



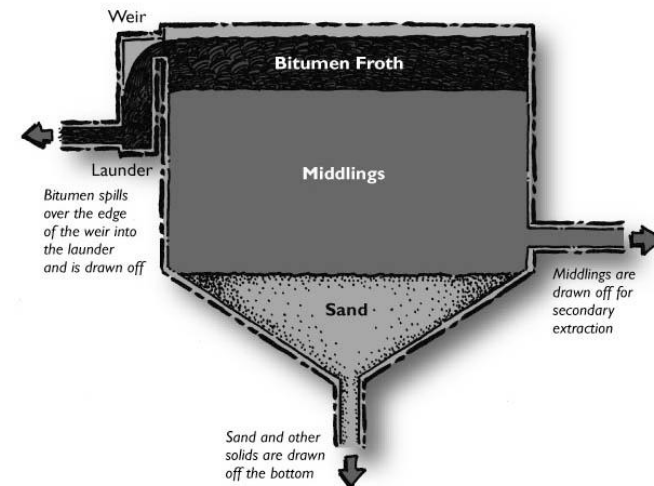
The *in vitro* endocrine disruptive effects of untreated and ozone treated oil sands process water

Y. He, S. B. Wiseman, X. Zhang, M. Hecker, P. D. Jones, M. G. El-Din, J. W. Martin, J. P. Giesy.

Oil Sand Process Water (OSPW)

- ❑ The extraction process results in the production of large volumes of wastewater, commonly referred to **oil sands process water (OSPW)**
 - Sands, clay, metals, unrecoverable bitumen
 - Polycyclic aromatic Hydrocarbons (PAHs)
 - **Naphthenic acids (NAs)**

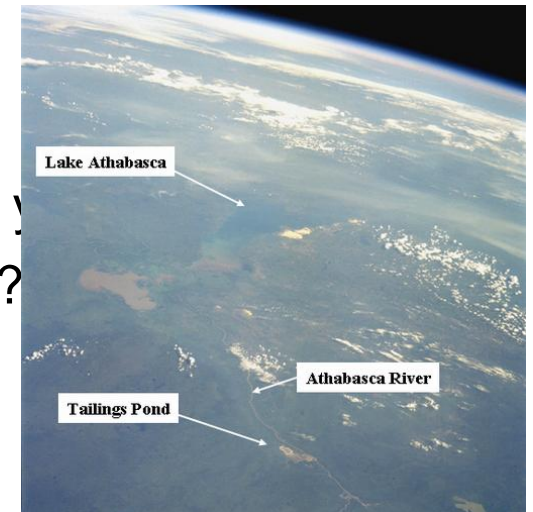
- ❑ NAs are thought to be a / the key contributor to OSPW toxicity.



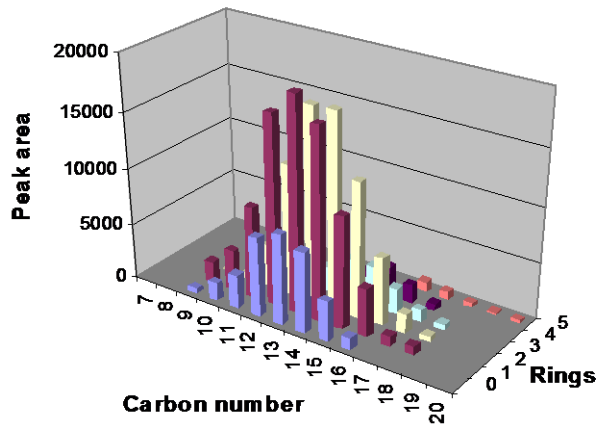
Reclamation

- ❑ Large quantity of OSPW
 - Zero discharge policy
 - Growing volume of stored OSPW
 - Over 10^9 m³ stored in various basins

- ❑ Reclamation
 - Aging reduces acute toxicity of OSPW
 - Chronic toxicity remains
 - Long half-life for natural degradation: ~12 y
 - Need more aggressive efforts: ozonation ?

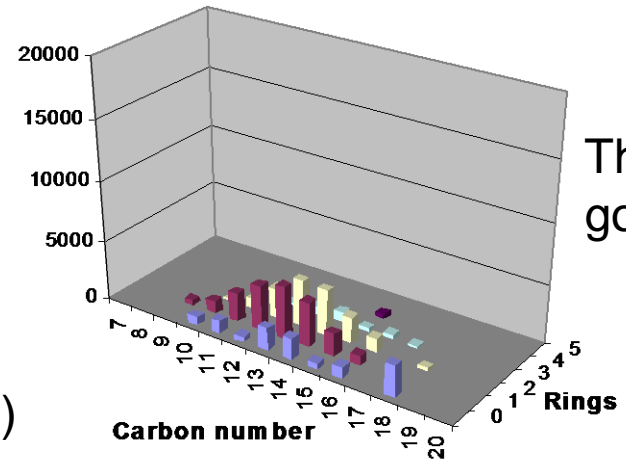


Need to be careful we don't create new toxic species.... Potentially endocrine disruptors?



