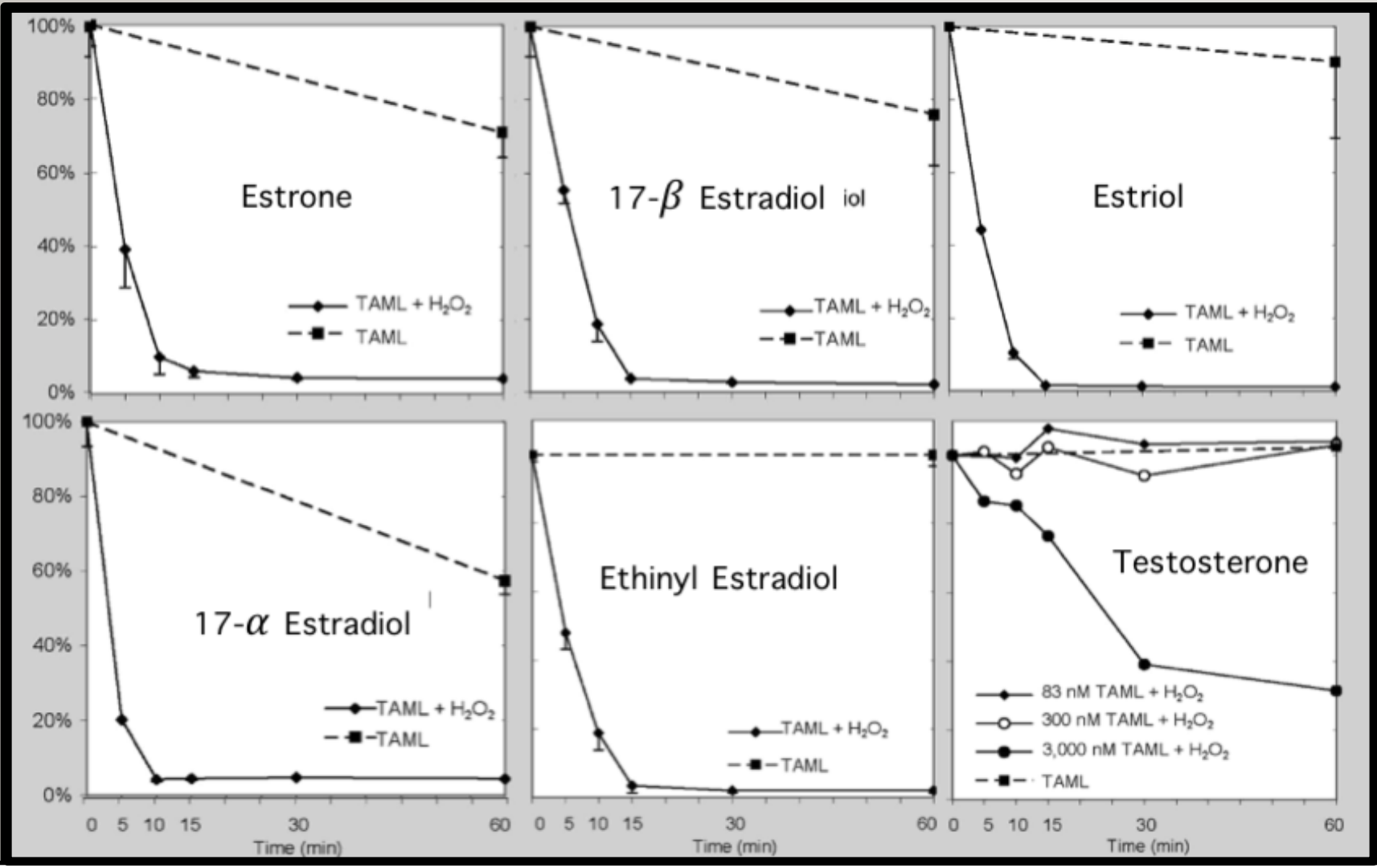


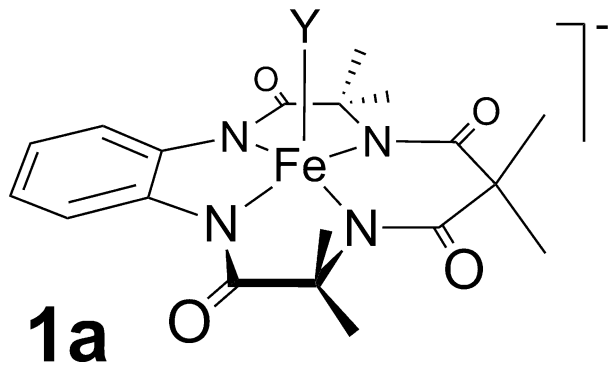
2a



2b

TAML Oxidation of Steroid Hormones





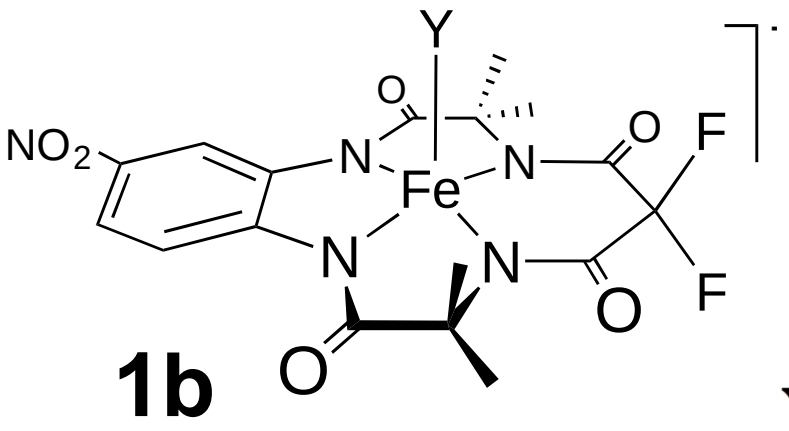
1a

- 80 μM Steroid
- 83 nM, **1a**
- 4 mM H_2O_2, pH 8
- pH = 8
- Catalase termination

In a preliminary experiment, incubations with a municipal wastewater sample (obtained prior to chlorination) resulted in >99% removal of estradiol (ELISA).

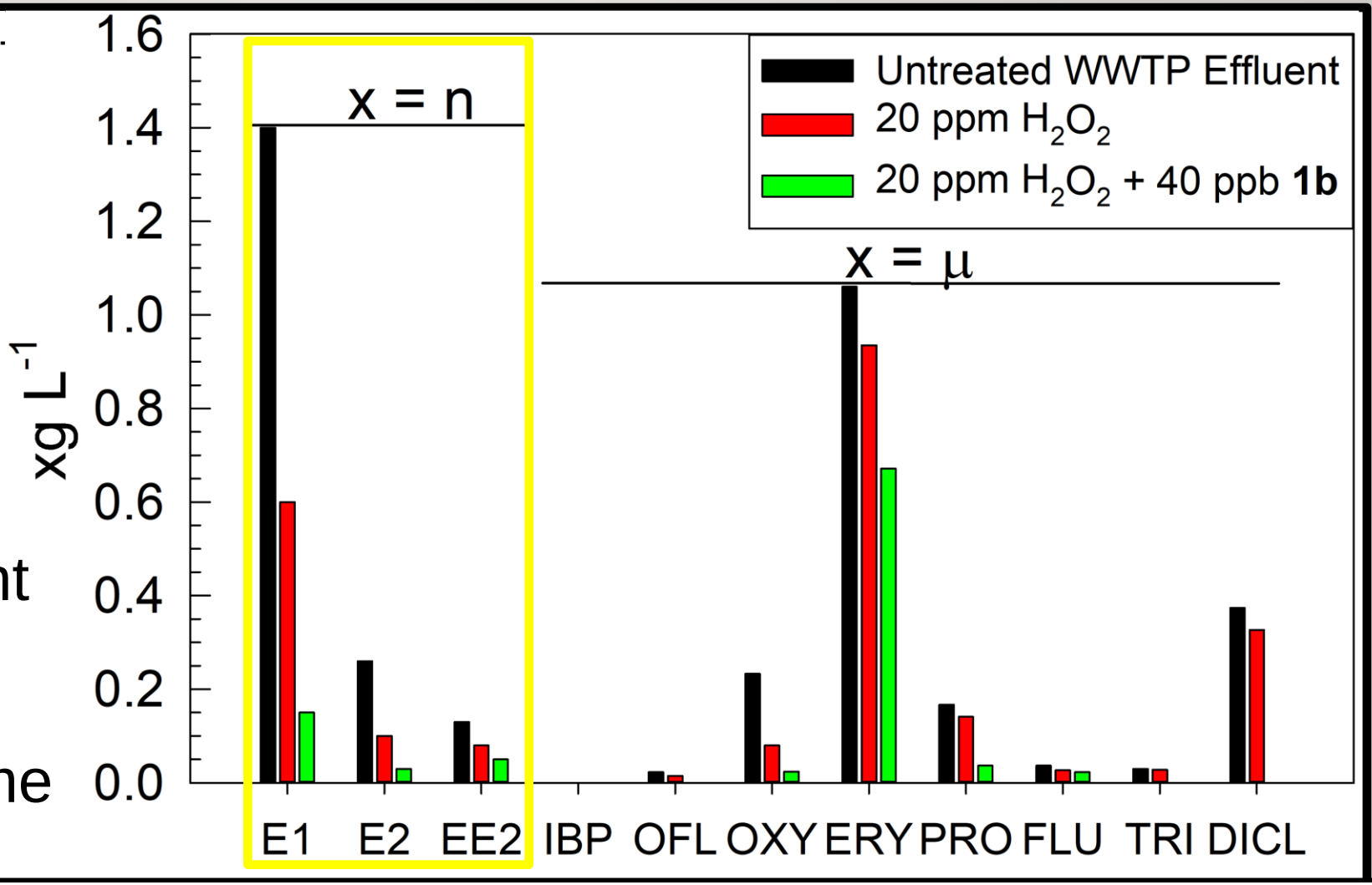
Shappell, N. W.; Vrabel, M. A.; Madsen, P. J.; Harrington, G.; Billey, L. O.; Hakk, H.; Larsen, G. L.; Beach, E. S.; Horwitz, C. P.; Ro, K.; Hunt, P. G.; Collins, T. J. *Environ. Sci. Technol.* **2008**, *42*, 1296-1300.

