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Review

The toxicity of oil sands process-affected water (OSPW): A critical review

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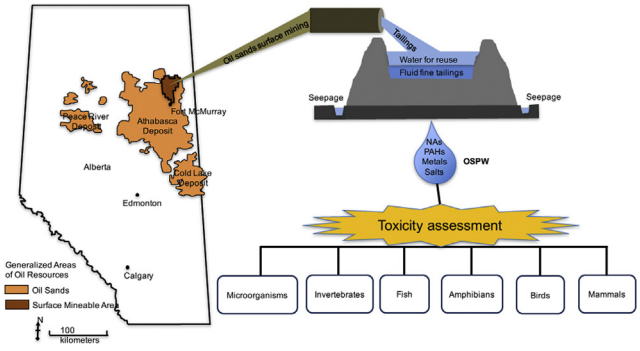
HIGHLIGHTS

- OSPW causes many physiological changes in a variety of organisms.
- OSPW toxicity depends on water chemistry, exposure duration and test organisms.
- Chemical compositions of OSPW from different tailing ponds are variable.
- NAs as well as other chemical constituents contribute to the overall toxicity of OSPW.

GRAPHICAL ABSTRACT



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ABSTRACT

Large volumes of oil sands process-affected water (OSPW) are produced by the surface-mining oil sands industry in Alberta. Both laboratory and field studies have demonstrated that the exposure to OSPW leads to many physiological changes in a variety of organisms. Adverse effects include compromised immunological function, developmental delays, impaired reproduction, disrupted endocrine system, and higher prevalence of tissue-specific pathological manifestations. The composition of OSPW varies with several factors such as ore sources, mining process, and tailings management practices. Differences in water characteristics have confounded interpretation or comparison of OSPW toxicity across studies. Research on individual fractions extracted from OSPW has helped identify some target pollutants. Naphthenic acids (NAs) are considered as the major toxic components in OSPW, exhibiting toxic effects through multiple modes of action including narcosis and endocrine disruption. Other pollutants, like polycyclic aromatic hydrocarbons (PAHs), metals, and ions may also contribute to the overall OSPW toxicity. Studies have been conducted on OSPW as a whole complex effluent mixture, with consideration of the presence of unidentified components, and the interactions (potential synergistic or antagonistic reactions) among chemicals. This review summarizes the toxicological data derived from *in vitro* and *in vivo* exposure studies using different OSPW types, and different taxa of organisms. In general, toxicity of OSPW was found to be dependent on the OSPW type and concentration, duration of exposures (acute *versus* sub chronic), and organism studied.

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Contents

1. Introduction

The oil sands in northern Alberta, have the third largest oil reserves in the world (after Venezuela and Saudi Arabia), containing an estimated 2.5 trillion barrels of recoverable bitumen (a heavy and viscous form of crude oil) (Penner and Foght, 2010; Jiang et al., 2016). There are three major oil sands deposits in northern Alberta including Athabasca, Cold Lake and Peace River, covering a surface area of >140,000 km^2 (Fig. 1) (Allen, 2008; Honarvar et al., 2011). The bitumen production by the Alberta's oil sands industry has reached 2.15 million barrels/day (bpd) in 2014, and is estimated to grow to 3.95 million bpd by 2030 (CAPP, 2015). The modified Clark water extraction process is commonly used to separate bitumen from oil sands in surface mining (Allen, 2008; Gao et al., 2010). The detailed description of the Clark extraction process, and the resulting tailings streams (a mixture of water, solids and unrecovered bitumen) can be found in previous publications (Allen, 2008; Mahaffey and Dube, 2016).

Fig. 1. Major oil sands areas in Alberta, Canada.
Source: Alberta Energy Regulator and Alberta Geological Survey.

Any water that has been in contact with oil sands is referred to as oil sands process-affected water (OSPW) (Natural Resources Canada-Canmet ENERGY, 2010). This broad definition encompasses a variety of water types, including fresh OSPW that is retained in active settling basins or tailings ponds, consolidated tailings (CT) released water that is released after treatment of fine tailings with chemical/physical separation techniques, seepage or dyke drainage water collected from surrounding the active settling basins, and aged or treated OSPW from wetlands and reclamation ponds (Mahaffey and Dube, 2016). Consequently, there is significant variability in the chemical composition of different OSPWs. Despite the differences in water chemistry, OSPW always contains several major classes of contaminants including: naphthenic acids (NAs), polycyclic aromatic hydrocarbons (PAHs), BTEX (benzene, toluene, ethyl benzene, and xylenes), phenols, heavy metals and ions (Allen, 2008; Puttaswamy and Liber, 2012; van den Heuvel et al., 2012; Mahaffey and Dube, 2016). NAs are postulated to be the primary source of OSPW toxicity in the early stages of investigating the OSPW toxicity (Madill et al., 2001; MacKinnon and Boerger, 1986; Allen, 2008; Clemente and Fedorak, 2005). Recent studies demonstrate that NAs account for <50% of all the compounds in the OSPW organic fraction (OSPW-OF) (Headley et al., 2009; Grewer et al., 2010; Garcia-Garcia et al., 2011a, 2011b; Garcia-Garcia et al., 2012; Hagen et al., 2012; Hagen et al., 2013). In addition to NAs, PAHs and other organic species, dissolved ions, and heavy metals may also contribute to the overall OSPW toxicity (Alharbi et al., 2016b; Garcia-Garcia et al., 2011a, 2011b; Garcia-Garcia et al., 2012; Kavanagh et al., 2012; Sansom et al., 2012; Leclair et al., 2013; Morandi et al., 2015).

Due to the complexity of OSPW, it is difficult to specifically assess the toxic effects of individual constituent(s). Consequently, most of the studies to date have examined the toxic effects using whole OSPW or its complex fractions (*i.e.*, organic fraction, NAs fraction). However, in the majority of studies, the OSPW types and sources are not often detailed. The inconsistent terminology and variance in different OSPW types have confounded comparisons of toxic effects induced by OSPW. In this review, a detailed summary of the exposures and endpoints for various organisms (*i.e.*, microorganisms, invertebrates, fish, amphibians, birds, and mammals) exposed to different OSPW types is provided.

2. Toxicity of OSPW

OSPW is toxic to both prokaryotes and eukaryotes. The main contributors of this toxicity appear to be the acid-extractable organic

compounds, known as NAs (Madill et al., 2001; MacKinnon and Boerger, 1986; Allen, 2008; Clemente and Fedorak, 2005). This organic fraction is comprised of NAs with an empirical formula $C_nH_{2n+z}O_2$, oxy-NAs ($C_nH_{2n+z}O_x$, x = 3–5), nitrogen and sulfur-containing species, and other organic acids (Kannel and Gan, 2012). The identification and synthesis of individual, structurally representative NA in OSPW (Rowland et al., 2011a, 2011b, 2011c; Bowman et al., 2014; Wilde and Rowland, 2015; Wilde et al., 2015) has enabled research on the relationships between structure and toxicity (Jones et al., 2011; Scarlett et al., 2012). NAs concentrations vary between ponds, ranging from ~20 to 80 mg/L in fresh settling basins (as reviewed by Mahaffey and Dube, 2016), and ~5 to 40 mg/L in reclamation ponds or experimental wetlands (Anderson et al., 2012; Hagen et al., 2013; Hersikorn et al., 2010; Kavanagh et al., 2013, 2011). Significant research efforts have focused on evaluation of NAs toxicity using OSPW NAs extracts (NAEs), although other organics are also present in this complex mixture. Multiple physiological changes have been caused by exposure to NAEs, including impaired reproduction, developmental delays, suppressed immune response, and histological alterations in aquatic species and mammals (Garcia-Garcia et al., 2011a, 2012; Kavanagh et al., 2012; Marentette et al., 2015a, 2015b; Nero et al., 2006a; Rogers et al., 2002; Rogers, 2003), suggesting that removal NAs from OSPW, would result in decreased toxicity.

The potential toxic effects caused by other constituents in OSPW (*e.g.*, PAHs, dissolved ions, heavy metals, *etc.*) have not received much attention. While specific information on the toxicity of these compounds present in OSPW is limited, they have been associated with a wide range of biological dysfunctions in exposed organisms, such as mutagenicity, carcinogenicity, immunotoxicity, and endocrine disruption (as reviewed by Li et al., 2014). It is probable that given the extreme complexity of OSPW composition, possible synergistic or antagonistic chemical interactions may be responsible for observed toxicity. For example, studies have demonstrated that the addition of salts decreased the NAEs toxicity in fish (Nero et al., 2006a; Kavanagh et al., 2012). However, this reduction in toxicity was likely achieved by decreasing toxicant entry (*i.e.*, NAs) through a reduced gill surface area caused by salts addition, which could also impact the efficiency of gas exchange and might lead to long-term issues in terms of fish health (Nero et al., 2006a).