Parent NAs in Commercial Mixture

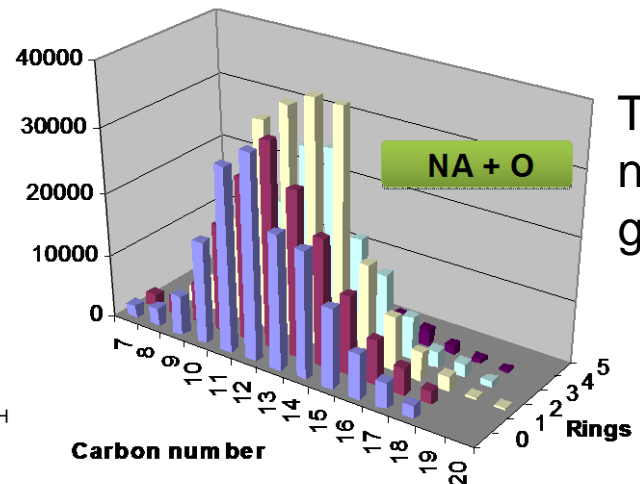
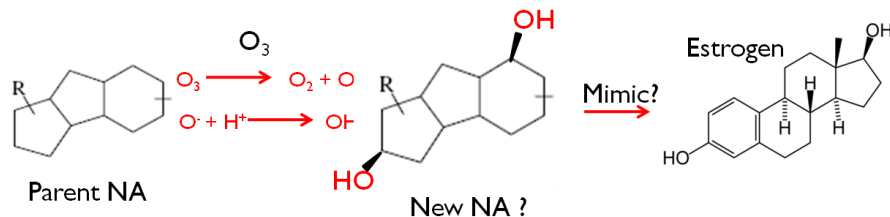
O₃ (20 ppm)



78% degradation of NAs

This is good!

Any low MW NAs formed during ozonation, or hydroxylated NAs resembling steroids, may become more bioavailable and consequently adversely affect aquatic organisms.



Production of New Oxidized NAs

This may not be good!



Endocrine Disruption in the Literature

□ Exposures to tailing pond OSPW

- Reduce *in vitro* production of E2 & T by ovarian and testicular tissue (slimy sculpin) (Tetreault *et al.*, 2003)
- Reduce plasma sex hormones in yellow perch (van de Heuvel *et al.*, 1999) & goldfish (Lister *et al.*, 2008)
- Cause longer time to produce first clutch of eggs, and fewer number of clutches of eggs (fathead minnow) (Siwik *et al.*, 2000)
- Reduce plasma T and 11-keto-T in male, and reduce spawning in female fathead minnow (Kavanagh, et al., 2010)