OldTAML 1b Treatment of WWTP Effluent



1b

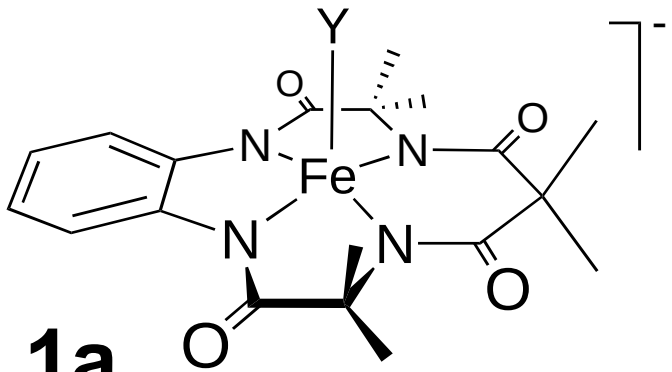
- 14.4–15.7 °C (ambient UK temp)
- 15 minute reaction time



Churchley, J.; Collins, T.; Jobling, S. *Catalytic Oxidation of Pharmaceutical Compounds in Wastewater Effluents*; UK Water Industry Research Limited: London, **2011**.

Iron *T*etra*A*mido *M*acrocylic *L*igand Complexes (TAML Activators)

‘OldTAML’



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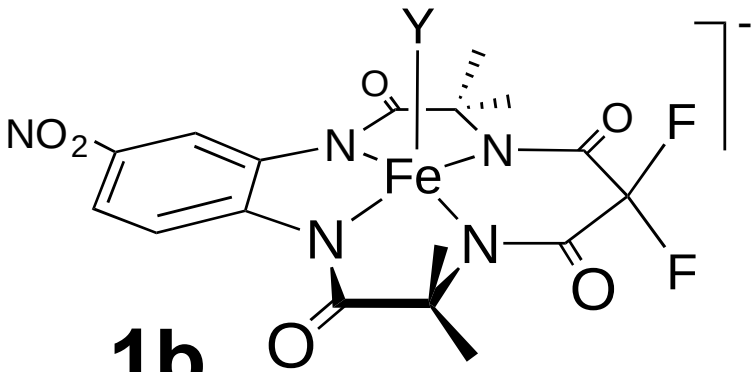

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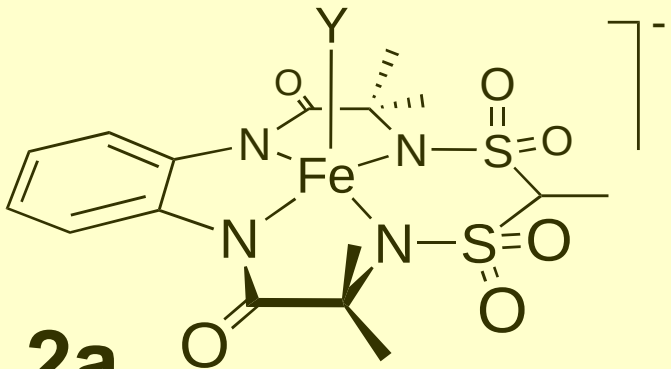
‘NewTAML’



