

Endocrine Disrupting Effects of Fresh OSPW: Mechanisms of Action and the Usefulness of Ozonation for Treating OSPW

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Endocrine Disruption in the Oil Sands

❑ Decreased plasma testosterone (T) and estradiol (E2) in goldfish (Lister et al., 2008) and yellow perch (van den Heuvel et al., 1999) exposed to OSPW.



❑ Reduced synthesis of T and E2 by ovarian and testicular tissues from goldfish (Lister et al., 2008).



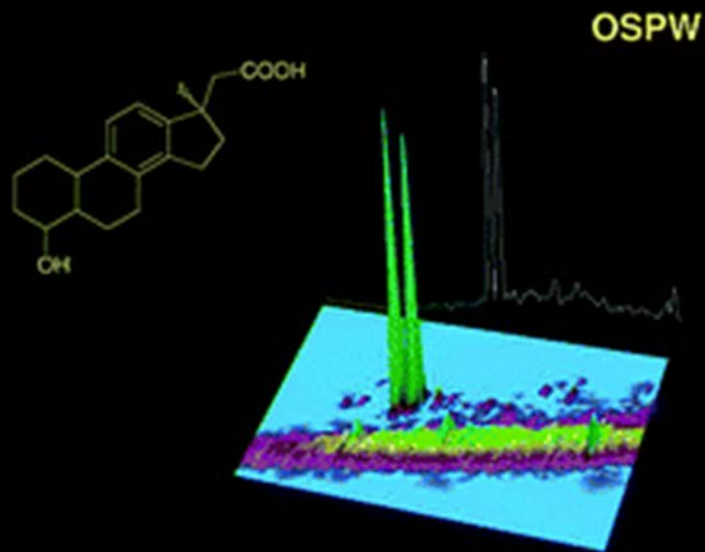
❑ Impaired reproduction of fathead minnows exposed to OSPW (Kavanagh et al., 2011).

- decreased fecundity in females.
- 2^o sex characteristics less pronounced in males.
- males had less T.
- females had less E2.

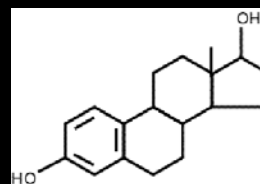
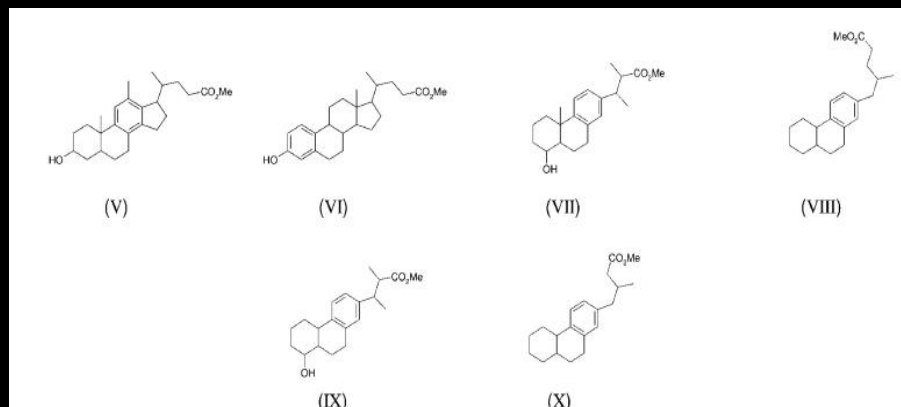


Steroid-Like 'Naphthenic Acids' in OSPW

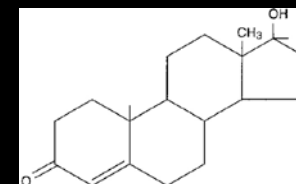
Some naphthenic acids have structures similar to sex steroid hormones.



Rowland et al., 2011

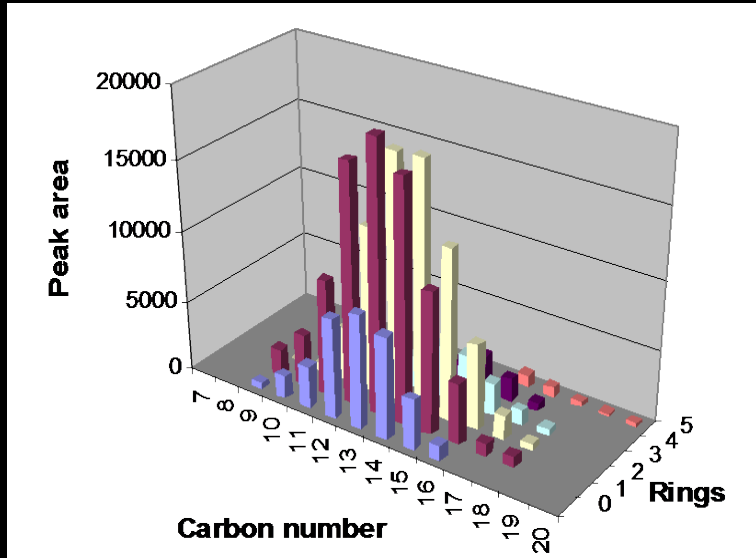


Estradiol (E2)

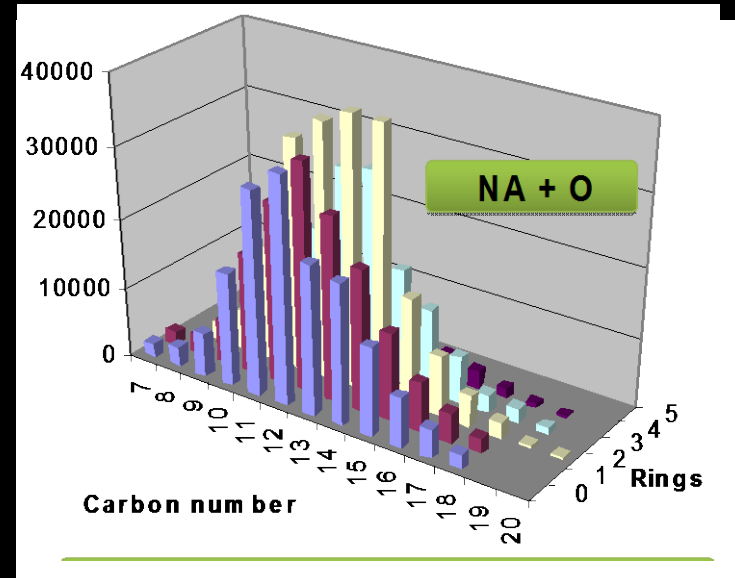
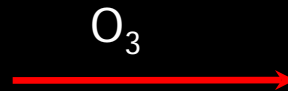