2.1. Assessment of OSPW toxicity using in vitro assays

Various *in vitro* model systems (*e.g.*, bacteria, immortalized cell lines, primary isolated cells, *etc.*) have been used to investigate the potential adverse effects of OSPW (Table 1). The *in vitro* toxicity is commonly demonstrated based on IC_{50} or IC_{20} values; concentrations causing 50% or 20% inhibition of a specific biological or biochemical function. IC_{50} (24%–67%) and IC_{20} (9%–33%) values (v/v) of fresh OSPW towards the marine bacterium *Vibrio fischeri* have been reported (Gamal El-Din et al., 2011; Holowenko et al., 2002; Scott et al., 2008; Wang et al., 2013; Zubot et al., 2012). The toxic effects were reduced following *in-situ* biodegradation, as shown by increased IC_{50} (64%–100%) and IC_{20} (11%–100%) values (v/v) of aged OSPW obtained from remediation ponds and wetlands (Holowenko et al., 2002). The reported amelioration of OSPW acute toxicity might be due to the altered structure and composition of NAs over time. Indigenous microbial populations seem to preferentially degrade NAs with <22 carbons, resulting in the increased proportions of C_{22+} with OSPW aging (Biryukova et al., 2007; Clemente et al., 2004; Quagraine et al., 2005). Similarly, NAs with higher molecular weight (MW) were shown to be less toxic to *V. fischeri* (Frank et al., 2008). These observations seemed to contradict the concept that, for typical NAs structures, the larger MW compounds have a greater hydrophobicity, are more likely to bioaccumulate, and thus should exhibit stronger narcotic potency (Frank et al., 2009a). However, Frank et al. (2009a, 2009b) hypothesized that the reduced toxicity of higher MW NAs was due to the presence of higher NAs —COOH content which decreased hydrophobicity and, consequently, reduced toxic effects.

The endocrine disruptive properties of fresh OSPW have been reported *in vitro*, shown by estrogenic and antiandrogenic effects observed in T47D-kbluc and MDA-kb2 cells, respectively, (He et al., 2011), as well as elevated 17β-estradiol (E2) and decreased testosterone (T) concentrations produced by H295R cells (He et al., 2010). OSPW has also been shown to induce immunotoxicity. The OSPW organic fraction (OSPW-OF) was reported to cause a dose-dependent decrease in nitric oxide and respiratory burst responses of mouse bone marrow-derived macrophages (BMDM), as well as suppressed phagocytosis (Garcia-Garcia et al., 2011b). Recently, the *in vitro* exposures of fish hepatocytes to OSPW and OSPW acid-extractable organics (OSPW-AEOs) were reported to induce genotoxic and mutagenic changes in the cells (Lacaze et al., 2014; Zetouni et al., 2016). That *in vivo* exposure to OSPW constituents, such as diamondoid naphthenic acids, can cause genetic damage in gills and haemocytes of marine mussels has also been reported (Dissanayake et al., 2016).

In vitro tests have become common approaches for evaluating OSPW toxicity due to their low cost, simplicity, high capacity, and reproducibility. These characteristics make them effective screening tools to quickly identify the priority pollutants in OSPW, and to evaluate the effectiveness of wastewater treatment methods. The bioluminescence is the endpoint of the Microtox assay using *V. fischeri*. It is a direct measure of narcosis (membrane disruption) that is hypothesized to be the primary mode of action for acute toxicity of NAs (Frank et al., 2008; Frank et al., 2009b). However, *V. fischeri* is a marine organism that is not an appropriate model to predict effects on freshwater species. The use of hormone-responsive cell lines and primary cells isolated from animal tissues have revealed more specific mechanisms (endocrine disrupting effects and immunotoxicity) affected by OSPW; however, the abnormal functions in cancerous cell lines, and the absence of biokinetics in *in vitro* models might lead to a misinterpretation of the data when extrapolated to the potential effects at the organismal level (Saeidnia et al., 2015). Therefore, interpretation and reporting of the effects from *in vitro* experiments requires careful consideration, and appropriate follow-up *in vivo* studies to assess whether the toxic effects observed *in vitro* are reproducible *in vivo* are needed.

2.2. Assessment of OSPW toxicity using animal models

2.2.1. Toxicity of OSPW in invertebrates

The lethal effects of fresh OSPW exposure of invertebrates are shown in Table 2. Early research demonstrated acute toxicity of fresh OSPW from Mildred Lake Settling Basin (MLSB, Syncrude Canada Ltd.) towards *Daphnia magna* and *Daphnia pulex*, with low 96 h-LC_{50} (the median lethal concentration) values of 10% (v/v) and 2%–10% (v/v), respectively (MacKinnon, 1986; MacKinnon and Boerger, 1986; MacKinnon and Retallack, 1982). A recent report indicated 48 h-LC_{50} value of >100% (v/v) for fresh OSPW from West In Pit (WIP) settling basin (Syncrude Canada Ltd.) for *D. magna* (Zubot et al., 2012). This low acute lethality of *D. magna* was also reported by Lari et al. (2016), however, exposure to sub-lethal concentrations of OSPW (1%) impaired feeding, growth, and reproduction of *D. magna*, which may threaten their survival (Lari et al., 2016). Results of studies using *Ceriodaphnia* bioassays indicated both acute (survival, 6-d LC_{50} of 65%), and chronic (fecundity, 6-d IC_{50} of >39%) toxicity for fresh OSPW (Zubot et al., 2012). It was suggested that OSPW salinity affected the ability of *Ceriodaphnia* to reproduce (Zubot et al., 2012). Multiple toxic effects of fresh OSPW were observed using *Chironomus dilutus* larvae, including reduced body mass, lower pupation levels, decreased rates of emergence, abnormal behavior, oxidative stress responses, and altered endocrine signaling (Anderson et al., 2012; Wiseman et al., 2013a). The suppressed larval growth might be due to oxidative stress and disruption of endocrine processes, as suggested by the changes in relevant gene expression (Wiseman et al., 2013a). While NAs were correlated strongly with toxic endpoints, it

Table 1
Studies demonstrating *in vitro* toxicity of OSPW.

Sample designation	OSPW type	Test organism	Duration of exposure	Endpoint & result	Reference
MLSB	Fresh OSPW	*Vibrio fischeri*	15 min	IC_{50}: 32%	(Holowenko et al., 2002)
Recycle water pond (Syncrude)	Fresh OSPW	*Vibrio fischeri*	15 min	IC_{50}: 100%; IC_{20}: 23%	(Scott et al., 2008)
WIP	Fresh OSPW	*Vibrio fischeri*	5 min	IC_{50}: 24%	(Gamal El-Din et al., 2011)
WIP	Fresh OSPW	*Vibrio fischeri*	15 min	IC_{20}: 9%; IC_{50}: 67%	(Zubot et al., 2012)
WIP	Fresh OSPW	*Vibrio fischeri*	15 min	IC_{20}: 32.6%	(Wang et al., 2013)
WIP	Fresh OSPW	H295R cell line	1 h (serial dilution)	E2 metabolism (−1.2-fold for 1% OSPW; −1.4-fold for 90% OSPW; −2.3-fold for 100% OSPW)	(He et al., 2010)
WIP	Fresh OSPW	H295R cell line	2, 4, 8 h (100% OSPW)	cyp19a mRNA expression (+1.8-fold, 2.0-fold, and 3-fold after 2 h, 4 h, and 8 h, respectively)	(He et al., 2010)
WIP	Fresh OSPW	H295R cell line	24 h (serial dilution)	Aromatase activity (+1.9-fold for 90% OSPW; + 2.5-fold for 100% OSPW)	(He et al., 2010)
WIP	Fresh OSPW	H295R cell line	48 h (100% OSPW)	T production (−0.45-fold); E2 production (+2-fold)	(He et al., 2010)
WIP	Fresh OSPW	T47D-kbluc cell line	24 h (serial dilution)	Estrogenic response (proportional to concentrations; +2.58 for 100% OSPW)	(He et al., 2011)
WIP	Fresh OSPW	MDA-kb2 cell line	24 h (100% OSPW)	Antiandrogenic response coexposed to low T levels; Androgenic response coexposed to moderate T levels	(He et al., 2011)
Experimental ponds (Syncrude)	7-year and 11-year aged OSPW	*Vibrio fischeri*	15 min	IC_{50}: 100% for both ponds	(Holowenko et al., 2002)
OSPW-impacted wetlands (Suncor)	Wetlands composed of CT discharge and/or seepage collection water	*Vibrio fischeri*	15 min	IC_{50}: 100%, 100%, 100%, 100%, 98%, and 64% for increasing NAs concentrations in wetlands; IC_{20}: 100%, 46%, 33%, 52%, 14%, and 11% for increasing NAs concentrations in wetlands	(Holowenko et al., 2002)
OSPW-NAEs (WIP)	NAEs isolated from fresh OSPW	*Vibrio fischeri*	15 min	IC_{50}: 41.9, 58.1, 43.5, 54.7, 64.9, and 52.7 mg/L for five fractionated NAs with increasing MW, and the original NAs mixture	(Frank et al., 2008)
OSPW-NAEs	NAEs isolated from fresh OSPW (source not specified)	Mouse embryonic stem cell	5 days	Up-regulated gene expression of early cardiac markers (*nkx2.5*: 0.0025–2.5 mg/L NAEs; *gata4* and *mef2c*: 0.025–2.5 mg/L NAEs; *nrg1α* and *nrg1β*: 0.25–2.5 mg/L NAEs)	(Mohseni et al., 2015)
OSPW-OF (WIP)	Organic fraction isolated from fresh OSPW; Organic fraction consists of neutral fraction and NAEs	Mouse BMDM	18 h (serial dilution)	Viability (NSD); Proliferation (+ for 6.25 and 12.5 mg/L NAs; −40%, for 50 mg/L NAs); RNI (less for 25 and 50 mg/L NAs);	(Garcia-Garcia et al., 2011b)

