Assessing effects of dissolved organic chemicals in OSPW by use of the fathead minnow reproductive bioassay

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INTRODUCTION

Oil Sands Process Affected Waters (OSPW):
- OSPW is a by-product of the extraction and separation of bitumen from oil sands in northern Alberta, Canada[1].
- Because OSPW has acute and chronic toxicity to a range of species[2-4], it is not released to the environment.
- The dissolved organic fraction of OSPW is responsible for acute toxicity[2-4] and is a super-complex mixture (>2190 empirical formulas detected by use of Orbitrap MS).
- An effects-directed analysis of the dissolved organic fraction identified acutely toxic chemical classes isolated to the fractions F3-NE2a (O₂-, naphthenic acid containing) and F3-NE2b (SO₄, O₂-, 50° and NO⁻ containing) as the most potent.[5,7]
- Some chemical species of the O₂- chemical class have been identified as bioaccumulative[5]
- Knowledge of the chronic toxicity of these chemicals remains unknown.

Objectives:
I. By use of the USEPA 21 day Fathead minnow reproductive assay, assess the endocrine disrupting potential of OSPW and samples of OSPW by assessing changes in reproductive pathology of fathead minnows (Pimephales promelas).
II. Compare reproductive measurements (no of eggs, fertilisation)
III. Compare morphological, histopathological, and molecular/biochemical responses of males and females.
IV. Assess maternal transfer of chemical classes identified by Zhang et al.[7]
V. Assess time to hatch of embryos collected on Day 7 and 14.

RESULTS

Figure 1.

Figure 2. Cumulative egg production of fathead minnows exposed to 25% (v/v) OSPW, 25% equ. (v/v) of F3-NE2a and F3-NE2b samples of OSPW. Cumulative number shown as eggs/female/day. Asterisk denote significant difference from other treatment groups (p<0.05; one-way ANOVA).

Figure 3. Percent fertilization of embryos collected during 21-day reproductive assay. Data presented as mean ± SEM. Asterisk denotes significant difference from control (p < 0.05, one-way ANOVA).

Figure 4. A) Condition factor, B) hepato-somatic (HSI), and C) gonado-somatic (GSI) indices of male and female fathead minnows after 21 days of exposure to 25% (v/v) OSPW, 25% equ. (v/v) F3-NE2a and F3-NE2b samples of OSPW. Data are shown as mean ± SEM. Asterisk denotes significant difference from control (p < 0.05, one-way ANOVA).

DISCUSSION

• There was no significant effect of OSPW or samples of OSPW on fathead minnow fecundity. Fertilization remained consistent regardless of treatment, demonstrating a lack of effect.
• Time to 50% hatch of embryos remained constant.
• A significant increase in HSI was observed in male fathead minnows exposed to 25% OSPW.
• A trend towards decreasing HSI was observed for females exposed to OSPW and samples of OSPW.
• A trend towards decreased GSI was observed for males exposed to OSPW and samples of OSPW.

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Table 1. Time to 50% hatch of embryos collected on day 7 and 14 of exposure (n=4). Data presented as mean ± SEM.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Control</th>
<th>S. Control</th>
<th>OSPW</th>
<th>F3-NE2a</th>
<th>F3-NE2b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 7</td>
<td>5.00 ± 0.35</td>
<td>4.33 ± 0.29</td>
<td>4.75 ± 0.25</td>
<td>4.00 ± 0.35</td>
<td>4.8 ± 0.63</td>
</tr>
<tr>
<td>Day 14</td>
<td>5.25 ± 0.29</td>
<td>5.25 ± 0.29</td>
<td>4.75 ± 0.25</td>
<td>4.00 ± 0.35</td>
<td>4.8 ± 0.63</td>
</tr>
</tbody>
</table>

MATERIALS AND METHODS

The study was conducted at the Aquatic Toxicology Research Facility in the Toxicology Centre, University of Saskatchewan.

- Fish were acclimated in a static renewal system for a one month pre-exposure period to establish baseline fecundity and fertility data.
- Tanks producing a minimum 10 eggs/female/day were selected for exposure
- OSPW was collected from a permanent platform on Base Mine Lake in June, 2015.
- Fractionation of OSPW was conducted by the research group of Dr. Jon Martin.
- An initial acute toxicity study was conducted to determine survival of fish at chosen concentrations:
  - Control, Solvent Control (S. Control), 0.00% EtOH), 25% (v/v) OSPW, 25% (v/v) equ F3-NE2a, and F3-NE2b.
  - Fish (1 Male and 2 Female, n=4) were exposed for 21 days to samples (50% static renewal).
  - Control, S. Control, 25% OSPW and 25% equ. (v/v) F3-NE2a and F3-NE2b
- Endpoints measured:
  - Fecundity and fertility (daily egg collection)
  - Collected eggs were snap frozen and collected for analysis of maternal transfer at a later date
  - Eggs (n=10) were collected on Day 7 and 14 for time to hatch assessment
  - Somatic indices (liver and gonad), tubercle score, and condition factor
  - Plasma, liver, brain, gill and gonad tissue were taken for future histological, molecular, and biochemical analysis.

Figure 1. A) Experimental set-up, B) Time to hatch C) male fathead minnow and D) female fathead minnow.

Figure 2. Cumulative egg production of fathead minnows exposed to 25% (v/v) OSPW, 25% equ. (v/v) of F3-NE2a and F3-NE2b samples of OSPW. Cumulative number shown as eggs/female/day. Asterisk denote significant difference from other treatment groups (p<0.05; one-way ANOVA).

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Figure 5. Cumulative no. of eggs/female/day.

Figure 6. Gonado-somatic index (%).

Figure 7. Condition factor.

Figure 8. Hepato-somatic index (%).

Figure 9. Gonado-somatic index (%).

Figure 10. Cumulative no. of eggs/female/day.

Figure 11. Gonado-somatic index (%).

Figure 12. Condition factor.

Figure 13. Hepato-somatic index (%).

Figure 14. Gonado-somatic index (%).

Figure 15. Cumulative no. of eggs/female/day.

Figure 16. Gonado-somatic index (%).

Figure 17. Condition factor.

Figure 18. Hepato-somatic index (%).

Figure 19. Gonado-somatic index (%).

Figure 20. Cumulative no. of eggs/female/day.