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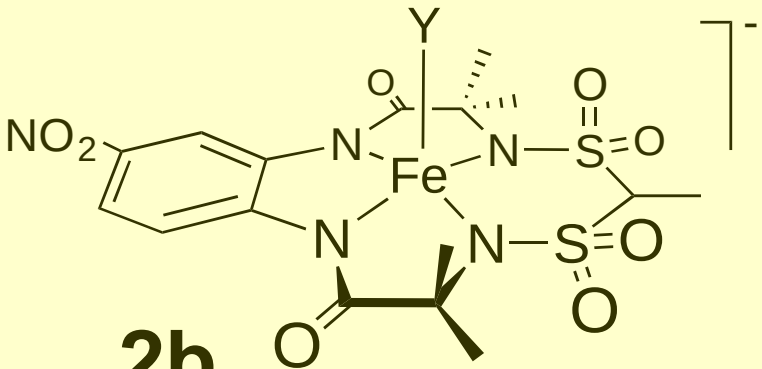
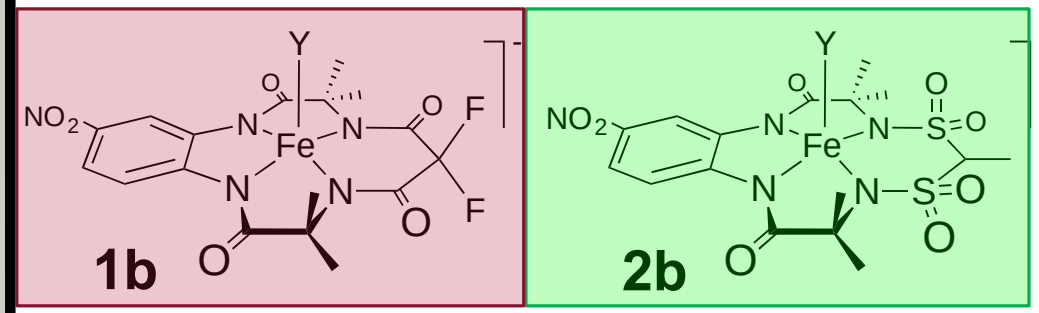
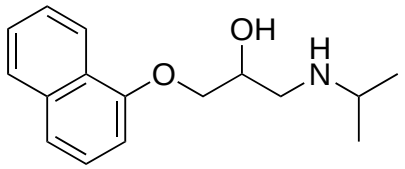


2b

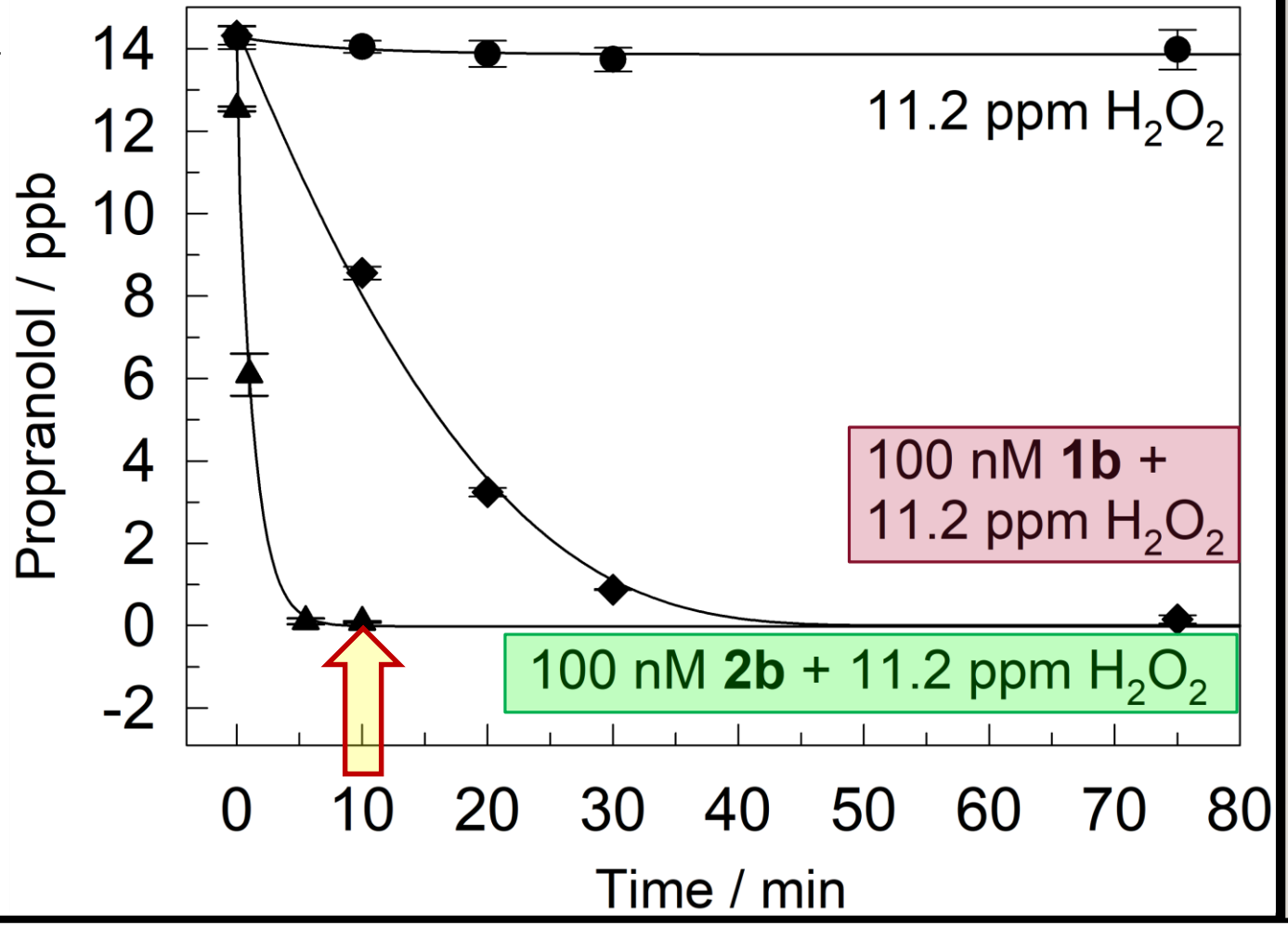
OldTAML 1b vs NewTAML 2b: oxidation of Propranolol in pH 7 lab water