Testosterone (T)

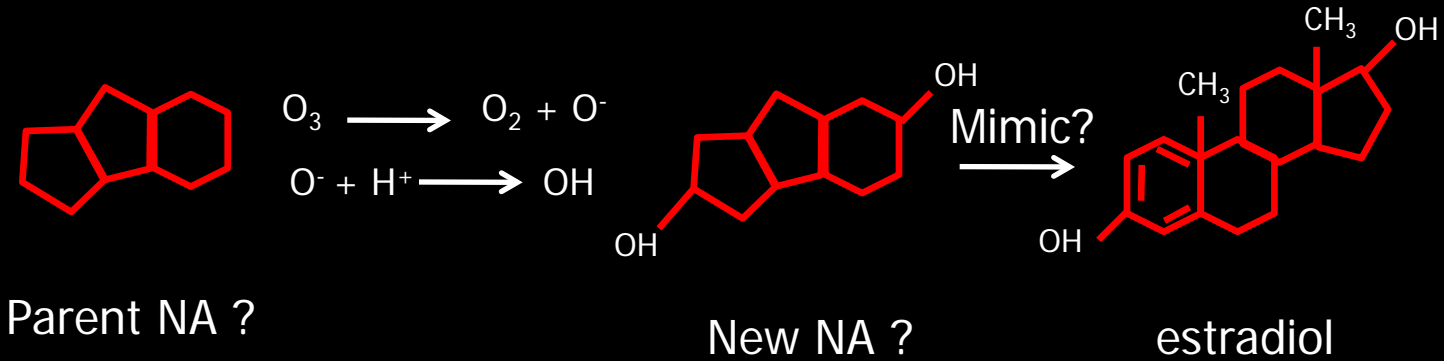
Endocrine disruption by ozonated OSPW?



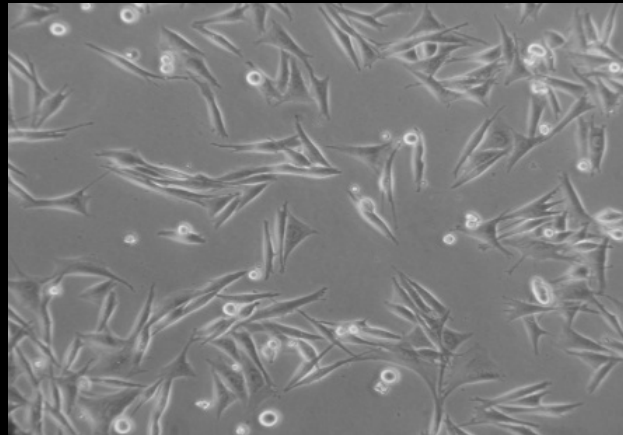
Parent NAs



Production of new NAs

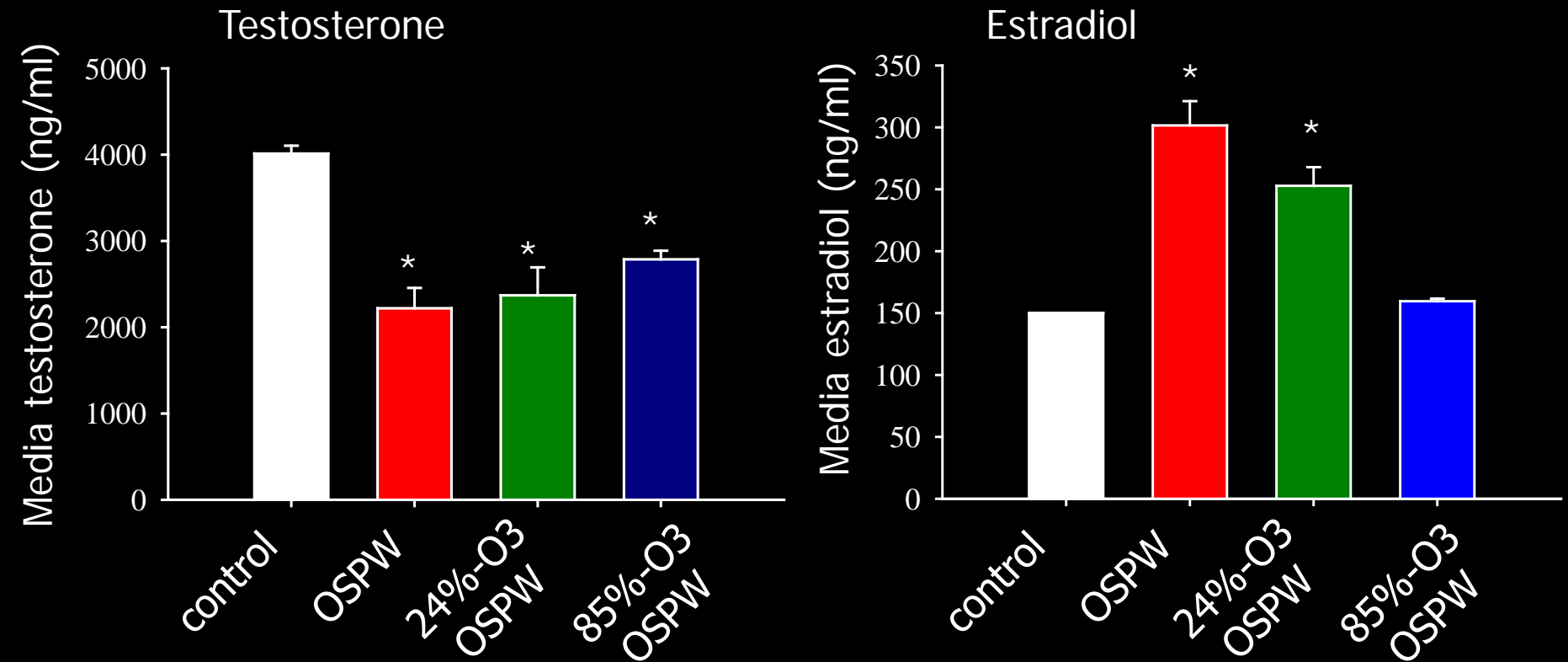


Effects of OSPW and Ozonated OSPW on Sex Steroid Synthesis in the H295R Cell Line



