Endocrine Disrupting Effects of Untreated and Ozone-treated Oil Sands Process-affected Water (OSPW) in vivo and in vitro

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Background

• Large volumes of oil sands process-affected water (OSPW) are produced by the oil sands industry in Alberta, Canada.
• Overall more than 10^6 m^3 of OSPW is stored on-site.
• Naphthenic acids (NAs) are a primary toxic organic constituent of OSPW.
• Ozonation decreases concentrations of NAs and attenuates some of the adverse effects of OSPW.

Objectives

• Investigate the endocrine disrupting effects of OSPW and ozone-treated OSPW in vivo and in vitro by quantifying:
  • Sex steroid hormone synthesis in the H295R cell line
  • Gonadotropins and receptors: LHβ, FSHβ, ERα, ERβ, βHSD
  • Steroidogenesis: StAR, CYP11A, CYP17, CYP19a, CYP19b, 3βHSD, 17βHSD
  • Sex steroid receptor signaling: Kiss1R, GnRHR2, GnRHR1, FSHβ, FSHγ, LHβ, LHβ
  • Transcriptional profile of hypothalamic-pituitary-gonad-liver (HPGL) axis

Methods

OSPW

Fresh OSPW was collected from the West In Pit during 2007 and 2010. Concentrations of NAs in all OSPW samples were 19.7 mg/L as determined by RP-HPLC/HRMS.

Sex Steroid Hormone Synthesis

H295R cell line

• Concentrations of testosterone (T) & 17β-estradiol (E2) in medium were measured by ELISA.
• OSPW was subjected to ozonation under two treatment conditions – 30 mg/L and 80 mg/L.

Sex Steroid Hormone Receptor Signaling

MDA-kb2 cell line

• Anti-androgenic effects were determined by co-exposure to OSPW and T.

Results 1 – Effects on T & E2 Production in the H295R Cell Lines

Figure 2: Fold change of T (left) and E2 (right) production by H295R cells exposed to control, OSPW and ozone-treated OSPW. Asterisks indicate significant differences from control (p < 0.05, one-way ANOVA with Dunnett’s tests).

Results 2 – Effects on AR & ER Signaling in the MDA-kb2 and T47D-kblue Cell Lines

Figure 3: Anti-androgenic response in the MDA-kb2 cell line (left) and estrogenic response in the T47D-kblue cell line (right). Different letters indicate significant differences between treatments (p < 0.05, one-way ANOVA with Tukey’s test).

Results 3 – Effects on Gene Expression in HPGL Axis in Male & Female Fish

Figure 4: Abundances of transcripts of genes of the HPGL axis in adult male (upper panel) and female (lower panel) FHMs exposed to a freshwater control, OSPW, and ozone-treated OSPW. A) Brain, B) Gonad, C) Liver. Different letters indicate significant differences between treatments (p < 0.05, Kruskal-Wallis test).

Conclusion

• OSPW had endocrine disrupting effects on sex steroid synthesis, sex steroid receptor signalling, and expression of genes at all levels of HPGL axis in male and female FHMs.
• OSPW had different effects on male and female FHMs. Effects were more prominent in males than females.
• Ozonation attenuated effects of OSPW on certain endocrine endpoints.
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