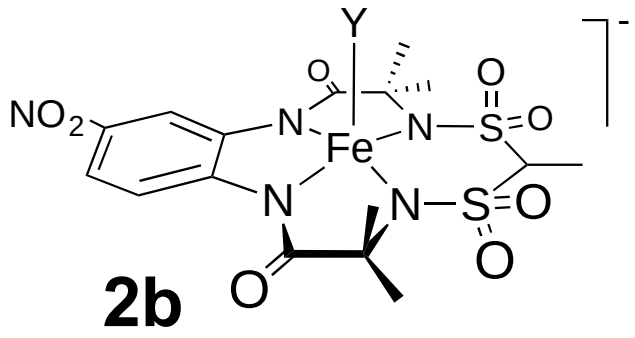
Propranolol

- pH 7 (0.01 M, phosphate) 25 °C
- 10 min to non-detect



Unpublished Results

NewTAML 2b Oxidation of Propranolol in Filtered, pH 7 adjusted Allegheny River Water



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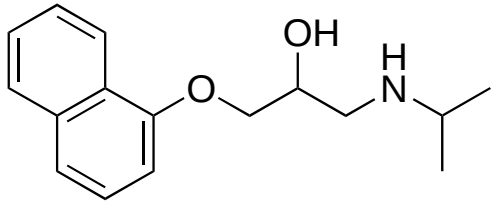
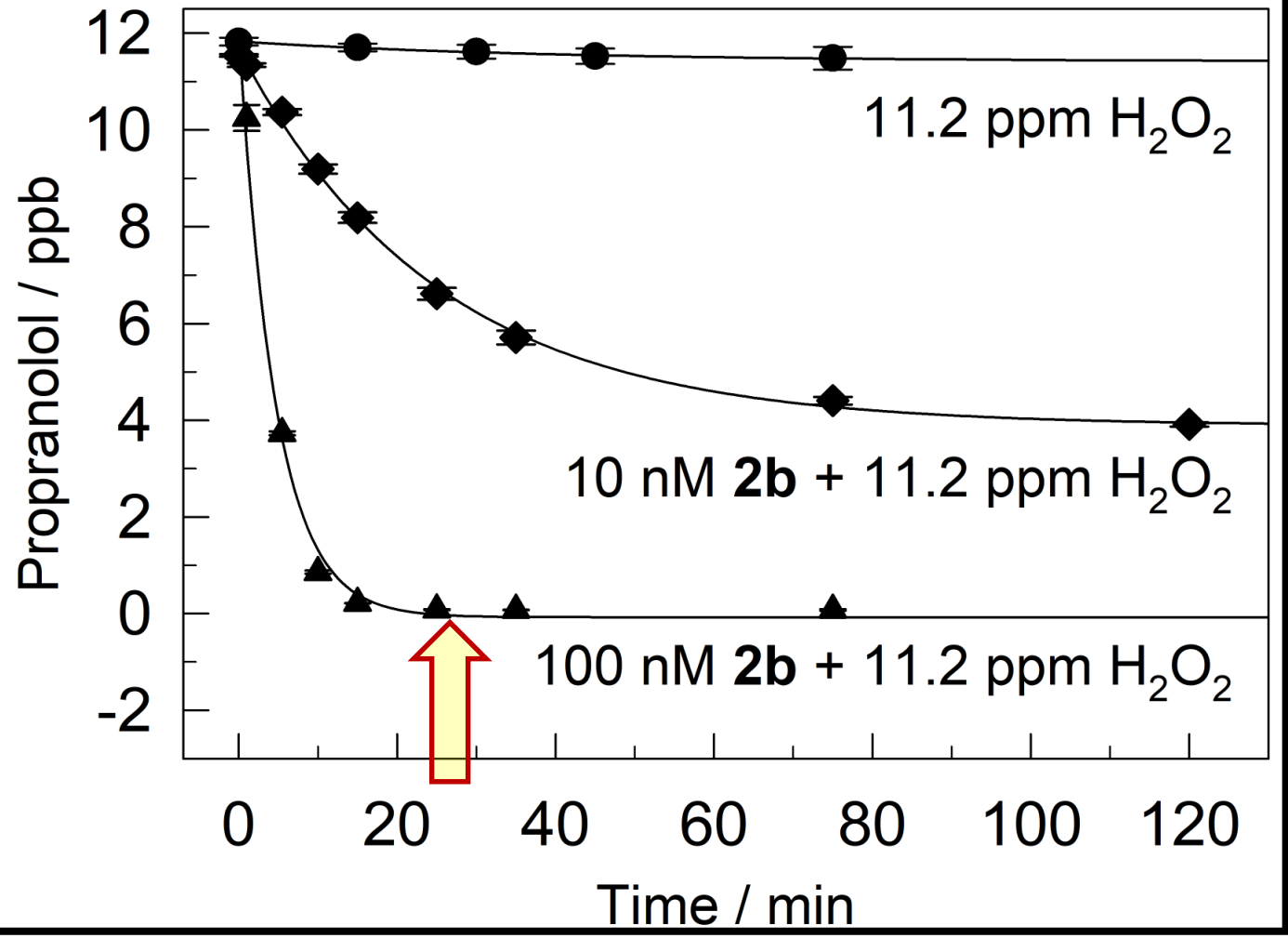