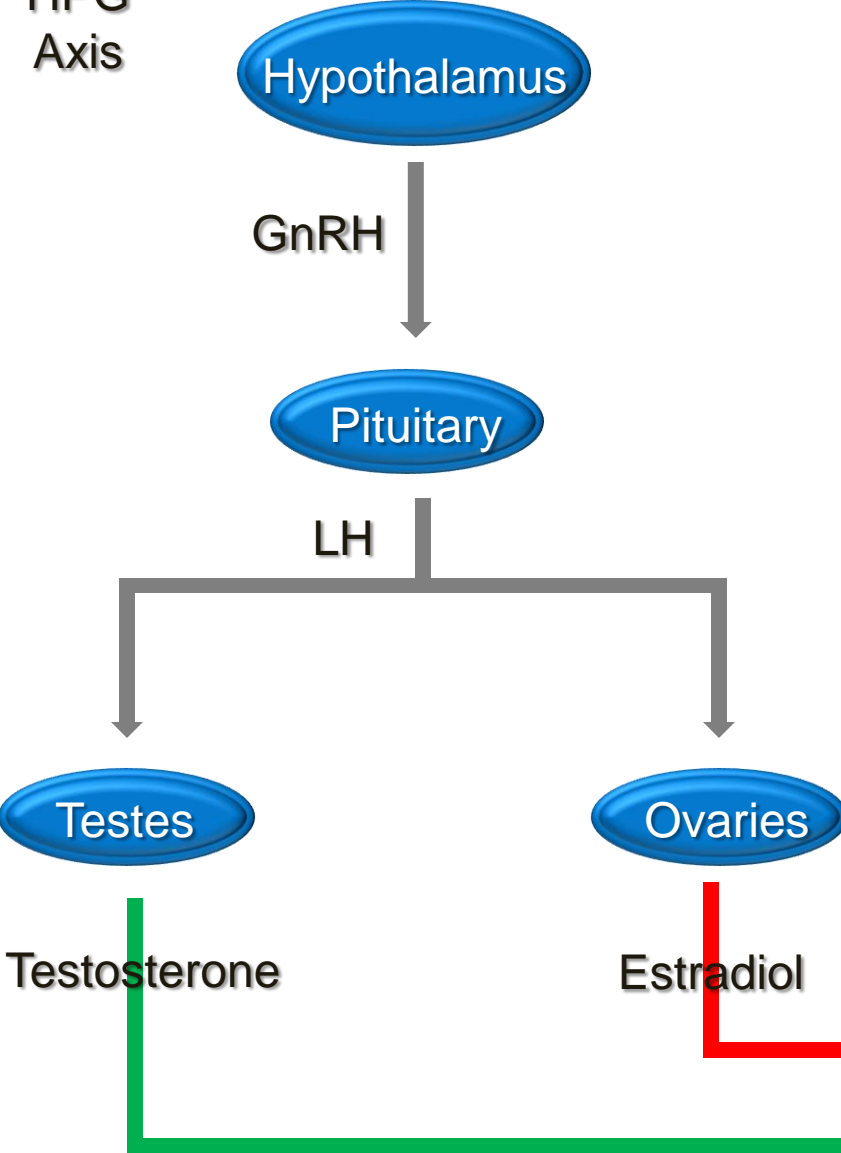


Objectives

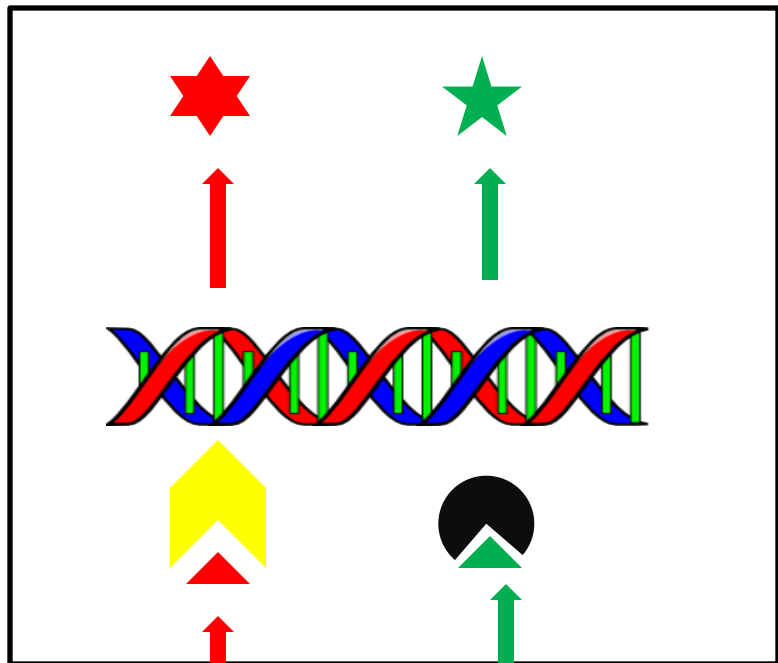
- ❑ To assess the endocrine disruptive effects of OSPW
- ❑ To determine the effects of ozonation on the endocrine disruptive properties of OSPW

