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Endocrine disruption and oxidative stress in larvae of *Chironomus dilutus* following short-term exposure to fresh or aged oil sands process-affected water

S.B. Wiseman[a,*,1], J.C. Anderson[a,1], K. Liber[a,b], J.P. Giesy[a,c,d]

[a] Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada
[b] School of Environment and Sustainability, University of Saskatchewan, Saskatoon, SK, Canada
[c] Department of Veterinary Biomedical Sciences, University of Saskatchewan, Saskatoon, SK, Canada
[d] Department of Biology and Chemistry and State Key Laboratory in Marine Pollution, City University of Hong Kong, Kowloon, Hong Kong Special Administrative Region

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ABSTRACT

Understanding the toxicity of oil sands process-affected water (OSPW) is a significant issue associated with the production of oil from the Alberta oil sands. OSPW is acutely and chronically toxic to organisms, including larvae of *Chironomus dilutus*. In this study, fresh OSPW ('WIP-OSPW') was collected from the West In-Pit settling pond and aged OSPW ('FE5-OSPW') was collected from the FE5 experimental reclamation pond, both of which are located on the Syncrude Canada Ltd. lease site near Fort McMurray, Alberta, Canada. Larvae of *C. dilutus* were exposed to a freshwater control, WIP-OSPW, or FE5-OSPW for 4 or 7 days and survival, growth, and markers of oxidative stress and endocrine disruption were assessed. Survival was not significantly different among treatment groups. Compared to masses of larvae exposed to freshwater, masses of larvae exposed to WIP-OSPW were 49% lesser on day 4 and 62% lesser on day 7. However, organisms exposed to FE5-OSPW did not have significantly lesser masses than controls. Abundances of transcripts of glutathione-s-transferase (*gst*), catalase (*cat*), and glutathione peroxidase (*gpx*), which are important for the response to oxidative stress, were significantly altered in larvae exposed to WIP-OSPW, but not FE5-OSPW, relative to controls. Peroxidation of lipids was greater in larvae exposed to WIP-OSPW, but not FE5-OSPW. Exposure to fresh OSPW might have caused endocrine disruption because abundances of transcripts of the steroid hormone receptors, ultraspiricle protein (*usp*), ecysteroid receptor (*esr*), and estrogen related receptor (*err*) were greater in larvae exposed to WIP-OSPW for 7 days, but not FE5-OSPW. These results suggest that lesser growth of larvae of *C. dilutus* exposed to fresh OSPW might be due to oxidative stress and disruption of endocrine processes, and that aging of OSPW attenuates these adverse effects.

1. Introduction

Global energy demands are expected to increase by 50% by the year 2030, mostly within non-OECD countries (National Energy Board of Canada, 2010). Although the majority of current energy needs are met by fossil fuels from conventional production, emphasis is shifting toward alternative sources of oil, such as oil sands (Government of Alberta, 2008). One of the largest reserves of petroleum hydrocarbons in the world is found in Canada within three primary deposits of bitumen in northern Alberta (Government of Alberta, 2006; Veil et al., 2009). Bitumen is extracted from surface-mined oil sands by use of the Clark hot water process. This results in saline, alkaline process water known as oil sands process-affected water (OSPW; Rogers et al., 2002; Hao et al., 2005). Because companies are currently held to a policy of zero-discharge to surrounding freshwater environments, OSPW is held in active settling basins where fine silts and clays slowly densify to form mature fine tailings. Greater than 1 billion m^3 of OSPW is stored on-site of several companies operating in this region and because this volume is increasing it represents a considerable management responsibility for the oil sands industry and expense for future reclamation (Del Rio et al., 2006; Veil et al., 2009).

The dissolved organic fraction of OSPW is the major driver of acute and chronic toxicity of OSPW (Anderson et al., 2012a, 2012b;

* Corresponding author at: University of Saskatchewan, Toxicology Centre, 44 Campus Drive, Saskatoon, SK, Canada S7N 5B3. Tel.: +1 306 966 4912; fax: +1 306 970 4796.
E-mail address: steve.wiseman@usask.ca (S.B. Wiseman).
[1] These authors contributed equally to this work.

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He et al., 2012a). The dissolved organic fraction is a complex mixture of compounds, including naphthenic acids (NAs) which are cyclic and alkyl-substituted aliphatic carboxylic acids with the formula $C_nH_{2n+z}O_2$, where n is the number of carbon atoms and z is zero or an even negative integer denoting the number of hydrogen atoms that are absent because of the presence of rings or double bonds (Holowenko et al., 2002; Headley and McMartin, 2004). In addition, numerous organic compounds containing oxygen, nitrogen, or sulfur have been identified in OSPW (Barrow et al., 2010; Pereira et al., 2013). Over time, concentrations of organic compounds dissolved in OSPW are depleted to some extent by biodegradation and this results in lesser acute toxicity of aged OSPW (Del Rio et al., 2006; Han et al., 2009).