Propranolol

- Water was spiked with propranolol and adjusted to pH 7 and 25 °C
- 25 min to non-detect (NOM)



Unpublished Results

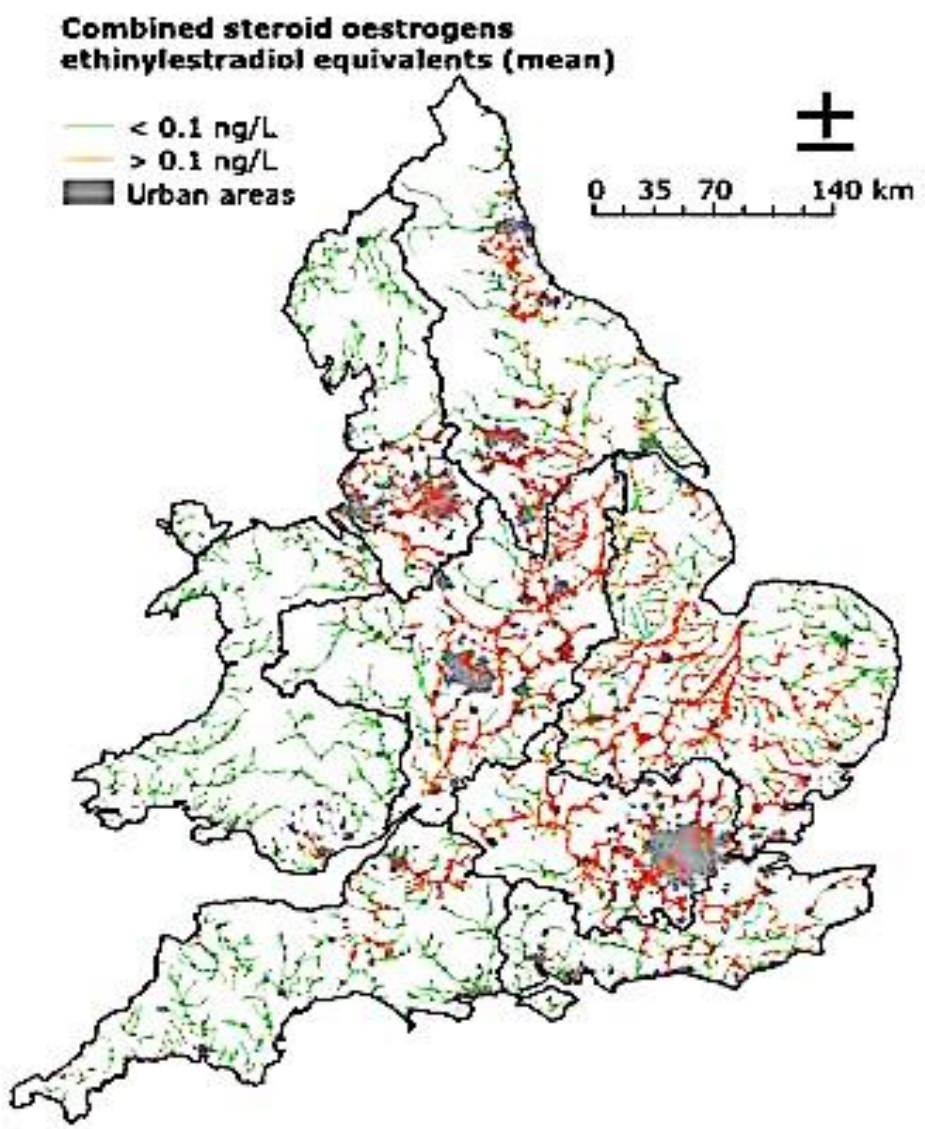
Main Points

- OldTAML catalysts degrade the estrogen hormones associated with incidences of TO and elevated serum VTG in WWTP effluent as well as many other MPs.
- The estrogenic activity of the treated solutions disappears.
- At application relevant catalyst concentrations, no toxicity has been detected in either receptor, zebrafish or mouse studies.
- We are currently testing NewTAML catalyst performance in American WWTP effluents.
- NewTAML catalysts are even better cost and technical performers—tens of thousands of tonnes of water per catalyst kg and very low peroxide concentrations.
- TAML/peroxide technologies are much cheaper than ozone, the least expensive approach currently available—see Dr. Kanda's slide next.

Micropollutants in Water – The European Approach to an Emerging Environmental Challenge

Priority Substance Group	Typical Sources