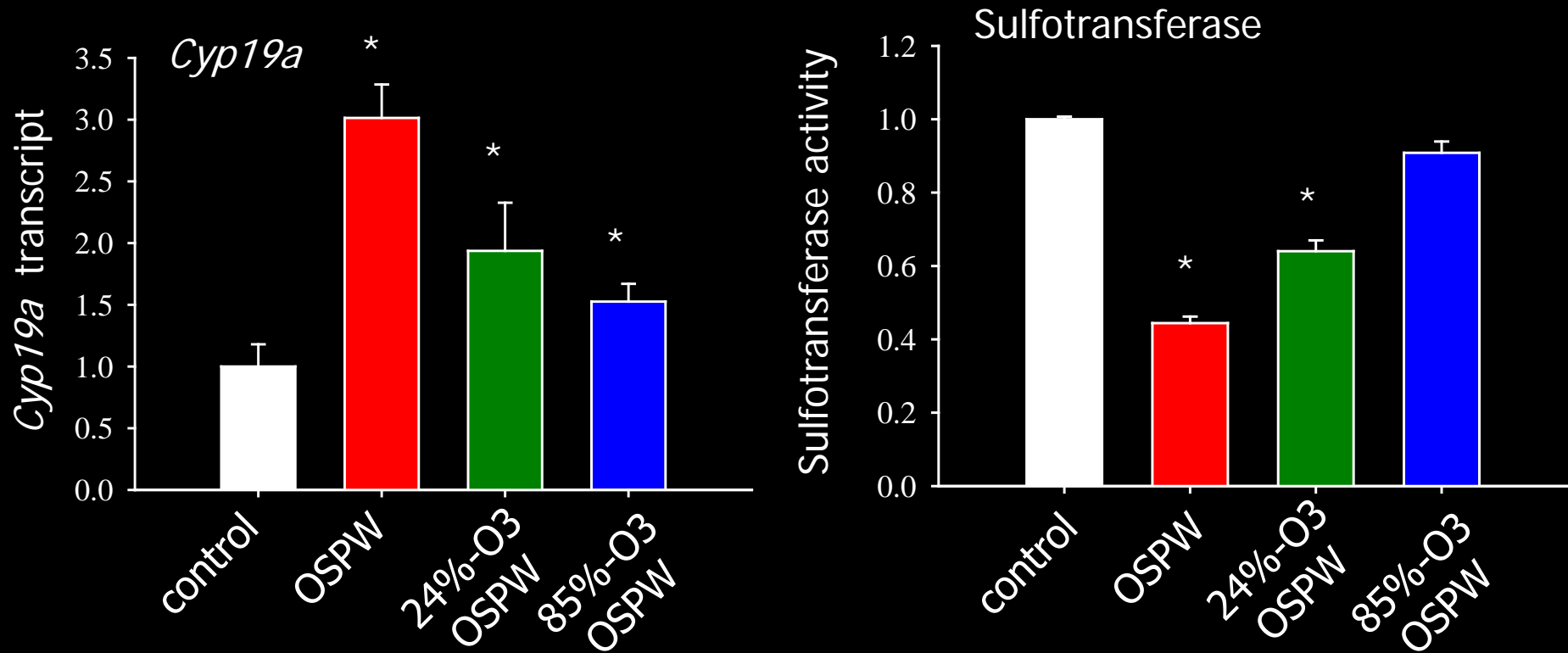
He, Y., Wiseman, S.B., Zhang, X., Hecker, M., Jones, P.D., El-Din, M.G., Martin, J.W., Giesy, J.P. 2010. Ozonation attenuates the steroidogenic disruptive effects of sediment free oil sands process water in the H295R cell line. *Chemosphere*. 80:578-584.

Sex steroid synthesis



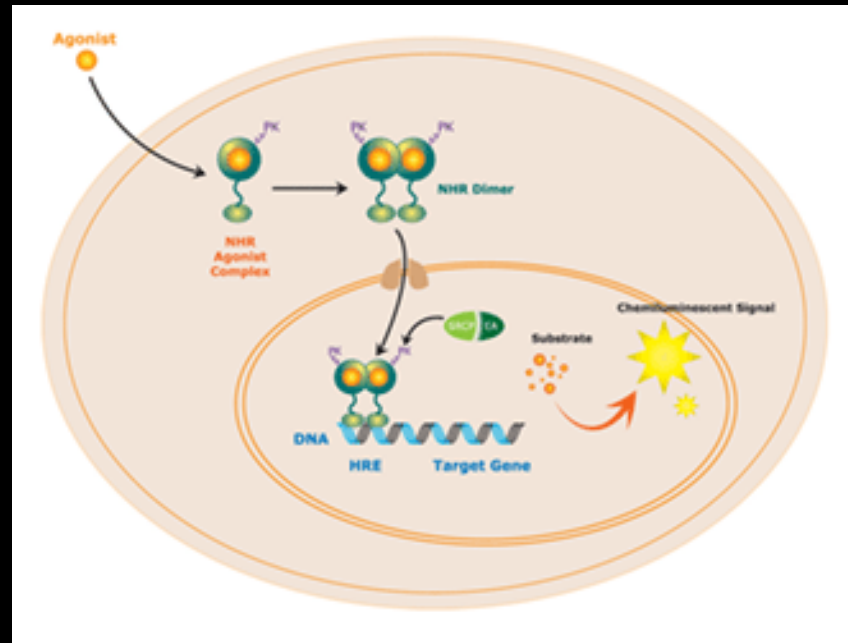
- ❑ OSPW decreased synthesis of T and increased synthesis of E2.
- ❑ Ozonation attenuates the effects.
- ❑ Dose-response effect of ozonation.