Critical mechanisms of toxicity of OSPW are not known. Because NAs have properties of surfactants, it has been proposed that acute toxicity of OSPW is due to narcosis (Roberts, 1991; Scarlett et al., 2013; Frank et al., 2009). However, this mechanism of toxicity has not been demonstrated. Several studies have provided evidence that exposure to OSPW might exert toxicity by oxidative stress (He et al., 2012a; Wiseman et al., 2013; Gagné et al., 2013). Other studies have shown that OSPW has endocrine disrupting effects in vitro (He et al., 2010, 2011) and in vivo (Van den Heuvel et al., 2012; Lister et al., 2008; Kavanagh et al., 2011, 2012, 2013; He et al., 2012b; Reinardyn et al., 2013). These effects of OSPW have been elucidated primarily from studies of fishes. Midges of the genus *Chironomus* (class Insecta) comprise a large proportion of the biomass found within aquatic ecosystems of the Athabasca oil sands region (Bendell-Young et al., 2000), which makes their larvae potentially important targets of toxic effects of OSPW. With the exception of studies showing that exposure to OSPW impairs growth and development of larvae of the non-biting midge, *Chironomus dilutus*, there is little known about adverse effects of OSPW on insects, and nothing is known about the associated mechanisms of effect(s) (Anderson et al., 2012a, 2012b). Therefore, the goal of the current study was to investigate whether oxidative stress or disruption of endocrine processes might play a role in the impaired growth and development of larvae of *C. dilutus*. By understanding the mechanism of action, it would be possible to conduct more appropriate assessments of risk, and develop both biomarkers of exposure and methods of treatment for remediation and restoration of OSPW.

2. Materials and methods

2.1. Test organisms

To obtain larvae for the exposure study, adults of *C. dilutus* from an in-house laboratory culture (Toxicology Centre, University of Saskatchewan) were collected and bred. Resulting egg masses were placed into 15-L aquaria maintained at $23 \pm 1\,^{\circ}C$ and raised to test age in an environmental chamber with a 16:8 h light:dark photoperiod. During rearing, larvae were kept in control freshwater, which was Saskatoon, SK, municipal water that was bio-filtered, carbon-filtered, and aerated for 24 h prior to placement into aquaria or test vessels. The characteristics of the control freshwater are provided in Table 1.

2.2. Exposure waters

Larvae of *C. dilutus* were exposed to one of three treatment waters: (1) freshwater control, (2) untreated fresh OSPW (designated 'WIP-OSPW'), or (3) aged OSPW (designated 'FE5-OSPW'). The freshwater for the control exposure was the same as that used for culturing *C. dilutus*. Both samples of OSPW were collected from sites located on the Syncrude Canada Ltd. lease site (near Fort McMurray, AB). The WIP-OSPW was collected in summer 2009 from West In-Pit (WIP). At the time of collection, the WIP was an active settling basin that was regularly fed from the main extraction plant and represented relatively fresh, untreated process water (as described by Han et al., 2009). The FE5-OSPW was collected in summer 2009 from an experimental reclamation pond comprised of mature fine tailings capped with OSPW in 1989 and represents OSPW that has been subjected to some degree of natural biodegradation (Han et al., 2009). The total concentration of NAs in WIP-OSPW was 71.7 mg/L and the total concentration of NAs in FE5-OSPW was 13.0 mg/L, as determined by Fourier transform infrared spectroscopy (FTIR; Anderson et al., 2012b). Although the FTIR method is not specific for NAs, there is a good correlation between concentrations of NAs measured by this method and concentrations of NAs measured by high pressure liquid chromatography/high-resolution mass spectrometry (HPLC/HRMS; Han et al., 2009). The WIP-OSPW and FE5-OSPW were stored in the dark at $4\,^{\circ}C$ from the time of collection until they were used in the exposure studies, which were performed in spring 2010. Characteristics of the WIP-OSPW and FE5-OSPW are provided in Table 1.

2.3. Experimental design

The study was conducted under the environmental conditions described in Section 2.1. A static-renewal assay was used to assess effects of acute exposure to the treatment waters. Ten 9 to 10-day post-hatch larvae were randomly assigned to each of six replicate 300-mL, tall-form beakers per treatment group per time-point (4 and 7 days). Approximately equal-sized larvae were randomly placed into each replicate beaker. The substratum in each beaker was comprised of 30 g of clean silica sand (particle size 200–400 μm). The initial mean wet mass was 1.17 ± 0.29 mg per individual, based on a representative subsample of 30 larvae collected on day 0. Over the course of the exposure period, larvae were fed 0.67 mg dry weight TetraFin® fish food (Tetra Company, Blacksburg, VA) per individual per day and 50% of the water volume was replaced every 2 days. Beakers were continually aerated to maintain concentrations of dissolved oxygen (DO) near 7 mg/L. Different subgroups of beakers, including at least one representative beaker from each treatment, were sampled each day to measure oxygen and temperature using an Orion 3-Star RDO Portable Meter and Probe (Thermo Fisher Scientific Inc., Nepean, ON). Water samples for analysis of conductivity, pH, dissolved oxygen, and total ammonia were collected from each beaker on days 0, 4, and 7. Values for each indicator of water quality on day 4 and day 7 did not deviate significantly from day 0. Larvae were sampled from each of the six beakers per treatment group after 4 and 7 days of exposure. Upon

Table 1
Measured water chemistry parameters for WIP-OSPW or FE5-OSPW over the duration of the exposure period. Values are presented as mean ± SD.

Water chemistry parameter					
Treatment water	NAs (mg/L)	pH	Conductivity (μS/cm)	DO (mg/L)	Ammonia (mg/L)
Freshwater	1.2 ± 0.1	7.9 ± 0.2	422 ± 41	6.7 ± 1.7	<1.0
FE5-OSPW	13 ± 3.0	8.9 ± 0.1	2790 ± 111	7.6 ± 1.0	<1.0
WIP-OSPW	72 ± 0.5	8.9 ± 0.3	3702 ± 232	7.2 ± 1.4	<1.0

Note: NA, naphthenic acids; DO, dissolved oxygen.