				iNOS gene expression (−20% for 50 mg/L NAs); ROI (less for 25 and 50 mg/L NAs); NADPH subunit - p91Phox gene expression (−20% for 50 mg/L NAs); Phagocytosis (inhibited for 50 mg/L NAs); pro-inflammatory cytokines gene expression (less IL-1β for both resting and activated cells at 50 mg/L NAs); Anti-inflammatory cytokines gene expression (less IL-10 for activated cells).	
OSPW-OF (WIP)	Organic fraction isolated from fresh OSPW; Organic fraction consists of neutral fraction and NAEs	Mouse BMDM	18 h (Serial dilution)	RNI (less for 25 and 50 mg/L NAs); iNOS gene expression (less for 50 mg/L NAs); ROI (less for 50 mg/L NAs); NADPH subunits - p47Phox & p67Phox gene expression (less for 50 mg/L NAs); Phagocytosis (inhibited for 50 mg/L NAs); Cytokines gene expression (less IL-1, IL-6, IL-12, TNF-α for resting cells at 50 mg/L NAs; less IL-1, more IL-12 and TNF-α for activated cells)	(Garcia-Garcia et al., 2011a)
OSPW-OF (Pond 10)	Organic fraction isolated from 17-year aged OSPW; four fractions tested: NAEs, neutral fraction, C18 MeOH fraction, C18 NaOH fraction;	H4IIE-luc cell line	24 h for cytotoxicity assay; 24, 48, and 72 h for AhR transactivation assay	Cytotoxic at 50 mg/L of each fraction; AhR agonist activity (5 mg/L of neutral fraction after 24 h, but dissipated at 48 and 72 h; NSD for other fractions)	(Leclair et al., 2015)
OSPW-OF (Pond 10)	Organic fraction isolated from 17-year aged OSPW; Four fractions tested: NAEs, neutral fraction, C18 MeOH fraction, C18 NaOH fraction	H295R cell line	48 h	Corticosterone production (+ for 5 mg/L NAEs; NSD for other fractions); progesterone production (+ for 0.05–0.5 mg/L C18 MeOH fraction; NSD for other fractions); androstenedione production (NSD for all fractions); Testosterone production (NSD for all fractions)	(Leclair et al., 2015)
OSPW-OF (Pond 10)	Organic fraction isolated from 17-year aged OSPW; four fractions tested: NAEs, neutral fraction, C18 MeOH fraction, C18 NaOH fraction	Yeast (*Saccharomyces cerevisiae*) cells	48 and 72 h for yeast androgen screen and yeast estrogen screen, respectively	No estrogen or androgen receptor agonists for all fractions; antiestrogenic potency for neutral fraction, NAEs and C18 MeOH fraction	(Leclair et al., 2015)

Note: (NSD) no significant difference relative to control; (+) significant increase relative to control; (−) significant decrease relative to control; MLSB: Mildred Lake Settling Basin built in 1989, an active settling basin on Syncrude's site; WIP: West-In-Pit, an active settling basin established in 1995, on Syncrude's site; Pond 10: a small tailings storage pond, containing 17-year old OSPW, on Syncrude's site; OSPW-NAEs: OSPW naphthenic acids extracts; OSPW-OF: OSPW organic fraction.

Table 2
Studies demonstrating OSPW toxicity in invertebrates.

Sample designation	OSPW type	Test organism	Duration of exposure	Endpoint & result	Reference
MLSB	Fresh OSPW	*Daphnia magna*	96 h	LC_{50}: 16–27%	(MacKinnon and Retallack, 1982)
MLSB	Fresh OSPW	*Daphnia pulex*	96 h	LC_{50}: 2%	(MacKinnon, 1986)
MLSB	Fresh OSPW	*Daphnia pulex*	96 h	LC_{50}: 10%	(MacKinnon and Boerger, 1986)
MLSB	Fresh OSPW	Daphnia	96 h	LC_{50}: 2%	(Boerger et al., 1986)
WIP	Fresh OSPW	*Daphnia magna*	48 h	LC_{25}: >100%; LC_{50}: >100%	(Zubot et al., 2012)
OSPW from three major oil sands companies	OSPW source not specified	*Daphnia magna*	24 and 48 h	Survival (48 h-LC_{50}: >100%); Feeding rate (24 h-IC_{50}: 5.34%); Chemosensory function (inhibited at ≥5% OSPW for 24 h); Total activity (inhibited at 10%–20% OSPW)	(Lari et al., 2016)
OSPW from three major oil sands companies	OSPW source not specified	*Daphnia magna*	21 days (1% -10% OSPW)	Feeding rate (−); reproductive capacity (−); growth (−)	(Lari et al., 2016)
WIP	Fresh OSPW	*Chironomus dilutus*	10 days (100% OSPW)	Survival (NSD); growth (−)	(Pourrezaei et al., 2011)
WIP	Fresh OSPW	*Chironomus dilutus*	4 days (100% OSPW)	Survival (NSD); growth (−49%); gene expression: oxidative stress response (*cat*: +1.9-fold; *gpx*: +2.7-fold; *gst* and *aif*: NSD); endocrine signaling (*err*: −1.8-fold; *esr* and *usp*: NSD)	(Wiseman et al., 2013a)
WIP	Fresh OSPW	*Chironomus dilutus*	7 days (100% OSPW)	Survival (NSD); growth (−62%); gene expression: oxidative stress response (*cat* and *gpx*: NSD; *gst*: −2.4-fold; *aif*: +1.6-fold); endocrine signaling (*err*: +4.2-fold; *esr*: +4.8-fold; *usp*: +8.9-fold); tissues concentrations of lipid hydroperoxides (+2.9-fold)	(Wiseman et al., 2013a)
WIP	Fresh OSPW	*Chironomus dilutus*	10 days (100% OSPW)	Survival (WIP-2009: -35%; WIP-2010: NSD); growth (WIP-2009: -64%; WIP-2010: -79%) Behavior: case building (smaller, fragile); case occupation activity (WIP-2009: less active on day 7& 9; WIP-2010: more active on day 3, less active on day 7& 9)	(Anderson et al., 2012)
WIP	Fresh OSPW	*Ceriodaphnia dubia*	6 days: renewal test	Survival (LC_{25}: 52%, LC_{50}: 65%); Fecundity (IC_{25}: 8%, IC_{50}: >39%)	(Zubot et al., 2012)
WIP	Fresh OSPW	*Chironomus dilutus*	60 days (100% OSPW)	Pupation (WIP-2009: -32%; WIP-2010: -70%); cumulative emergence (WIP-2009: -84%; WIP-2010: -90%)	(Anderson et al., 2012)
Recycle water pond (Syncrude)	Fresh OSPW	*Ceriodaphnia dubia*	6–8 days	Survival (LC_{50}: 70.7%); reproduction (IC_{50}: 49.4%)	(Puttaswamy et al., 2010)

TPW	Aged OSPW	*Chironomus dilutus*	10 days (100% OSPW)	Survival (NSD); Growth (−23%); Behavior: case building (slightly smaller); case occupation activity (more active)	(Anderson et al., 2012)
TPW	Aged OSPW	*Chironomus dilutus*	60 days (100% OSPW)	Pupation (−43%); cumulative emergence (−72%)	(Anderson et al., 2012)
FE5	Aged OSPW	*Chironomus dilutus*	10 days (100% OSPW)	Survival (NSD); Growth (NSD); Behavior: case building (smaller); case occupation activity (more active)	(Anderson et al., 2012)
FE5	Aged OSPW	*Chironomus dilutus*	4 days (100% OSPW)	Survival (NSD); growth (NDS); gene expression: oxidative stress response (cat, gpx, gst, and aif: NSD); endocrine signaling (err, esr, and usp: NSD)	(Wiseman et al., 2013a)
FE5	Aged OSPW	*Chironomus dilutus*	7 days (100% OSPW)	Survival (NSD); growth (NDS); gene expression: oxidative stress response (cat, gpx, and aif: NSD; gst: −2-fold); endocrine signaling (err, esr, and usp: NSD); tissues concentrations of lipid hydroperoxides (NSD)	(Wiseman et al., 2013a)
FE5	Aged OSPW	*Chironomus dilutus*	60 days (100% OSPW)	Pupation (NSD); cumulative emergence (NSD)	(Anderson et al., 2012)
Big Pit	Aged OSPW	*Chironomus dilutus*	10 days (100% OSPW)	Survival (NSD); growth (−19%); Behavior: case building (slightly smaller); case occupation activity (more active)	(Anderson et al., 2012)
Big Pit	Aged OSPW	*Chironomus dilutus*	60 days (100% OSPW)	Pupation (NSD); cumulative emergence (NSD)	(Anderson et al., 2012)
OSPW-impacted wetlands (Suncor & Syncrude)	Oil sands process-impacted wetland waters	*Chironomus riparius*	10 days: laboratory bioassays (100% OSPW)	Larvae size (−); when reared in water mimicking combinations of salts and NAs, there was an antagonistic interaction between the two components.	(Kennedy, 2012)
OSPW-impacted wetlands (Suncor)	Oil sands process-impacted wetland waters	Chironomids	NA	Density and biomass (+); incidence of mentum deformities (NSD or +); mutagenicity (NSD)	(Bendell-Young et al., 2000)

Note: (NSD) no significant difference relative to control; (+) significant increase relative to control; (−) significant decrease relative to control; MLSB: Mildred Lake Settling Basin built in 1989, an active settling basin on Syncrude's site; WIP: West-In-Pit, an active settling basin established in 1995, on Syncrude's site; Big Pit, FE5 and TPW are three on-site experimental reclamation ponds that have been aged by different approaches; Big Pit: have been aging since 1993 and is comprised of fluid fine tailings (FFTs) capped with freshwater; FE5 pond: created in 1989 by capping MFTs with OSPW; TPW: OSPW that has been aging since 1993.

was suggested that some metals (*e.g.*, nickel, manganese, and uranium) present in OSPW may also contribute to the reported toxic effects (Anderson et al., 2012).