Mechanism of effect



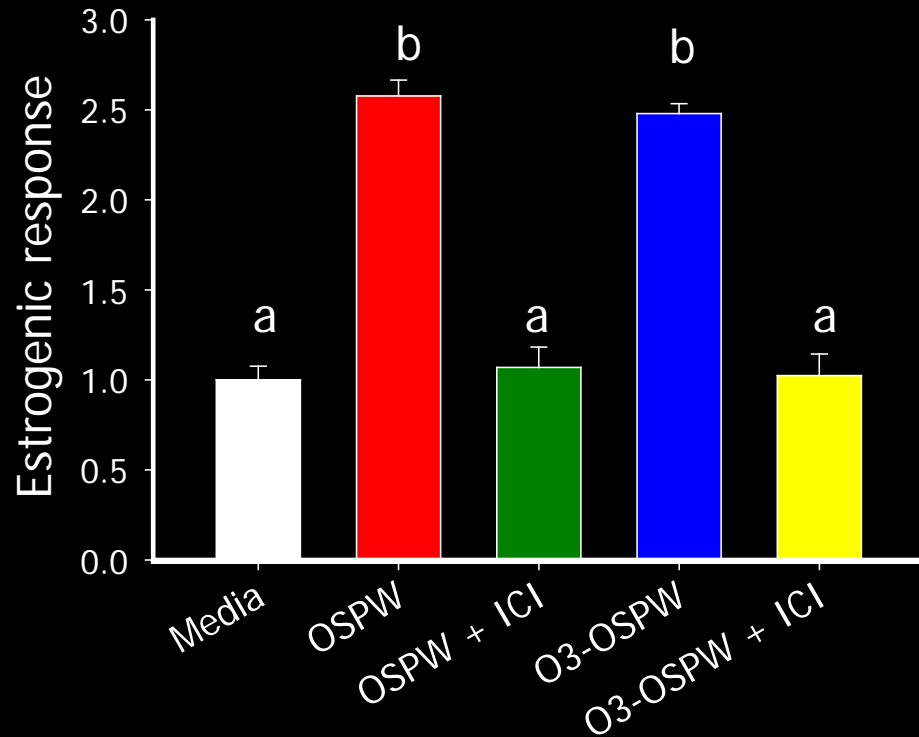
- ❑ Greater E2 results from greater *CYP19a* transcript abundance and less metabolism of E2.
- ❑ Incomplete attenuation of effects by ozonation of OSPW.

Effects of OSPW and Ozonated OSPW on Sex Steroid Receptor Signaling *in Vitro*



He, Y., S.B. Wiseman, M. Hecker, X. Zhang, N. Wang, L.A. Perez, P.D. Jones, M. Gamal El-Din, J.W. Martin, and J. P. Giesy. 2011. Effect of Ozonation on the Estrogenicity and Androgenicity of Oil Sands Process-Affected Water. *Environ Sci. Technol.* 45:6268–6274

Hormone signaling - estrogenicity

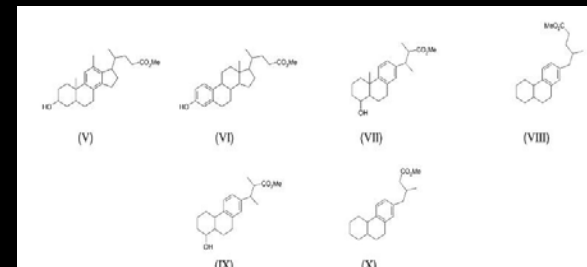


☐ OSPW and ozonated OSPW are estrogenic.

☐ Ozonation neither attenuated nor potentiated estrogenicity of OSPW.

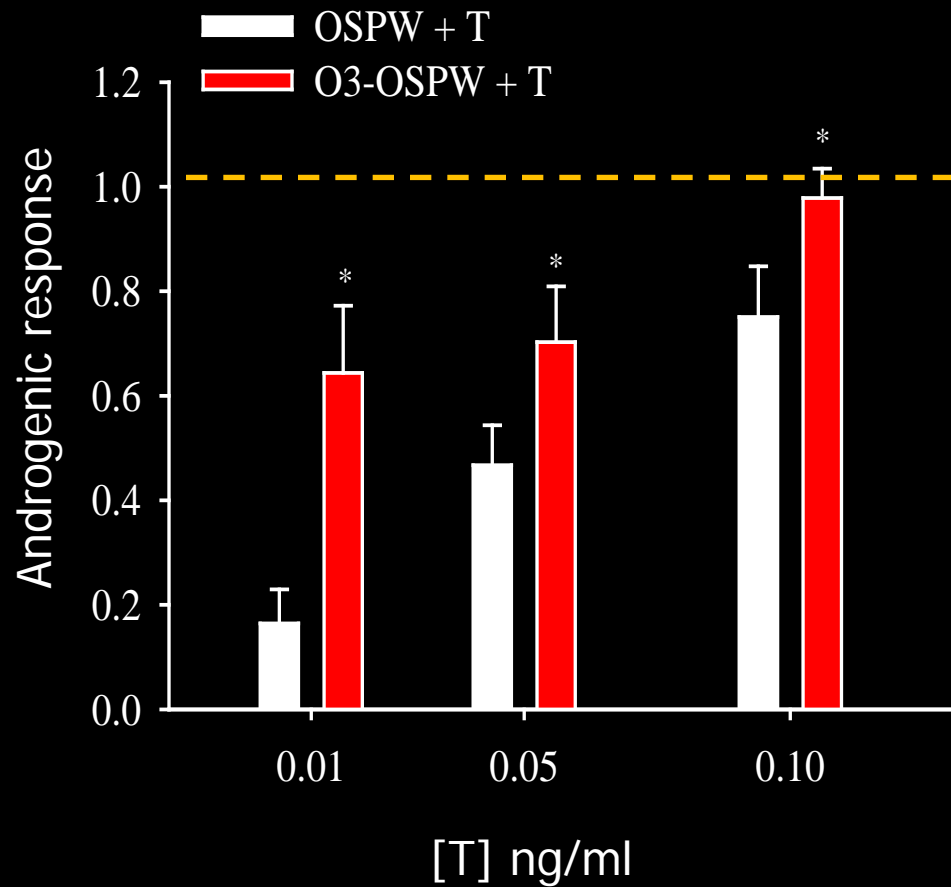
☐ Chemical(s) in OSPW and ozonated OSPW bind to the ER.

☐ Neither OSPW nor ozonated OSPW are anti-estrogenic.