Chemical compounds	Found in hand creams, and personal cleaning products. Kitchen, bathroom and industrial cleaning products. Flame retardants (which get onto clothing)
Chemicals used in plastics	These are present in everyday plastics such as shower curtains, gutters, down pipes and sewer pipes.
Metals such as, copper, nickel, zinc and lead.	These materials are common in household plumbing, building products such as roof flashing and are used in industrial processes.
Hormones such as oestrogen	Human waste – both naturally occurring hormones and those associated with birth control products are present in waste water.
Pharmaceuticals such as ibuprofen.	Human waste – medicines are not fully absorbed into the body so some passes through and into the waste water.

UK Rivers and Streams



- EE2 (17α-Ethinylestradiol, the active ingredient in the female birth control pill)
- UK rivers and streams exceed the PNEC for EE2

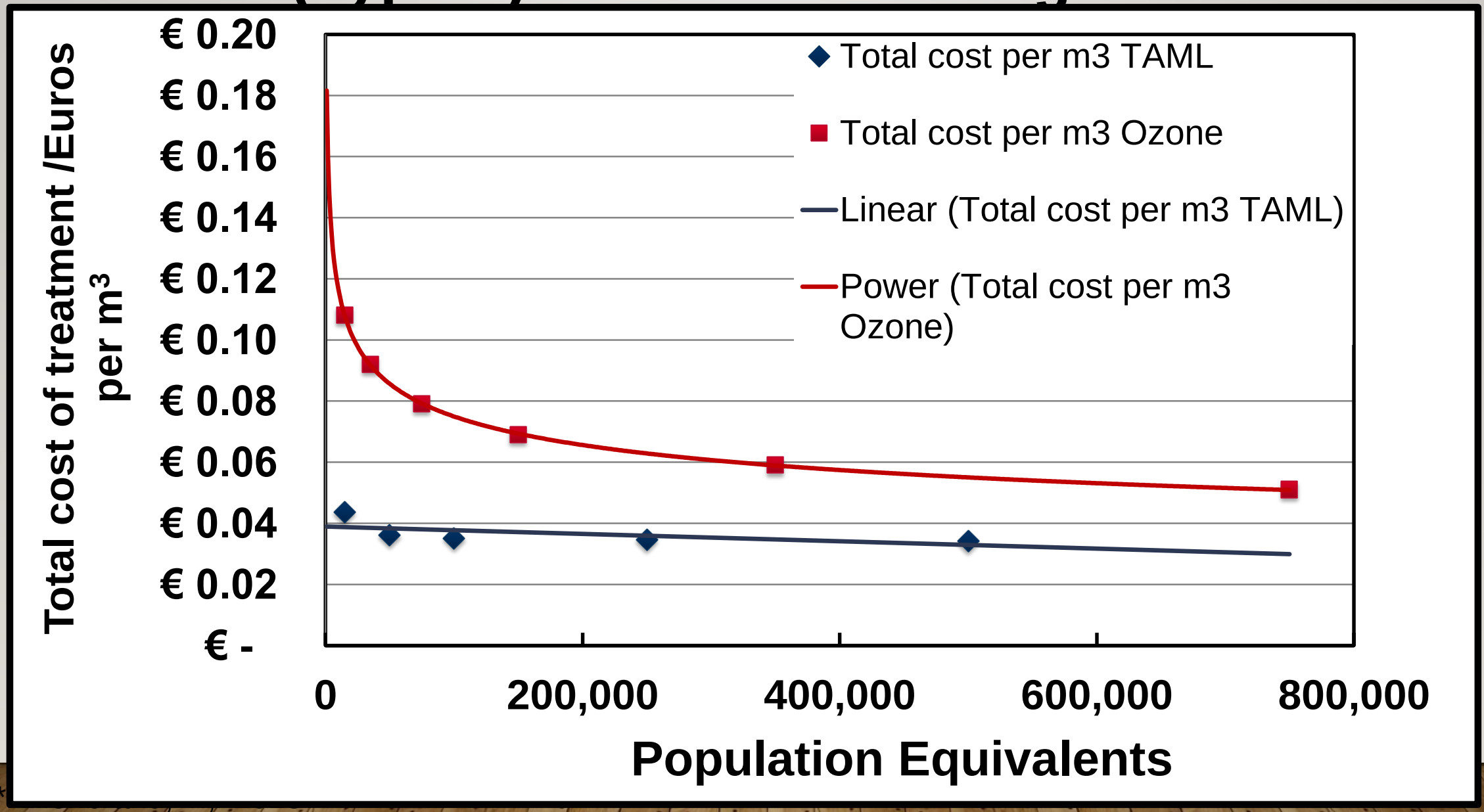
European Union: Water Framework Directive