Sex Hormone Production and Action

HPG Axis



Site of Hormone Action



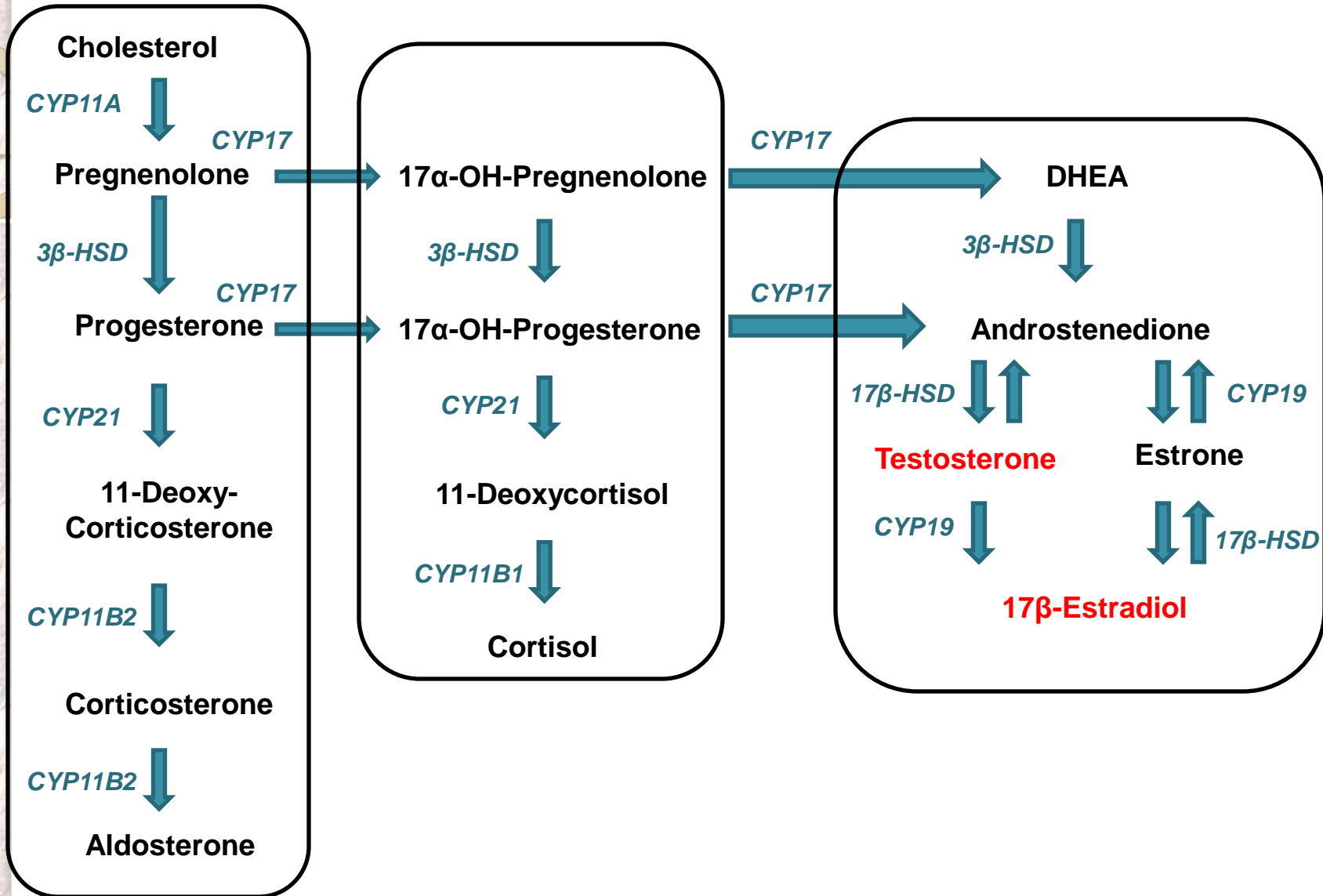


° Study 1: Hormone Production

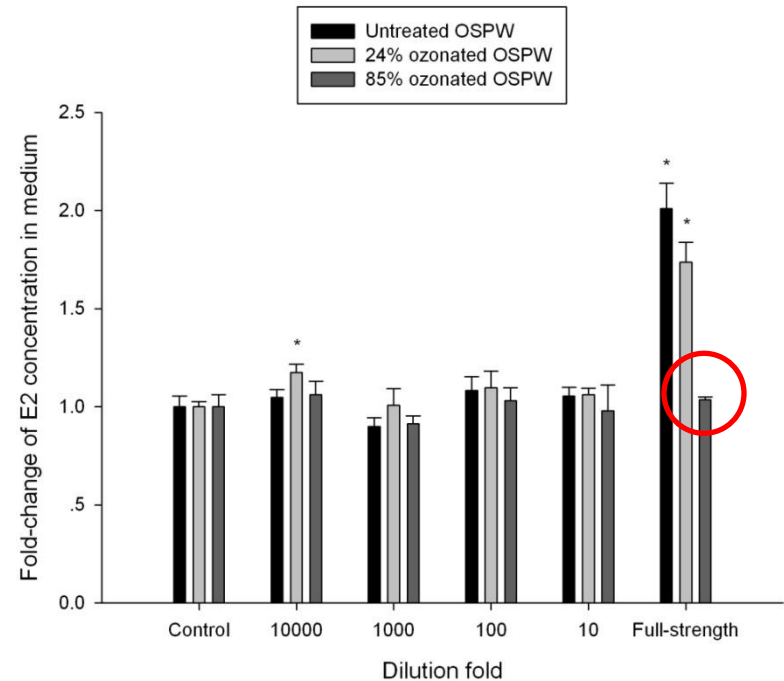
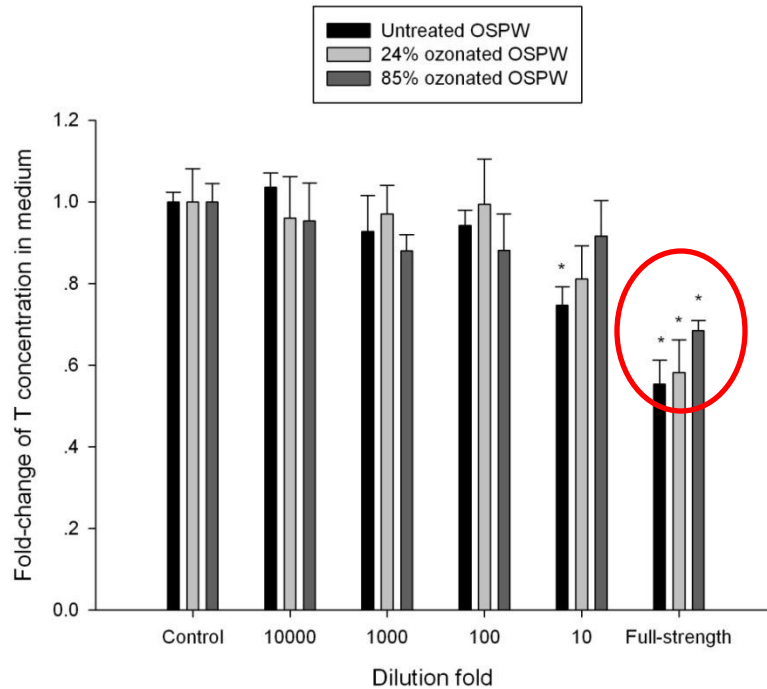
Ozonation attenuates the steroidogenic disruptive effects of sediment free oil sands process water in the H295R cell line

Y. He, S. B. Wiseman, X. Zhang, M. Hecker, P. D. Jones, M. G. El-Din, J. W. Martin, J. P. Giesy. 2010. Ozonation attenuates the endocrine disruptive effects of sediment-free oil sands process water in the H295R cell line. Chemosphere. 80: 578-584.