Rowland et al., 2011

Hormone signaling – anti-androgenicity



❑ Neither OSPW nor ozonated OSPW are androgenic.

❑ OSPW and ozonated OSPW are anti-androgenic.

❑ Ozonation attenuated but did not abolish the anti-androgenicity.



Effects of OSPW and Ozonated OSPW on the Sex Steroid Synthesis and Signaling in Sexually Maturing Fathead Minnows



Sex Steroid Synthesis and Signaling

Hormone production

Hypothalamus



GnRH

Pituitary



LH / FSH

LH / FSH

Testes

Ovaries

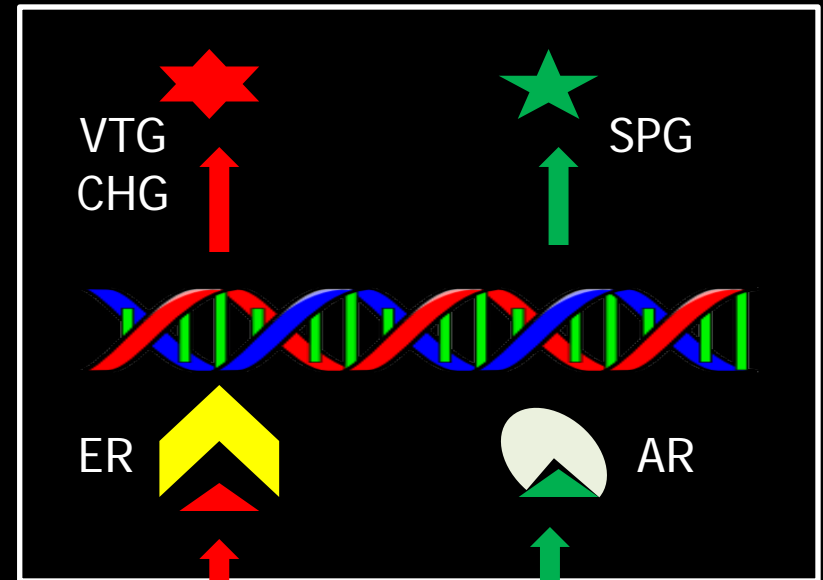


Estradiol

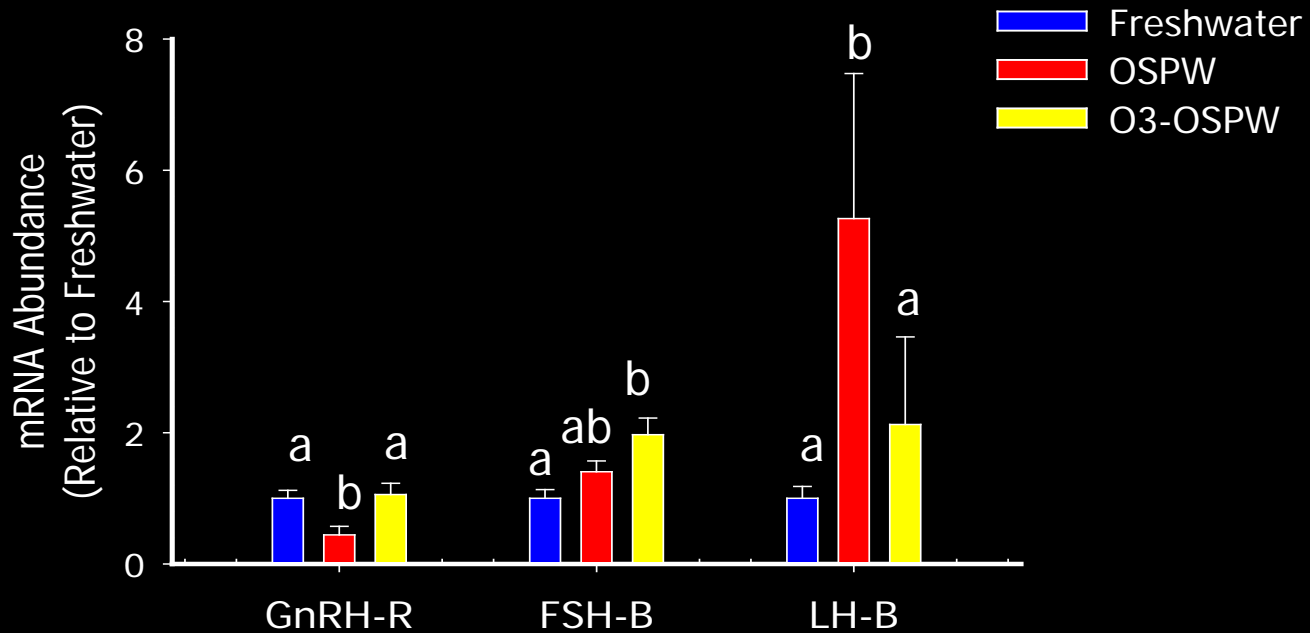
Testosterone

Hormone action

Liver



Pituitary - Females

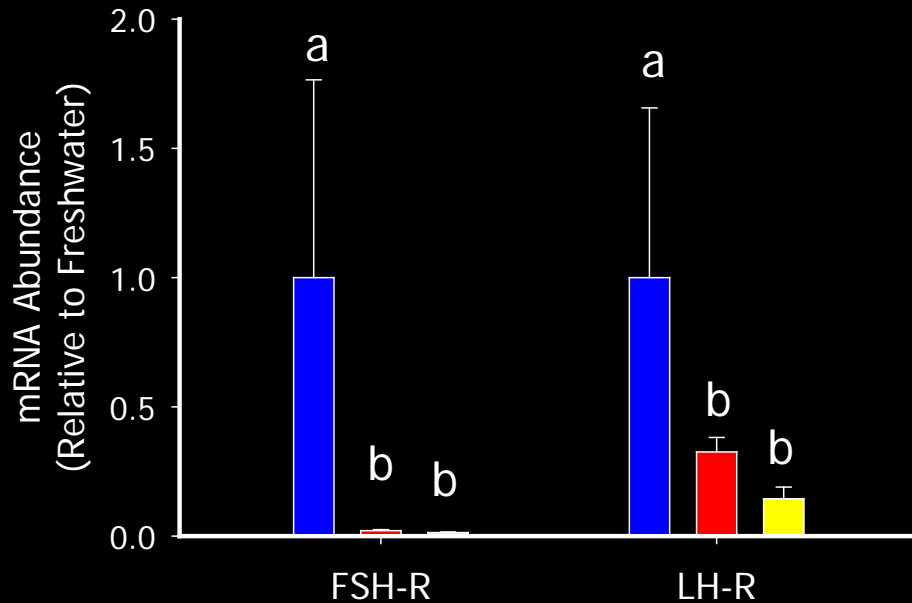


- Greater synthesis of LH but not FSH in OSPW.
 - LH promotes final oocyte maturation and stimulates E2 synthesis by ovaries
- Ozonation attenuates effects of OSPW on LH-B but stimulates FSH-B synthesis.