- The WFD is the single most important environmental regulation in the EU
- Requires member states to achieve good status of all water bodies **by 2015**.
- 50% of all European waters still fail to comply with proposed water quality standards (in 2017)
- Many WWWTP still require upgrades to existing facilities (3rd or 4th stage) wastewater treatment
- In Europe Switzerland is proposing to upgrade 100 of the 700+ sewage treatment plants
  - Investment: ca. 1 billion Euro within 20 years
  - subsidy: 75% investment from wastewater fee per inhabitant
- The EC estimates that the total costs of implementation of WFD in the EU to be in the region of **210-330 billion EUR or 8-15 billion EUR/year (Pers. comm M, Wenning, European Commission)**.

Limitations of Non-TAML Solutions and Barriers to Innovation

- Affordability of water services is a significant factor in the adaptation and implementation of WFD treatment infrastructure
- Even in wealthy countries such as Switzerland, high-tech advanced wastewater solutions are not economical options for smaller sewage works in rural and agricultural areas
- Alternative lower cost, lower energy alternatives to advanced existing treatment solutions are required

TAML (Type 2) vs Ozone 4th Stage



TAML Activators as a Technical Alternative

- **TAML activators provide a technical alternative to 4th stage treatment** (ozone, PAC, GAC)
- **Ozone treatment is not appropriate for all sewage treatment** (Federal Council of the Swiss Government)
- If the water contains, high contamination of contaminants for example, high concentrations of bromide, ozone can sometimes produce too high a concentration of potentially carcinogenic bromates
- Type 2 TAML activators provide significant advantage over ozone and GAC plants especially at low PE plants (a large percentage of plants in Europe)
- Two multi million € proposals with partners across Europe to demonstrate TAML treatment in the UK, Belgium, Norway have twice been **first reserve** for funding

Main Points

- Most of the WWTPs in the CB watershed are relatively small as would be any infrastructure installed at agricultural sites
- TAML is a very cost-effective choice for removal of estrogens and other micropollutants at small treatment plants
- TAML catalysis possesses an ideal combination of technical, environmental, and cost performances for the removal of the micropollutants associated with intersex in Chesapeake Bay Smallmouth Bass and other known EDs

Future Directions

- We would like to work with the Chesapeake Bay Commission and plants and facilities that you cover—could we think about a CBC/IGS case study?
- We could start with lab studies of wastewaters from a selection of CBC plants—we need support **($0.5 M would get us going**).
- We have the water analysis collaborations functioning, but we would also be delighted to work on-site with any plant that has the MP analytical facilities and expertise
- We need a pilot trial as soon as possible**—$2 M would enable this and set the stage for demonstration plants.**
- We envision a major new American industry with many American jobs (catalyst production, plant design and production, expert water services) with a huge improvement in water quality worldwide.

Acknowledgements

- TJC gratefully acknowledges the Heinz endowments and the Heinz Family Foundation for support