The wetland reclamation represents a passive treatment method under the assumption that NAs concentrations and OSPW toxicity will eventually diminish through *in-situ* biodegradation (Kavanagh et al., 2011). The characteristics of OSPW-impacted wetlands have been studied. They appeared to display a lower overall benthic invertebrate community diversity (Bendell-Young et al., 2000; Whelly, 2000), a greater chironomid diversity (Bendell-Young et al., 2000), and decreased growth of *Chironomus riparius* larvae (Kennedy, 2012). The mouthpart deformities of chironomids were also examined as evidence of teratogenic effects, and no significant difference was found between wetlands (Bendell-Young et al., 2000). The toxicity of OSPW may decline by aging, as indicated by less diverse invertebrate populations in young (<7-year old) OSPW-impacted wetlands than older ones (Leonhardt, 2003). This reduction in toxicity might be explained by the NAs biodegradation in aged wetlands, but may also be due to the colonization by more tolerant species over time (Kennedy, 2012). Recent study using invertebrates showed that NAs from fresh OSPW were less toxic than those from aged OSPW (Bartlett et al., 2017). These results were contradictory from previous research reporting decrease in the OSPW toxicity occurring with degradation processes associated with aging (MacKinnon and Boerger, 1986; Holowenko et al., 2002; Anderson et al., 2012). These different observations raised the possibility that the reduction in OSPW toxicity with age was the result of a difference in whole OSPW composition, including potential interactions with organic and inorganic compounds, other than NAs degradation. In fact, the laboratory experiments have indicated the antagonistic interaction between NAs and salts, when *C. riparius* was exposed to water mimicking combinations of these two constituents (Kennedy, 2012).

Benthic invertebrates are commonly used as indicators of the water quality, as they spend all or most of their life cycle in water, and many of them are sensitive to the pollutants. The toxicity of OSPW in this species has been demonstrated in terms of effects on the ecologically relevant endpoints of survival, growth, pupation, and emergence, as well as community characteristic such as abundance and diversity.

2.3. Toxicity of OSPW in fish

Fish are continuously exposed to the wastewater, and serve as valuable aquatic vertebrate models for toxicological research, owing to their relatively small size, rapid growth and development, short generation time, and externally developing embryos that facilitate experiments in developmental toxicology (Hinton et al., 2009). OSPW is widely reported to be toxic to fish by affecting a variety of endpoints (Table 3). Early studies reported the 96 h-LC_{50} values of <35% (v/v) for fresh OSPW collected from various tailings ponds in rainbow trout and fathead minnows, and in some cases, low to ~3% v/v caused mortality (MacKinnon, 1981, 1986; MacKinnon and Retallack, 1982; Nix and Martin, 1992). The mortality of rainbow trout exposed to fresh OSPW (100% mortality of rainbow trout after exposure to 50% of MLSB-OSPW for 96 h) was also reported by Rogers et al. (2007). Fathead minnow survived a 96-h exposure with altered hematology in CT water and dyke seepage water, but all died in a prolonged period (28 days) (Farrell et al., 2004). The different responses to OSPW exposures could be associated with the different sensitivities of fish species to pollutants, and the variance in composition of OSPW tested. Research on OSPW organic fractions demonstrated that the fraction containing alicyclic, 'classical' NAs were acutely toxic to larval zebrafish, with 96 h-LC_{50} of 13.1 mg/L (Scarlett et al., 2013). Interestingly, an aromatic NAs fraction, containing compounds like dehydroabietic acid, was more toxic (96 h–LC_{50} 8.1 mg/L) (Scarlett et al., 2013). These observations suggest that NAs are at least partially responsible for the lethal effects of OSPW on fish, and that these effects are composition- and structure- dependent.

The non-lethal effects of fresh OSPW have also been extensively studied in fish, including reduced fertilization success, premature hatching, increased embryo deformities, and elevated transcript of genes associated with xenobiotics metabolism, oxidative stress and apoptosis (He et al., 2012a; Peters et al., 2007). Oxidative stress could result in damage to mitochondria and promote activation of caspase enzymes and apoptotic cell death (He et al., 2012a). Some of these toxic effects in fish embryos were also caused by OSPW-NAEs (Marentette et al., 2015a, 2015b; Wang et al., 2015). Studies at molecular levels indicate that OSPW-NAEs negatively impacted the development and endocrine function of fish, likely *via* altering the expression of endocrine-disrupting biomarker genes (Wang et al., 2015). Hagen et al. (2013) reported on the immunotoxic effects of both acute and sub-chronic exposures of goldfish to fresh OSPW. Fish acutely exposed to OSPW had higher transcripts of pro-inflammatory cytokine genes, and enhanced ability to control parasites infection (Hagen et al., 2013). However, this does not necessarily mean that fish are healthy, and in fact, prolonged exposure resulted in significant down-regulations of pro-inflammatory cytokine genes that may influence the susceptibility of fish to infection diseases (Hagen et al., 2013).

Similar to the results obtained using microorganisms and invertebrates, the acute toxicity of OSPW appears to decline in aged OSPW ponds (Nero et al., 2006b; Hagen et al., 2013). However, aged OSPW still induced toxic effects, manifested by histological changes in liver and gill (Nero et al., 2006b), dysregulation of immune genes expression (Hagen et al., 2013), decreased plasma levels of steroid hormones (Kavanagh et al., 2011; Lister et al., 2008), and impaired growth and reproduction performance (Kavanagh et al., 2011) in different fish species. Although the exact causative pollutants in OSPW are not fully known, there is evidence that these toxic effects are due to the NAs. For example, a 21-day exposure of yellow perch to OSPW-NAEs caused histopathological changes in liver and gill (Nero et al., 2006a), and lower fecundity, spawning and plasma steroid concentrations in fathead minnows (Kavanagh et al., 2012).

Recently, increasing efforts have been made to discover the endocrine disruptive properties of OSPW. An *in vivo* investigation on fathead minnow indicated that fresh OSPW had endocrine-disrupting effects at all levels of brain-gonad-liver axis (He et al., 2012b). The compounds responsible for the activities were not identified, but some NAs have been implicated as the candidate endocrine disrupting compounds (EDCs). For instance, the transcription of estrogen receptor (ERα), and vitellogenin were significantly induced by OSPW-NAEs on the early life stage of zebrafish that might negatively impact the development and endocrine functions (Wang et al., 2015). The endocrine disruptive effects seem to be structure-dependent. For example, studies have demonstrated that some aromatic NAs in OSPW are structurally similar to estrogens (Rowland et al., 2011b), and some polycyclic NAs with a single aromatic ring may possess human estrogenic and androgenic activity (Scarlett et al., 2012). Reinardy et al. (2013) reported the vitellogenin-inducing effects of esterifiable OSPW NAs, particularly the aromatic NAs in zebrafish larvae. These results suggest that some NAs, especially aromatic NAs, might account for some endocrine disrupting activities reported in OSPW and OSPW-NAEs. EDCs could disrupt synthesis, secretion, transport, binding, or elimination of hormones and steroids in organisms. The hormones and steroids or their receptors are often involved in homeostasis, reproductive capacity, development, or behavior (CEPA, 1999; Hagen, 2013). Interestingly, the reduce plasma levels of E2 witnessed in fish (Lister et al., 2008; van den Heuvel et al., 1999) were in contrast to the increased E2 production by H295R cells exposed to OSPW (He et al., 2010). These variable results may be due to the different chemistry of waters tested (*i.e.*, fresh OSPW *vs.* aged OSPW), and the difference of specificity and sensitivity of endocrine disruptive properties between fish and human cell lines.