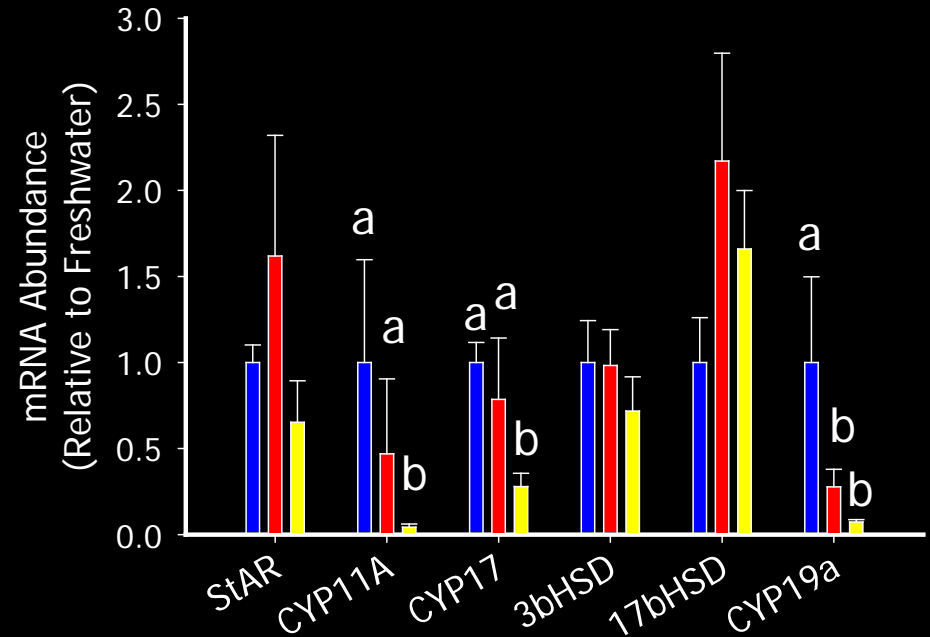
Gonad - Females



Gonadotropin Receptors



Steroidogenic Enzymes

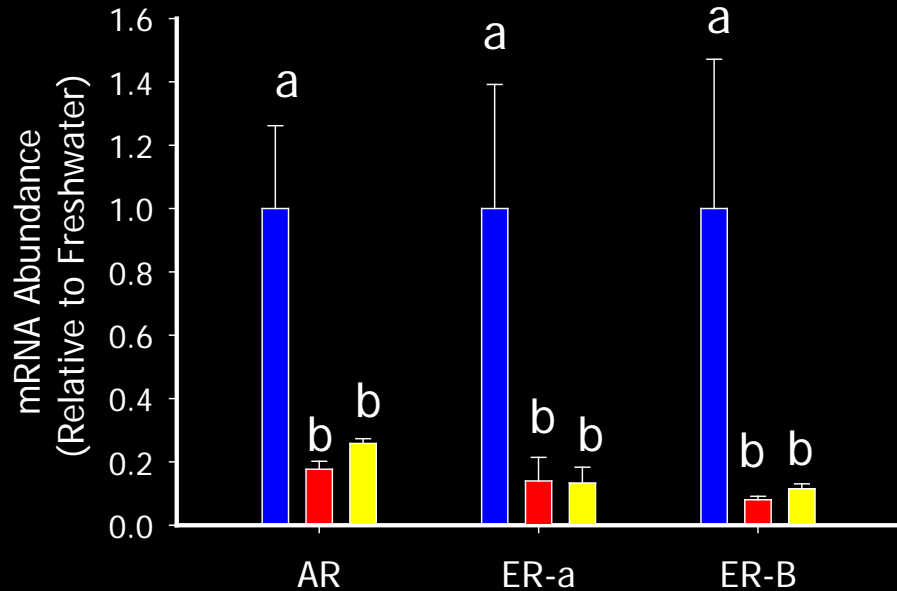


❑ Significantly less abundance of transcripts of FSH-R , LH-R and CYP19a (aromatase) in OSPW and ozonated OSPW
- could result in impaired synthesis of E2.

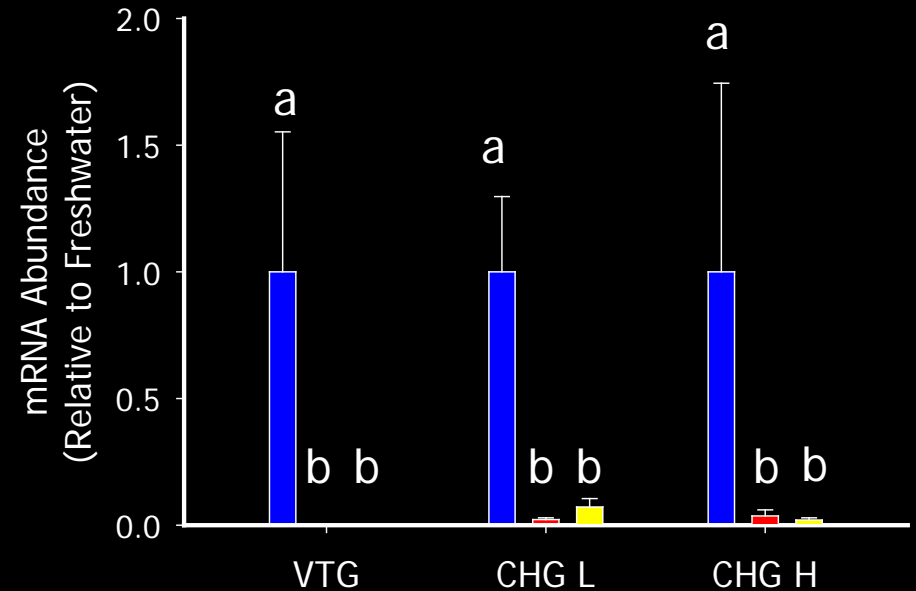
❑ Ozonation does not attenuate effects on transcript abundances.