The H295R Cell Line

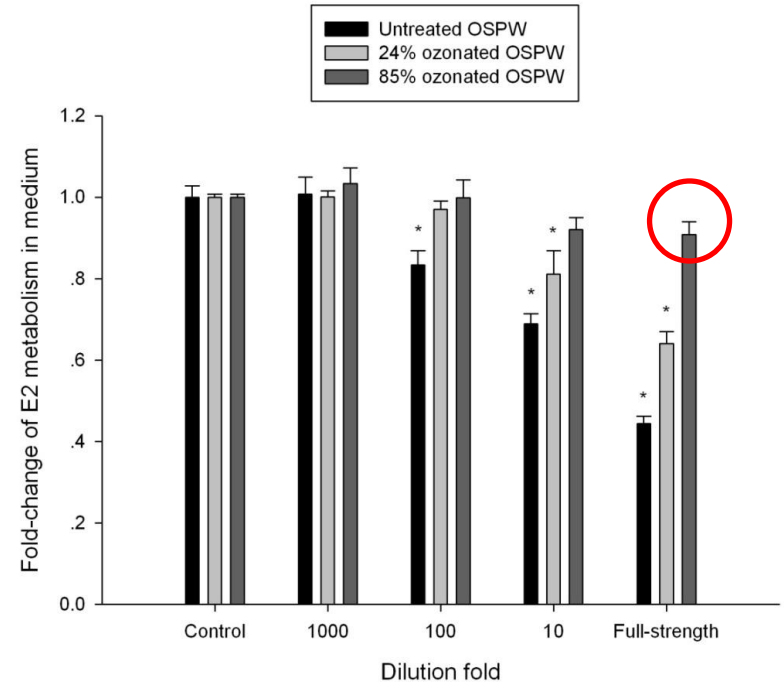
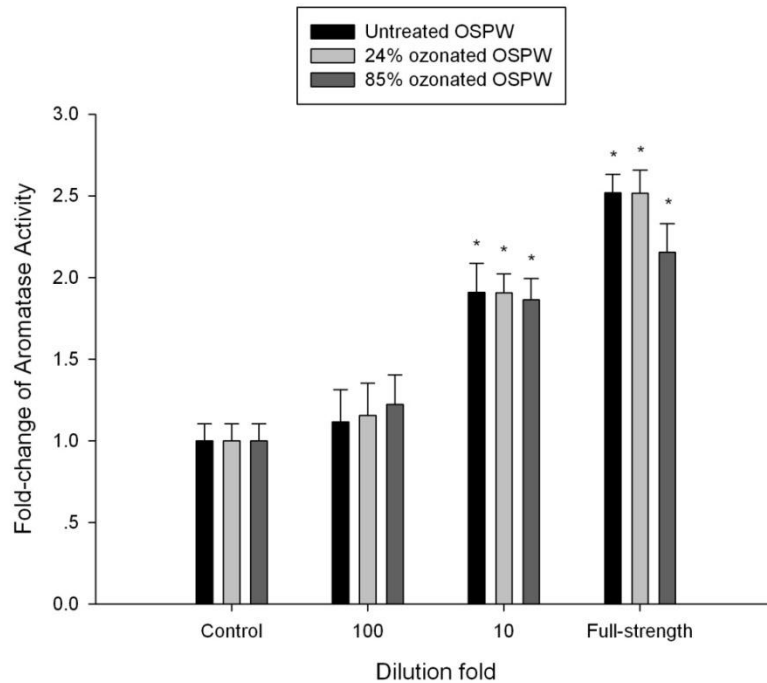


Results: T & E2 production



- ❑ T production decreased
- ❑ E2 production increased
- ❑ Ozonation attenuates the effects

Results: Aromatase & E2 metabolism



- ❑ Aromatase activity increased
- ❑ E2 metabolism decreased
- ❑ Ozonation eliminates the adverse effects at 85%

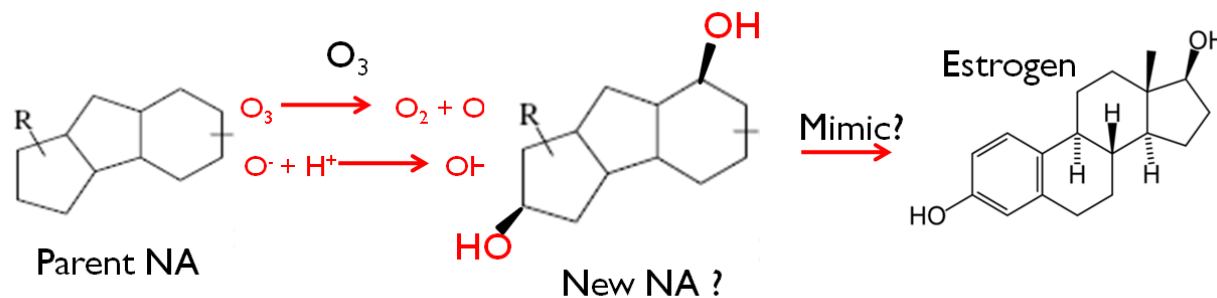
Conclusion – Study 1

- ❑ Ozonation attenuated the adverse effects of OSPW
 - T and E2 production
 - E2 metabolism
 - Aromatase enzyme activity