Given the high complexity of OSPW, other contaminants in OSPW (PAHs, salts, *etc.*) likely contribute to the overall toxicity in aquatic organisms. While PAHs can be reduced by volatilization, degradation,

and sediment absorption, an average concentration of 0.01 mg/L in OSPW substantially exceeds environmental guidelines of 0.00001–0.00006 mg/L (Allen, 2008; Beck et al., 2015; Parajulee and Wania, 2014). There is significant evidence that PAHs from different oil sources cause toxicity in organisms. Many PAHs have been shown to induce teratogenic, mutagenic, carcinogenic, endocrine disruptive, and immunotoxic properties (reviewed by Collier et al., 2013), however, the information on OSPW-derived PAHs is limited. Alharbi et al. (2016b) reported the inhibition of ATP-binding cassette (ABC transport proteins) in Japanese medaka exposed to water-soluble organic fraction isolated from synthetic OSPW (prepared by extraction of bitumen from oil sands in the laboratory). ABC transporters are important for excretion of PAHs and their metabolites. The inhibition of the protein activity might exacerbate accumulation and effects of PAHs and/or their bio-activated metabolites in cells, resulting in greater toxicity (Alharbi et al., 2016b). Further research on the effects of OSPW on toxicity of retene (the model alkyl-PAH) provided additional evidence that dissolved organic pollutants in OSPW might increase exposure and uptake of PAHs by fish (Alharbi et al., 2016a).

During the bitumen extraction process, salts leaches from oil sands, leading to elevated total dissolved solids (TDS) concentrations (~1200–2500 mg/L) and high conductivity of OSPW (~1000 to 4000 μS/cm) (Mahaffey and Dube, 2016), which may induce ionic imbalances in fish and cause osmotic stress and mortality. While the sensitivity is organism-specific, conductivity above 2000 μS/cm or TDS above 1340 mg/L represent concentrations sufficient to cause toxicity in fish (Goodfellow et al., 2000; Leah, 2012).

The heavy metals present in OSPW may also contribute to the overall toxicity of this complex chemical matrix, since they are recalcitrant and are known to bioaccumulate. The concentrations and toxicity risk of heavy metals in OSPW have been described previously, and the target heavy metals that exceeded the Canadian Council of Ministers of the Environment (CCME) water quality guidelines include arsenic, copper, cadmium, lead, and chromium (Li et al., 2014; Zhang, 2016). Information on toxicity of OSPW-derived heavy metals is scarce, however, research on heavy metals present in other industrial wastewaters has demonstrated their toxic effects on cardiovascular, respiratory, gastrointestinal, nervous, hepatic, hematopoietic, immunological, endocrine, and reproductive systems in fish and mammals (Li et al., 2014; Hagen, 2013). All of these results emphasize the importance of continued research to investigate interactions among chemicals co-existing in OSPW rather than focusing on one specific group of pollutants.

2.4. Toxicity of OSPW in amphibians

Amphibian larvae are very sensitive to contaminants in the aquatic environments, and it is not surprising that the OSPW exposures could affect their health. Both laboratory and field studies have examined the toxicity of OSPW-impacted wetlands towards amphibians, and the findings of these studies are summarized in Table 4. Higher mortality, stunted growth and development, elevated ethoxyresorufin-*o*-deethylase (EROD) activity, as well as alterations in hormones production were observed in tadpoles of *Bufo boreas* or *Lithobates sylvaticus* after exposure to OSPW-impacted wetlands waters (Pollet and Bendell-Young, 2000; Hersikorn et al., 2010; Hersikorn and Smits, 2011). EROD measures the activity of the cytochrome CYP 450 enzyme family, and has been a well established biomarker of contaminants exposure (Whyte et al., 2000; Havelková et al., 2007; Hersikorn and Smits, 2011). Elevated EROD activity in tadpoles from OSPW-impacted wetlands indicated increased detoxification efforts by the animals, to some extent, reflecting greater concentrations of pollutants (Hersikorn and Smits, 2011). Hersikorn and colleagues have also provided evidence that detoxification occurs in OSPW-impacted wetlands by aging, since "old" OSPW-impacted wetlands showed markedly lower toxicity than "young" wetlands (Hersikorn et al., 2010; Hersikorn and Smits, 2011).

2.5. Toxicity of OSPW in birds

Reclaimed wetlands receiving oil sands tailings have been constructed by some companies on their mining leases, with the aim to eventually return the lands disturbed by oil sands mining to self-sustaining ecosystems (Gentes et al., 2007a). Tree swallows (*Tachycineta bicolor*) inhabiting the reclaimed sites are good indicator species for evaluating the potential toxicity of OSPW and the sustainability of wet landscape reclamation strategy. They could be exposed to the OSPW compounds through food-web transfer, because 80% of their diets are the aquatic insects whose larvae develop in OSPW and sediments (Smits et al., 2000; Gentes et al., 2007a). The toxic effects of OSPW-impacted wetlands on tree swallows are summarized in Table 5. It was found that tree swallows from OSPW-impacted wetlands had reduced reproductive performance and increased mortality of nestlings, though data were obtained during harsh weather (Gentes et al., 2006). When the weather was less challenging, the mortality rates were low, but less weight and higher hepatic EROD activity of nestlings were recorded (Gentes et al., 2006). More recently, there was evidence of endocrine disrupting effects and immunotoxicity resulting from OSPW exposures, including elevated thyroid hormones (Gentes et al., 2007a) and heavy blow fly infestation (Gentes et al., 2007c). The altered thyroid function might compromise the post-fledging survival by negatively impacting the metabolism, behavior, feather development, and molting (Gentes et al., 2007a), and higher blow fly burdens indicated impaired host resistance to parasites (Gentes et al., 2007c). Another study by Gentes et al. (2007b) reported minimal sub-acute toxicity of commercial NAs mixture that had comparable chemistry to NAs extracted from OSPW. However, it was also suggested that though nestling tree swallows might tolerate short-term exposures to environmentally realistic concentrations of NAs, chronic toxicity of NAs still needs to be determined, because birds breed on these reclaimed sites lengthening their exposure to pollutants (Gentes et al., 2007b). A experiment with mallard (*Anas platyrhynchos*) ducklings reared on reclaimed wetlands suggested that such water is not acutely toxic, though the observed differences (lower body mass and skeletal size) might be related to the decreased survival of juvenile waterfowl (Gurney et al., 2005). The negligible adverse physiological effects were also documented in domestic mallards (*Anas platyrhynchos domestica*) after repeated, short-term exposures to OSPW from a recycled water pond (Beck et al., 2014).

So far, the direct assessments of toxicity in birds from OSPW exposures used those either held on reclaimed wetlands (Gentes et al., 2006, 2007a, 2007b, 2007c) or subjected to OSPW from a recycle water pond (Beck et al., 2014). When evaluating the OSPW toxicity from reclaimed wetlands, the interaction of birds and their environment should be considered, given the potential cumulative effects of other stressors such as harsh weather and additional routes of contaminant exposures (*e.g.*, toxins in sediments, plants, and invertebrates). The relatively low toxicity documented in current literature could not rule out the possibility of adverse health effects on birds exposed to other OSPW sources, especially fresh OSPW; therefore, further studies are required.

2.6. Toxicity of OSPW in mammals

There are limited toxicity data on mammals after exposure to OSPW, which have focused primarily on rodents (Table 6). An early report by Rogers et al. (2002) demonstrated the toxicity of OSPW-NAEs in rats. It was shown that exposure to a high dose (300 mg/kg bw) of NAEs caused liver and heart damage and heavier ovaries and spleens in female rats, and brain hemorrhage and heavier testes in male rats (Rogers et al., 2002). It should be noted that animals were exposed to the OSPW dose that was 50 times higher than the "worst-case single-day exposure for wild animals" (Rogers et al., 2002). However, in a sub-chronic (90-day) toxicity test, lower dose (60 mg/kg bw/d NAEs) exposure caused adverse health effects in rats (Rogers et al., 2002). Although this dose was 10 times the estimated worst-case daily exposure,

Table 3
Studies demonstrating OSPW toxicity in fish.