Liver - Females

Sex Hormone Receptors



Egg Envelope Proteins

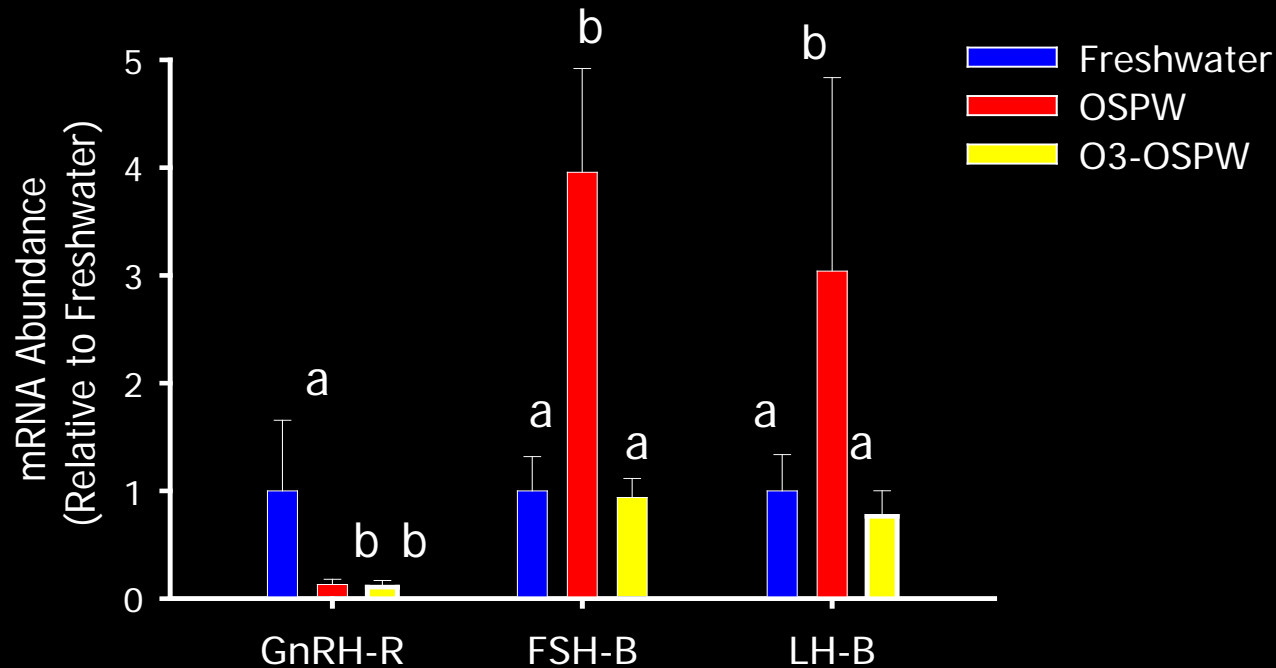


❑ Impaired E2 synthesis could lead to less ER expression.

❑ Alternatively, estrogen-like chemicals (naphthenic acids) in OSPW and ozonated OSPW could down regulate ER signaling pathways by negative feedback

❑ Could explain the decreased fecundity in female fathead minnows (Kavanagh et al., 2011).

Pituitary - Males

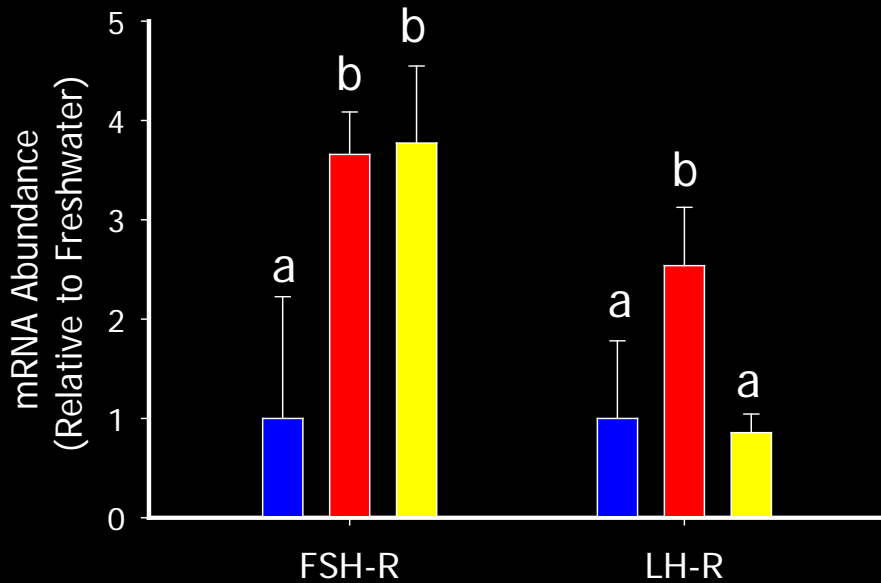


- Greater synthesis of FSH and LH in OSPW.
 - FSH promotes early maturation of testes.
 - LH stimulates final maturation of testes and spermatogenesis.
 - Both stimulate synthesis of T by testis.
- Ozonation of OSPW attenuates the effect of OSPW.