- ❑ Inhibition of E2 breakdown is the major reason for increased E2 production

Study 2: Receptor signaling

- ❑ To determine whether OSPW affects androgenicity and estrogenicity through receptor mediated signaling pathways *in vitro*
- ❑ To evaluate the effects of ozonation of OSPW on the receptor mediated signaling pathway



Background Information

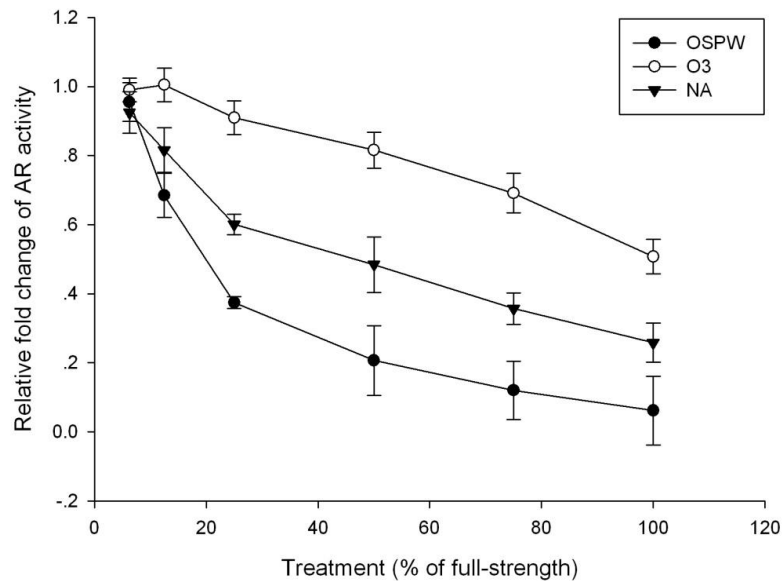
- ❑ Androgen receptor (AR) and Estrogen receptor (ER)
 - Play important roles in sexual differentiation of the reproductive tract, accessory reproductive organs, and other tissues during fetal development

- ❑ Test systems used are rapid, sensitive, and convenient assays for detecting AR/ER agonist/antagonist :
 1. *MDA-kb2*: Transformed with an androgen-responsive luciferase reporter plasmid

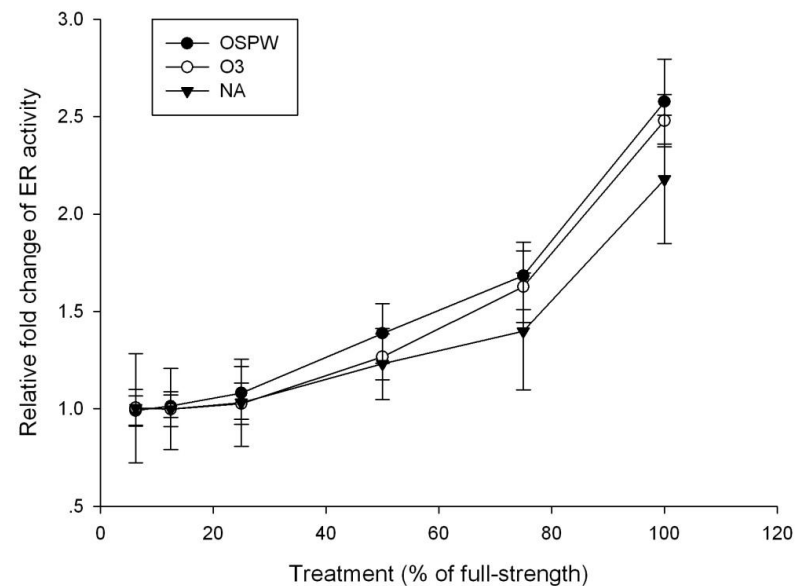
 2. *T47D-kbluc*: Transformed with a luciferase reporter gene containing ERE