Sample designation	OSPW type	Test organism	Duration of exposure	Endpoint & result	Reference
MLSB	Fresh OSPW	Rainbow trout	96 h	LC_{50}: <4%	(MacKinnon, 1981)
MLSB	Fresh OSPW	Rainbow trout	96 h	LC_{50}: 4–6%	(MacKinnon and Retallack, 1982)
MLSB	Fresh OSPW	Rainbow trout	96 h	LC_{50}: 7%	(MacKinnon, 1986)
MLSB	Fresh OSPW	Rainbow trout	96 h	LC_{50}: 8%	(Boerger et al., 1986)
MLSB	Fresh OSPW	Rainbow trout (fingerlings)	96 h	Survival: 5% OSPW (11%), 10% OSPW (13%), 20% OSPW (5%), 50% OSPW (0%)	(Rogers et al., 2007)
MLSB	Fresh OSPW	Fathead minnow	96 h	LC_{50}: 6–8.5%	(MacKinnon and Retallack, 1982)
MLSB	Fresh OSPW	Yellow perch	Early life stages (serial dilution)	Fertilization success (NSD for 0.16%–20% OSPW; 0% for 100% OSPW); Incidence of embryo deformities (+, optic-cephalic & spinal deformities, calculated threshold: 7.52 mg/L OSPW-NAs); Larval hatch length (−, calculated threshold: 1.92 mg/L OSPW-NAs)	(Peters et al., 2007)
MLSB	Fresh OSPW	Japanese medaka	Early life stages (Serial dilution)	Fertilization success (NSD at all OSPW dilutions tested); incidence of embryo deformities (+, circulatory and head region deformities, calculated threshold: 30 mg/L OSPW-NAs); larval hatch length (−, calculated threshold: 6.18 mg/L OSPW-NAs)	(Peters et al., 2007)
WIP	Fresh OSPW	Rainbow trout	96 h	LC_{25}: >25%; LC_{50}: 35%	(Zubot et al., 2012)
WIP	Fresh OSPW	Rainbow trout	End of bioassay	Mortality (100%)	(Zubot et al., 2012)
WIP	Fresh OSPW	Goldfish	1 week	Cytokine gene expression in gill (more IFN γ and IL-1-β1, less TNFα-2 for 25% OSPW; more IL-1-β1, less TNFα-2 for 50% OSPW); Cytokine gene expression in kidney (more IFN γ, IL-1-β1 and TNFα-2 for 25% OSPW; more IFN γ and IL-1-β1 for 50% OSPW); Cytokine gene expression in spleen (more IL-1-β1 for 25% OSPW; more IL-1-β1 and TNFα-2 for 50% OSPW)	(Hagen et al., 2013)
WIP	Fresh OSPW	Goldfish	12 weeks (serial dilution)	Cytokine gene expression in gill (more IL-1-β1 for 25% OSPW; less TNFα-2 for 50% OSPW); cytokine gene expression in kidney (less IFN γ and TNFα-2, more IL-1-β1 for 25% OSPW; less IFN γ and TNFα-2, more IL-1-β1 for 50% OSPW); cytokine gene expression in spleen (less IFN γ and TNFα-2, more IL-1-β1 for 25% OSPW; less IFN γ and TNFα-2, more IL-1-β1 for 50% OSPW)	(Hagen et al., 2013)
WIP	Fresh OSPW	Fathead minnow	Early life stages (100% OSPW)	Larval survival (−55.3%); spontaneous embryo movement (+92.1%); premature hatching rate (+); incidence of deformities (hemorrhage: +50%; pericardial edema: +56.2%; spinal malformations: +37.5%); transcript of genes related to the metabolism of xenobiotics (cyp1a: NSD; cyp3a: +2.35-fold), oxidative stress (gst: +2.15-fold; sod: +3.08-fold), and apoptosis (casp9: +3.26-fold; apopen: +2.38-fold); ROI generation (+1.68-fold)	(He et al., 2012a)
WIP	Fresh OSPW	Fathead minnow (males)	7 days (100% OSPW)	Gene expression in brain (erβ, ar, gnrh2, gnrh3: NSD; erα: +5.14-fold; kiss1r: +6.11-fold; fshβ: +3.96-fold; lhβ: +3.04-fold); cyp19b: +3.44-fold; gnrhr: −0.13-fold); gene expression in gonads (star, 17βhsd, cyp19a: NSD; fshr: +3.7-fold; lhr: +2.5-fold; cyp11a: +8-fold; 3βhsd: +7-fold); gene expression in liver (erα: +4.1-fold; vtg: +4.9-fold; chg-l: +5.4-fold; chg-h: +3.4-fold)	(He et al., 2012b)
WIP	Fresh OSPW	Fathead minnow (females)	7 days (100% OSPW)	Gene expression in brain (gnrh2, gnrh3, kiss1r, cyp19b, erα, erβ, ar: NSD; lhβ: +5.3-fold; gnrhr: -); gene expression in gonads (star, 3βhsd, 17βhsd, cyp11a, cyp17: NSD; fshr: −0.02-fold; lhr: 0.33-fold; cyp19a: −0.28-fold); gene expression in liver (ar: −0.18-fold); erα: −0.14-fold; erβ: −0.08-fold; vtg: −0.002- fold; chg-l: −0.022-fold; chg-h: −0.036-fold)	(He et al., 2012b)
WIP	Fresh OSPW	Fathead minnow (males)	7 days (100% OSPW)	Gene expression in liver: phase I biotransformation/detoxification (cyp1a: +2.1-fold; cyp2j28: +2.2-fold; cyp2ad2: +2.7-fold; cyp2k6: +10.1-fold; cyp2k19: +11.7-fold; ao1: +3.1-fold; aldh2: +3.6-fold; moa: +3.2-fold; eh: +2.0-fold); phase II & III biotransformation/detoxification (gstm: +4.5-fold; gstc: +>23.3-fold; ugt2a3: +6.3-fold; sult1,3: +1.8-fold; ugt5f1: −4.3-fold); oxidative stress response	(Wiseman et al., 2013b)

Table 3 (*continued*)

Sample designation	OSPW type	Test organism	Duration of exposure	Endpoint & result	Reference
				(gs: +3.1-fold; gr: +3.2-fold; gpx: +1.7-fold; tk: +2.4-fold; 6-pgdh: +10.1-fold; g6pdh: +2.7-fold; trx: +2.5-fold; trxr3: +2.7-fold; pdi p5: +2.2-fold; pdi a3: +1.5-fold; grx5: +1.7-fold); apoptosis (aif-3: +4.3-fold; aif m2: +4.1-fold; parp: +4.8-fold; pdcd4a: +1.5-fold; dram2: +>23.3-fold; cthpb: +1.5-fold; bnip3: −1.8-fold; foxo3a: −3.3-fold); immune response (c8β: −2.1-fold; c1q4c: −19.7-fold; c3: −7.6-fold; c3-h1: −2.1-fold; c4–2: −2-fold)	
CT water pond (Suncor)	OSPW released through MFT consolidation	Fathead minnows	96 h (100% OSPW)	Mortality (0%); Hematocrit (+38.8%); Leucocrit (−50.6%); Lymphocrit (−74%); Gill histology (NSD)	(Farrell et al., 2004)
CT water pond (Suncor)	OSPW released through MFT consolidation	Fathead minnows	28 days (100% OSPW)	Mortality (100%)	(Farrell et al., 2004)
DS pond (Suncor)	Dyke seepage water	Fathead minnows	96 h (100% OSPW)	Mortality (0%); Hematocrit (+36.6%); Leucocrit (−50.6%); Lymphocrit (−80%); Gill histology (less basal epithelial thickening); Critical swimming speed (−)	(Farrell et al., 2004)
DS pond (Suncor)	Dyke seepage water	Fathead minnows	28 days (100% OSPW)	Mortality (100%)	(Farrell et al., 2004)
Suncor Pond 1	Fresh OSPW	Rainbow trout	96 h	1981 (LC_{50}: 17%), 1982 (LC_{50}: 7.5–10.2%), 1984 (LC_{50}: 4.5%), 1989 (LC_{50}: 3.2%)	(Nix and Martin, 1992)
Suncor Pond 1A	Fresh OSPW	Rainbow trout	96 h	1981 (LC_{50}: 27%), 1982 (LC_{50}: 24%), 1984 (LC_{50}: 5.8%), 1989 (LC_{50}: 3.2%)	(Nix and Martin, 1992)
Suncor Pond 2	Fresh OSPW	Rainbow trout	96 h	1981 (LC_{50}: 16%), 1982 (LC_{50}: 4.2–5.1%), 1984 (LC_{50}: 4.2%), 1989 (LC_{50}: 3.2%)	(Nix and Martin, 1992)
Suncor Pond 3	Fresh OSPW	Rainbow trout	96 h	1989 (LC_{50}: 3.2%)	(Nix and Martin, 1992)
Syncrude Pond 9	Aged OSPW (>15 years)	Goldfish	1 week (100% OSPW)	Cytokine gene expression in gill (NSD); Cytokine gene expression in kidney (more IFN γ, TNFα-2); Cytokine gene expression in spleen (more IFN γ, TNFα-2)	(Hagen et al., 2013)
Syncrude Pond 9	Aged OSPW (>15 years)	Goldfish	12 weeks (100% OSPW)	Cytokine gene expression in gill (more IFN γ, IL-1-β1, TNFα-2); cytokine gene expression in kidney (more IL-1-β1, TNFα-2); cytokines gene expression in spleen (more IL-1-β1)	(Hagen et al., 2013)
Remediation Pond 3 (Syncrude)	Aged OSPW (>12 years); MFT capped with freshwater	Yellow perch	22 days (100% OSPW)	Mortality (0%); gill pathology (NSD); liver pathology (NSD)	(Nero et al., 2006b)
Remediation Pond 3 (Syncrude)	Aged OSPW (>12 years); MFT capped with freshwater	Goldfish	19 days (100% OSPW)	Mortality (1 fish dead); gill pathology (NSD); liver pathology (NSD)	(Nero et al., 2006b)
Remediation Pond 3 (Syncrude)	Aged OSPW (>12 years); MFT capped with freshwater	Goldfish	19 days (100% OSPW)	Plasma levels of hormones (T: -; E2: -); *in vitro* basal T production by gonadal tissues (NSD); hCG-stimulated T production by gonadal tissues (NSD); plasma cortisol levels in males (+)	(Lister et al., 2008)
Remediation Pond 5 (Syncrude)	Aged OSPW (>12 years); MFT capped with OSPW	Yellow perch	22 days (100% OSPW)	Mortality (0%); gill pathology (increased cell proliferation of epithelial and mucous cells); liver pathology (hepatocellular degeneration, inflammatory cell infiltration)	(Nero et al., 2006b)
Remediation Pond 5 (Syncrude)	Aged OSPW (>12 years); MFT capped with OSPW	Goldfish	19 days (100% OSPW)	Mortality (1 fish dead); gill pathology (epithelial cell necrosis); liver pathology (hepatocellular degeneration, hypertrophic hepatocytes)	(Nero et al., 2006b)
Remediation Pond 5 (Syncrude)	Aged OSPW (>12 years); MFT capped with OSPW	Goldfish	19 days (100% OSPW)	Plasma levels of hormones (T: -; E2: -); *in vitro* basal T production by gonadal tissues (−); hCG-stimulated T production by gonadal tissues (NSD); plasma cortisol levels in males (+)	(Lister et al., 2008)
Remediation Pond 5 (Syncrude)	Aged OSPW (>15 years); MFT capped with OSPW	Fathead minnows	21 days: laboratory bioassays (100% OSPW)	GSI (NSD); LSI (NSD); fecundity rate (−21.9%); mean spawn number (NSD); plasma steroid concentration in males (T and 11-KT: NSD); plasma steroid concentration in females (E2 and T: NSD)	(Kavanagh et al., 2011)