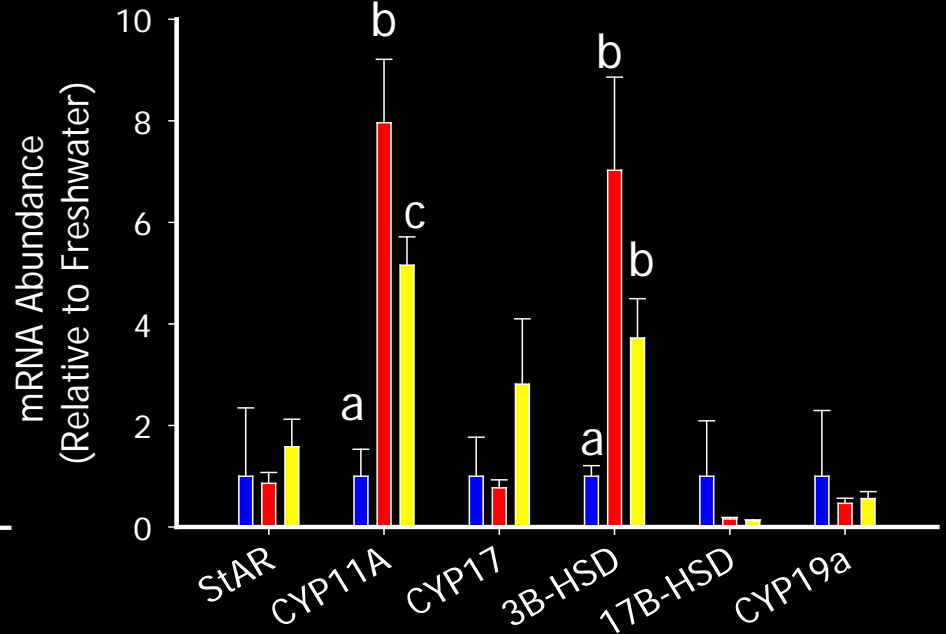
Gonad - Males

Freshwater
OSPW
O3-OSPW

Gonadotropin Receptors



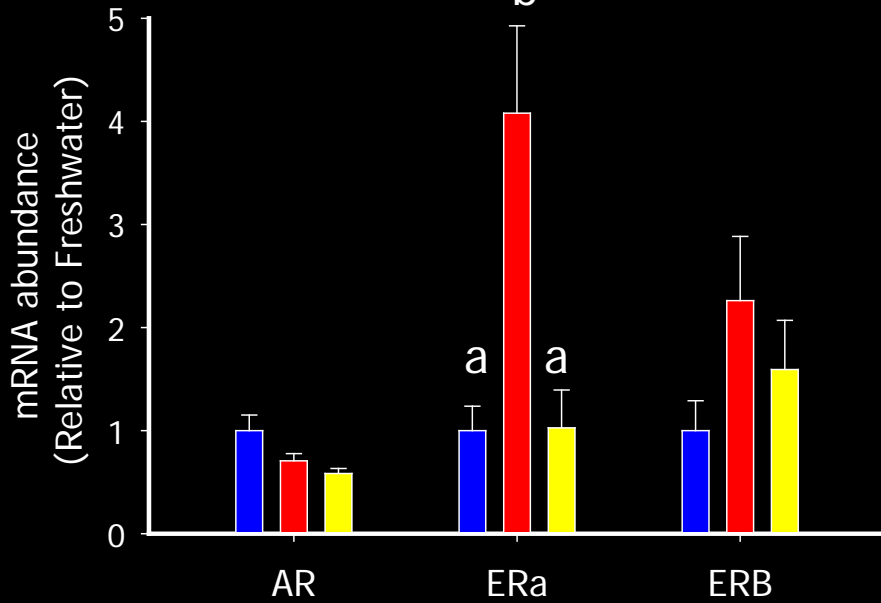
Steroidogenic Enzymes



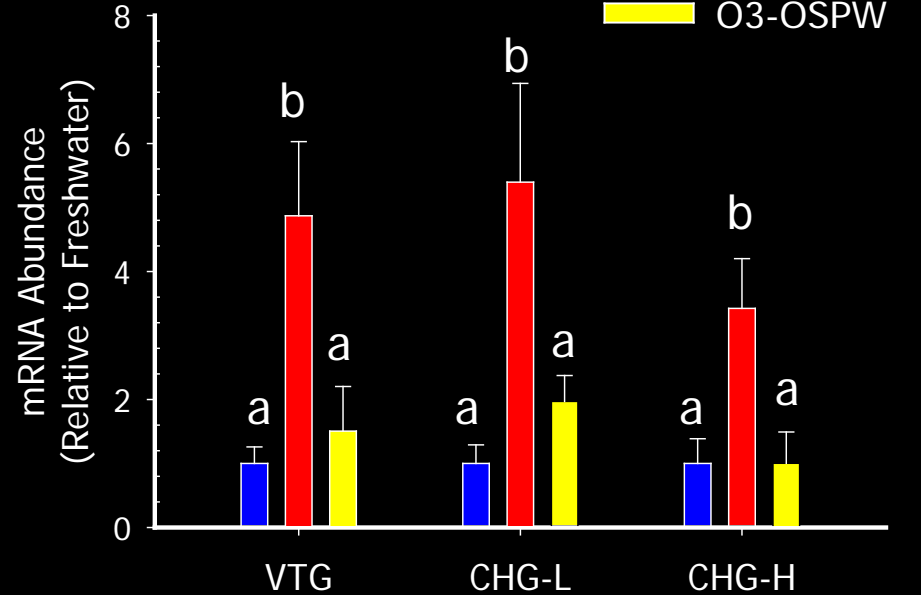
- Greater abundance of FSH-R and LH-R may be a response to greater FSH and LH Released from the pituitary in response to OSPW.
- Activation of FSH-R and LH-R may be stimulating greater sex steroid (T) production.
- Ozonation of OSPW partially attenuates effects of OSPW.

Liver - Males

Sex Hormone Receptors



Egg Envelope Proteins



Freshwater
OSPW
O3-OSPW

Greater abundance of ER-a transcripts could be due to greater concentrations of E2 secreted from ovaries or due to estrogenic chemicals in OSPW.

Effect of OSPW on VTG and CHG could be due direct stimulation of ER by either estrogenic compounds in OSPW (as shown in cell line reporter assay) or E2.

Could explain the less prominent male secondary sexual characteristics in male fathead minnows (Kavanagh et al., 2011).

- Could also be a result of anti-androgenic effects of OSPW.

Conclusions



- ❑ OSPW has endocrine disrupting effects on fathead minnows.
- ❑ OSPW has different effects in male and female fathead minnows.
 - effects may provide mechanistic basis for results from other studies.
- ❑ Endocrine disrupting effects appear to be at all levels of the HPG-L axis.
- ❑ Effects of ozonation on endocrine disrupting effects of OSPW less clear than in other studies.
 - Ozonation attenuates effects of OSPW on some endocrine endpoints but effect is more prominent in males than in females.



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