OSPW shows anti-androgenicity and estrogenicity

Anti-androgenicity of OSPW, O3, and NA



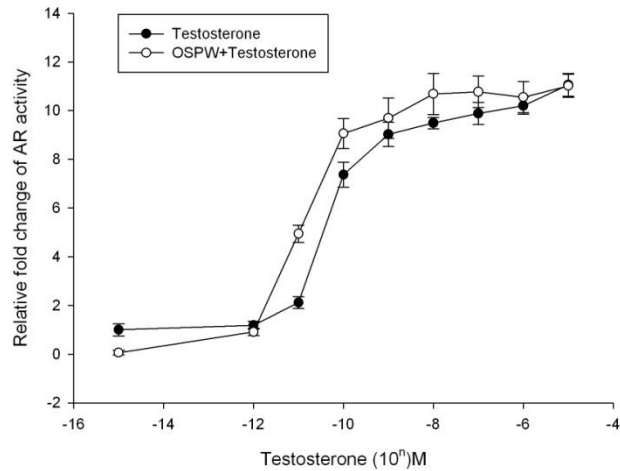
Estrogenicity of OSPW, O3, and NA



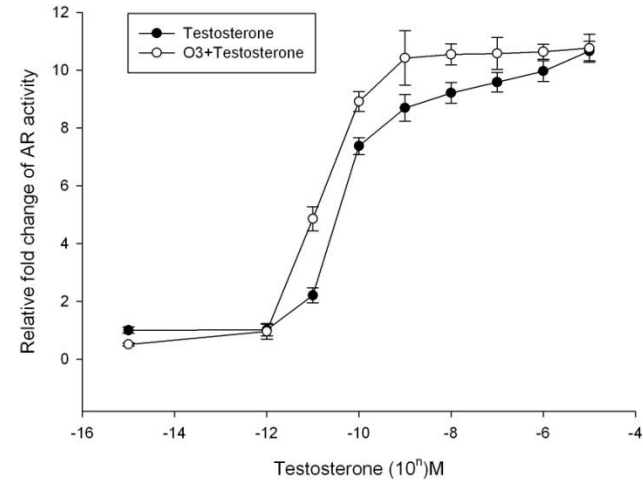
- ❑ OSPW shows anti-androgenicity, and ozonation attenuates the effect
- ❑ OSPW shows estrogenicity, and ozonation does not attenuate the effect

Anti-androgenicity

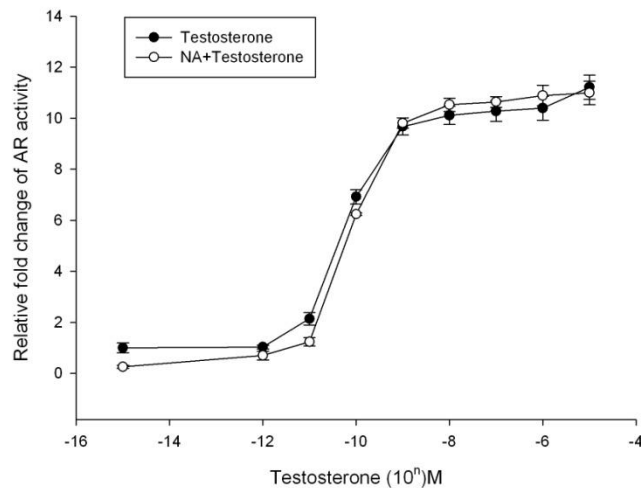
100% OSPW co-exposure with T



100% O3 co-exposure with T



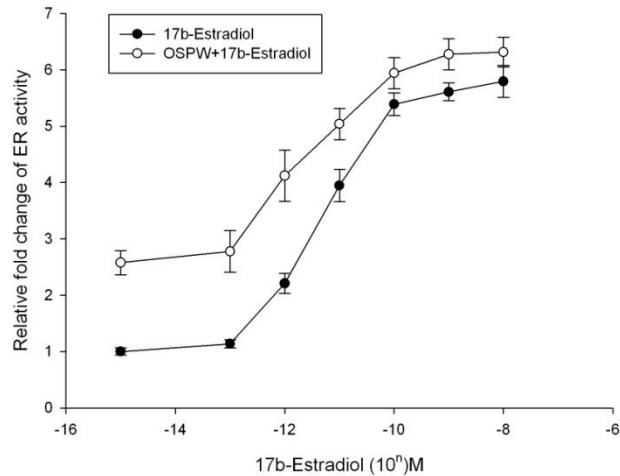
100% NA co-exposure with T



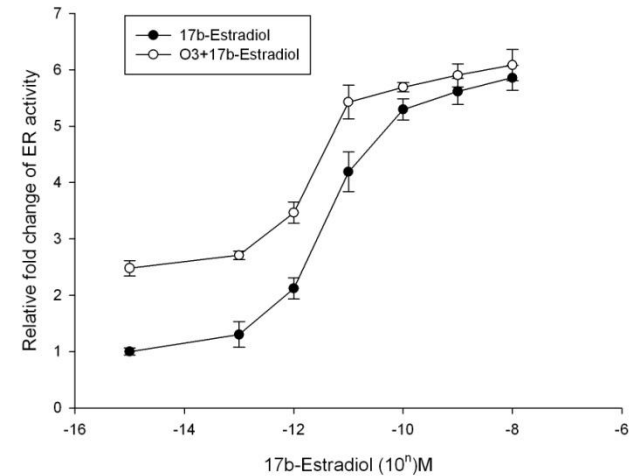
- OSPW is weak AR antagonist
- OSPW potentiates the effect of T at lower concentrations..