(*continued on next page*)

Table 3 (*continued*)

Sample designation	OSPW type	Test organism	Duration of exposure	Endpoint & result	Reference
Remediation Pond 9 (Syncrude)	Aged OSPW (>15 years)	Fathead minnows	21 days: laboratory bioassays (100% OSPW)	GSI (males: NSD; females: -); LSI (NSD); fecundity rate (−78.1%–100%); mean spawn number (−71.7%–100%); plasma steroid concentration in males (T: -; 11-KT: -); plasma steroid concentration in females (E2: -; T: NSD)	(Kavanagh et al., 2011)
Demonstration Pond (Syncrude)	Aged OSPW (>15 years); MFT capped with freshwater	Fathead minnows	21 days: laboratory bioassays (100% OSPW)	GSI (NSD); LSI (NSD); Fecundity rate (−18.9%); mean spawn number (NSD)	(Kavanagh et al., 2011)
Demonstration Pond (Syncrude)	Aged OSPW (>15 years); MFT capped with freshwater	Fathead minnows	Fish collected at various time in 2006–2008 (Jun-06, Jul-07, Aug-07, May-08, Jun-08)	Males: length (+, Jul-07; −, May-08); mass (+, Jul-07 and Jun-08; −, May-08); condition factor (+, Jun-06, Jul-07, and Jun-08; −, Aug-07); GSI (+, 2006–2008); LSI (+, 2007); SSI (−, 2006–2007); number of tubercles (−, Jun-06 and May-08; +, Aug-07); plasma steroid concentration in Jun-06 and Jul-07 (T: NSD; 11-KT: -) Females: length (+, Aug-07; −, Jun-08); mass (+, Jun-06 and Aug-07) Condition factor (+, Jun-06, Jul-07, Aug-07 and Jun-08; −, May-08); GSI (+, 2006–2007); LSI (+, 2006–2008); SSI (−, 2006–2007); plasma steroid concentration in Jun-06 and Jul-07 (T: NSD; 11-KT: NSD)	(Kavanagh et al., 2013)
Suncor North MFT Pond	Aged OSPW (>15 years); MFT capped with OSPW	Fathead minnows	21 days: laboratory bioassays (100% OSPW)	GSI (NSD); LSI (NSD); fecundity rate (−77.5%); mean spawn number (−68.4%); plasma steroid concentration in males (T and 11-KT: -); plasma steroid concentration in females (E2 and T: NSD)	(Kavanagh et al., 2011)
Suncor South MFT Pond	Aged OSPW (>15 years); MFT capped with OSPW	Fathead minnows	21 days: laboratory bioassays (50% and 100% OSPW)	50% OSPW: GSI (NSD); LSI (NSD); fecundity rate (−14.8%); Mean spawn number (−26.3%); plasma steroid concentration in males (T and 11-KT: NSD); plasma steroid concentration in females (E2 and T: NSD); 100% OSPW: GSI (NSD); LSI (NSD); fecundity rate (−57.4%); mean spawn number (−50%); plasma steroid concentration in males (T: NSD; 11-KT: -); plasma steroid concentration in females (E2 and T: NSD)	(Kavanagh et al., 2011)
OSPW-NAEs (WIP)	Fresh OSPW	Zebrafish	96 h	Whole acid extract (LC_{50}: 8.4 mg/L); esterifiable NAs (de-esterified with alkal) (LC_{50}: 5.4 mg/L); de-esterified alicyclic acids/classical NAs (LC_{50}: 13.1 mg/L); aromatic NAs (LC_{50}: 8.1 mg/L)	(Scarlett et al., 2013)
OSPW-NAEs (WIP)	Fresh OSPW	Yellow perch	21 days	Mortality: NAEs-6.8 mg/L (100% in ≤96 h); gill pathology: NAEs-1.7 mg/L (high levels of gill proliferative changes: epithelial, mucous, and chloride cell); Liver pathology: NAEs −1.7 mg/L (NSD)	(Nero et al., 2006a)
OSPW-NAEs (WIP)	Fresh OSPW	Fathead minnows	21 days (5 and 10 mg/L NAEs)	NAEs-5 mg/L: fecundity rate (NSD); mean spawn number (NSD); GSI (males: +; females: NSD); plasma steroid concentration in males (T: NSD; 11-KT: -); plasma steroid concentration in females (E2 and T: NSD) NAEs-10 mg/L: fecundity rate (−68%); mean spawn number (−68.2%); GSI (males: +; females: NSD); plasma steroid concentration in males (T and 11-KT: -); plasma steroid concentration in females (E2 and T: NSD)	(Kavanagh et al., 2012)

Table 3 (*continued*)

Sample designation	OSPW type	Test organism	Duration of exposure	Endpoint & result	Reference
OSPW-NAEs	NAEs isolated from fresh and aged OSPW	Fathead minnow	Early life stages : from <1 dpf to hatch day	Hatch success (EC_{50}: 5–10.6 mg/L for fresh OSPW-NAEs; EC_{50}: 12.4 mg/L for aged OSPW-NAEs); deformities at hatch (cardiovascular abnormalities)	(Marentette et al., 2015a)
OSPW-NAEs	NAEs isolated from fresh OSPW	Fathead minnow	Early life stages : from <1 dpf to 21 dpf	Hatch success (EC_{50}: 9.5–11 mg/L); growth (IC_{10}: 24.7–25.8 mg/L for total length; 14.7–15.8 mg/L for total mass); deformities at hatch (+ at 33.3 mg/L, predominated by cardiovascular abnormalities)	(Marentette et al., 2015b)
OSPW-NAEs	NAEs isolated from fresh OSPW	Walleye	Early life stages: from <1 dpf to 19–21 dpf	Hatch success (EC_{50}: 21.8–24.5-11 mg/L); deformities at hatch (dose-responsive increase at 0–33 mg/L), predominated by spinal curvature, followed by cardiovascular and craniofacial defects)	(Marentette et al., 2015b)
OSPW-NAEs	NAEs isolated from fresh OSPW	Walleye	Early life stages: from <1 dpf to hatch day	Gene expression: AhR-cytochrome P450 pathway (cyp1a1: +2.11-fold at 4.2 mg/L and +1.95-fold at 8.3 mg/L; arnt: NSD); oxidative stress response (gpx1b: −1.56-fold at 4.2 mg/L; cat, gst, sod1: NSD); Apoptosis (bax, casp3, p53: NSD); growth factor signaling (igf1, igf1b, igf1bp: NSD); tissue differentiation (vim: NSD)	(Marentette et al., 2017)

Note: (NSD) no significant difference relative to control; (+) significant increase relative to control; (−) significant decrease relative to control; MLSB: Mildred Lake Settling Basin built in 1989, an active settling basin on Syncrude's site; WIP: West-In-Pit, an active settling basin established in 1995, on Syncrude's site; OSPW-NAEs: OSPW naphthenic acids extracts.

there exists the possibility that indigenous mammals might be more sensitive to NAs than rats used in laboratory assessment, since some uncertainty (*e.g.*, age, season, diet, health status, contaminant interaction, *etc.*) in field toxicity testing could influence the response to NAs (Rogers et al., 2002). Recently, research using mice reported the immunotoxic effects of OSPW-OF manifested by alterations in various macrophage microbicidal functions, and immune gene expression in different organs (Garcia-Garcia et al., 2011a, 2011b, 2012). The doses that induced toxicity in these studies reflected environmentally realistic concentrations of NAs in OSPW. It is also quite possible that other organic compounds present in OSPW-OF, may also contribute to the observed immunotoxicity.

While there is a significant dataset on the effects of OSPW exposure on reproduction and development in fish, there is very little information on the acute and sub-chronic effects of OSPW exposure on development and reproduction of mammals. The exposure of mammals (rats) to OSPW-NAEs caused impaired embryonic implantation, which was likely associated with the changes in cholesterol availability and a parallel decrease in progesterone levels (Rogers, 2003). Recently, research using mouse embryonic stem cells (ESCs) showed that that OSPW-NAE affected the expression of cardiac specific markers in differentiating mouse ESCs, which may potentially cause developmental abnormalities (Mohseni et al., 2015). These findings suggest that OSPW organic compounds (including NAs) may affect mammalian reproduction and development, and emphasize the importance of testing organisms during sensitive developmental stages when establishing an environmental risk assessment of OSPW exposures.