Estrogenicity

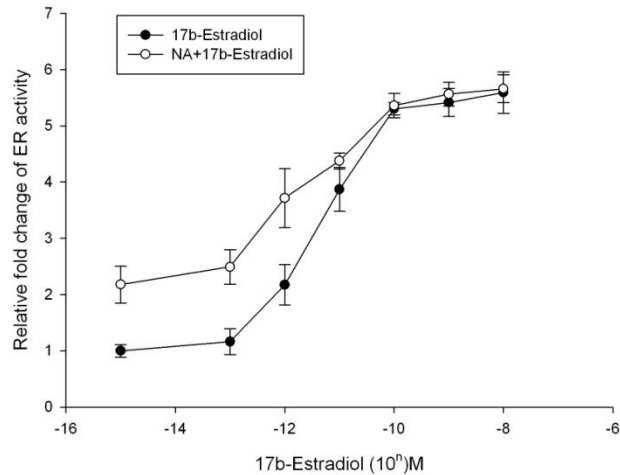
100% OSPW coexposure with E2



100% O3 coexposure with E2

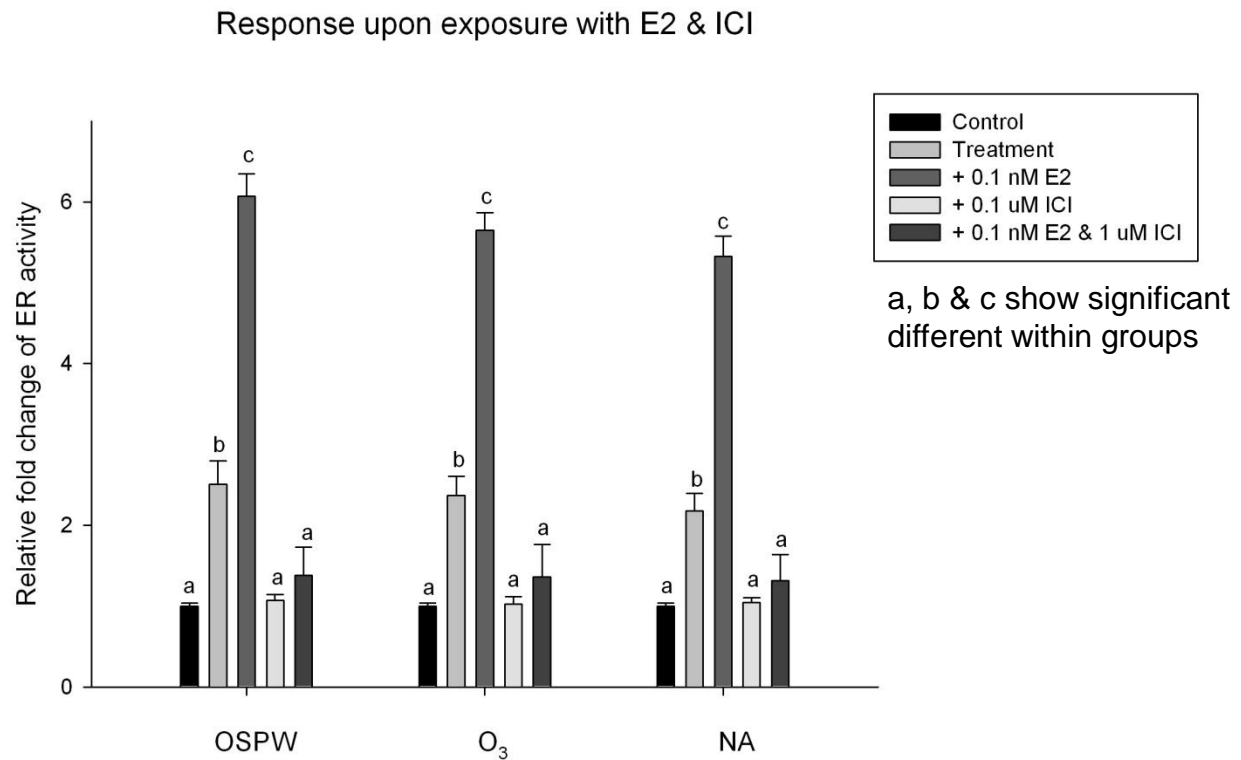


100% NA coexposure with E2



- ❑ Co-exposure with OSPW & E2 shows additive effects on ER activity
- ❑ Ozonation does not attenuate the effect

Estrogenicity is receptor mediated



- ❑ OSPW, ozonated OSPW, and commercial NAs are estrogenic
- ❑ The estrogenicity act through estrogen receptor-mediated pathway as the effect can be inhibited by a strong antagonist (ICI 182,780)
- ❑ Ozonation does not attenuate the effect

Conclusion – Study 2

- ❑ Both full-strength and ozonated OSPW can disrupt receptor mediated androgen and estrogen signaling
 - Both full-strength and ozonated OSPW show anti-androgenicity through weak competitor binding
 - Both full-strength and ozonated OSPW show estrogenicity
- ❑ Ozonation attenuates the effect of OSPW on anti-androgenicity, but not on estrogenicity

Summary

- ❑ OSPW affects sex hormones production (T↓&↑E2)
- ❑ Ozonation attenuates the adverse effects of OSPW
 - T and E2 production
 - E2 metabolism
 - Aromatase enzyme activity
- ❑ OSPW affects receptor mediated signaling (AR↓ & ER↑)
- ❑ Ozonation attenuates the effect of OSPW on anti-androgenicity, but not on estrogenicity

- ❑ Future studies on fathead minnow
 - Gene expression profile
 - Circulating hormones level
 - Reproduction and embryo development

Acknowledgements

- ❑ A research grant from the Alberta Water Research Institute.
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