3. Discussion and conclusions

Although the term 'OSPW' is commonly used in toxicology studies, the water chemistry of different OSPW-types varies considerably, depending on the ore sources, extraction and processing methods, and tailings pond characteristics. Different OSPW-types induce different

Table 4
Studies demonstrating OSPW toxicity in amphibians.

Sample designation	OSPW type	Test organism	Duration of exposure	Endpoint & result	Reference
Natural wetland (Suncor)	Wetland receiving dyke seepage water	Boreal toad (*Bufo boreas*)	Posthatch to complete metamorphosis (laboratory bioassays)	*B. boreas* tadpoles: Mortality (0%); Delayed metamorphosis (24-d compared to 21-d in reference wetland); Mass change after 96-h exposure (no change)	(Pollet and Bendell-Young, 2000)
Hummock wetland (Suncor)	Wetland receiving CT water (intentional release).	Boreal toad (*Bufo boreas*)	Posthatch to complete metamorphosis (laboratory bioassays)	*B. boreas* tadpoles: Survival (47% died before completing metmorphosis); delayed metamorphosis (31-d compared to 21-d in reference wetland); mass change after 96-h exposure (−)	(Pollet and Bendell-Young, 2000)
OSPW-impacted wetlands	Young wetland (≤7 years old); old wetlands (>7 years old)	Wood frog (*Lithobates sylvaticus*)	75 days	Tadpoles in young OSPW-impacted wetlands showed 41.5%, 62.6%, and 54.7% higher mortality than old OSPM-impacted, young reference, and old reference wetlands, respectively. Old OSPW-impacted wetlands had similar effects in tadpoles compared to reference wetlands.	(Hersikorn et al., 2010)
OSPW-impacted wetlands	Young wetland (≤7 years old); old wetlands (>7 years old)	Wood frog (*Lithobates sylvaticus*)	75 days	Tadpoles in young OSPW-impacted wetlands showed delayed metamorphosis (up to 75 d) compared to reference and old OSPM-impacted wetlands (50–60 d). Tadpoles in young OSPW-impacted wetlands had highest T4 concentration, and lowest T3:T4 ratio. Tadpoles in young OSPW-impacted wetlands had highest EROD activity.	(Hersikorn and Smits, 2011)

Note: (NSD) no significant difference relative to control; (+) significant increase relative to control; (−) significant decrease relative to control.

Table 5
Studies demonstrating OSPW toxicity in birds.

Sample designation	OSPW type	Test organism	Duration of Exposure	Endpoint & Result	Reference
OSPW-impacted wetland	CT water	Zebra finch (*Taeniopygia guttata*)	4 days: laboratory bioassays (70 μL/day orally)	No effects on immunosuppression of T-lymphocyte immune response, or on hematocrit, white blood cell differential and body mass. Had larger bursa of Fabricius.	(Smits and Williams, 1999)
Demonstration Pond (Syncrude)	MFT capped with freshwater	Tree swallows (*Tachycineta bicolor*)	Over two breeding seasons	Clutch size (NSD); Clutch mass (NSD); Hatching success (NSD); Fledging success (NSD); Immune response (+ in 1997; NSD in 1998); Hepatic EROD activity (NSD)	(Smits et al., 2000)
Demonstration Pond (Syncrude)	MFT capped with freshwater	Tree swallows (*Tachycineta bicolor*)	Late May to mid July of 2003 and 2004	In 2003, harsh weather: Mortality (58.8%); Reproductive performance (−) In 2004, less challenging weather: Mortality (0%); Hepatic EROD activity (+1.2-fold); Fledging size (−)	(Gentes et al., 2006)
Demonstration Pond (Syncrude)	MFT capped with freshwater	Tree swallows (*Tachycineta bicolor*)	May 19–July 15, 2014	Plasma hormones (T3 and T4: NSD); thyroid weight (NSD)	(Gentes et al., 2007a)
Natural wetland (Suncor)	Wetland receiving dyke seepage water	tree swallows (*Tachycineta bicolor*)	Over two breeding seasons	clutch size (NSD); clutch mass (NSD); hatching success (−); fledging success (−); immune response (NSD); hepatic EROD activity (+)	(Smits et al., 2000)
Natural wetland (Suncor)	Wetland receiving dyke seepage water	Mallard (*Anas platyrhynchos*) ducklings	33 days	Body mass (day 2, 5, 9 and 13: −; after 13 days: NSD); body size (on day 2, 5, 9 and 13: −; after 13 days: NSD); plasma triglyceride level (NSD); plasma glycerol level (day 13: +; day 33: NSD); EROD activity (NSD); PAH metabolite levels in the bile (pyrene: +; BαP: NSD; naphthalene: +; phenanthrene: NSD)	(Gurney et al., 2005)
Natural wetland (Suncor)	Wetland receiving dyke seepage water	Tree swallows (*Tachycineta bicolor*)	Late May to mid July of 2003 and 2004	In 2003, harsh weather: mortality (89.3%); reproductive performance (−) In 2004, less challenging weather: mortality (3.6%); hepatic EROD activity (+1.9-fold); fledging size (−)	(Gentes et al., 2006)
Natural wetland (Suncor)	Wetland receiving dyke seepage water	Tree swallows (*Tachycineta bicolor*)	May 19–July 15, 2014	Plasma hormones (T3: +; T4: NSD); Thyroid weight (NSD)	(Gentes et al., 2007a)
CT wetland (Suncor)	Wetland with consolidated tailings	Tree swallows (*Tachycineta bicolor*)	Late May to mid July of 2003 and 2004	In 2003, harsh weather: mortality (100%) In 2004, less challenging weather: mortality (0%); hepatic EROD activity (+2-fold); fledging size (−)	(Gentes et al., 2006)
CT wetland (Suncor)	Wetland with consolidated tailings	Tree swallows (*Tachycineta bicolor*)	May 19–July 15, 2014	Plasma hormones (T3: +; T4: NSD); thyroid weight (NSD)	(Gentes et al., 2007a)
Hummock wetland (Suncor)	Wetland receiving CT water (intentional release).	Mallard (*Anas platyrhynchos*) ducklings	33 days	Body mass (on day 2, 5, 9 and 13: -; after 13 days: NSD); body size (on day 2, 5, 9 and 13: -; after 13 days: NSD); plasma triglyceride level (NSD); plasma glycerol level (NSD)	(Gurney et al., 2005)

Note: (NSD) no significant difference relative to control; (+) significant increase relative to control; (−) significant decrease relative to control.

toxic effects both *in vivo* and *in vitro*. A lack of information on water sources, inconsistent analytical methods for water chemistry, and different procedures for OSPW fraction preparations likely accounts in part for the difficulties in the interpretation of toxicological data between taxonomic groups. Studies on whole OSPW and OSPW-derived fractions have identified the constituents with potential toxicity including NAs, PAHs, metals, salts, and other organic or inorganic compounds. NAs are the most widely reported contributors to OSPW toxicity that may induce toxic effects *via* multiple modes of action such as narcosis, endocrine disruption, immunotoxicity and carcinogenicity. NAs extracts from different water sources elicit different responses, and are dependent on both concentrations and composition. The great advances in analytical methodology for NAs have improved our understanding of individual NA compounds, and enabled the studies of structure-toxicity relationships. Future research on NAs-induced toxicity should focus on the compounds that potentially may be more harmful to exposed organisms, such as the more hydrophobic molecules with greater narcotic potency, the diamondoid NAs that causes genotoxicity, and some aromatic NAs that exhibits endocrine disruptive properties. The overall toxicity of OSPW is due to complex interaction between the compounds

Table 6
Studies demonstrating OSPW toxicity in mammals.

Sample designation	OSPW type	Test organism	Duration	Endpoint & Result	Reference
OSPW-NAEs (MLSB)	Fresh OSPW	Wistar rats	14 days	300 mg/kg/d, 5d a week: brain hemorrhage in males; cardiac periarteriolar necrosis and fibrosis in females; significant incidence of pericholangitis in both sexes	(Rogers et al., 2002)
OSPW-NAEs (MLSB)	Fresh OSPW	Wistar rats (females)	90 days	60 mg/kg/d, 5d a week: increased liver weight; elevated blood amylase; reduced hypocholesterolemia; excessive hepatic glycogen accumulation	(Rogers et al., 2002)
OSPW-NAEs (MLSB)	Fresh OSPW	Wistar rats (females)	Throughout pre-breeding, breeding and gestation	60 mg/kg/d: hypocholesterolemia; poor reproductive success; reduced litter size	(Rogers, 2003)
OSPW-OF (WIP)	Fresh OSPW	Mice	1 week	Cytokine gene expression in liver (less TNF α, IFN γ, IL-1, CSF-1, CCL3, and CCL4 at 100 mg/kg/week); cytokine gene expression in spleen (NSD at100 mg/kg/week); cytokine gene expression in MLN (less IL-1 at 100 mg/kg/week)	(Garcia-Garcia et al., 2011a)
OSPW-OF (WIP)	Fresh OSPW	Mice	1 and 2 weeks	Body weight (NSD); pro-inflammatory cytokines gene expression in liver (less IL-1β at 50 mg/kg/week for 1 week; less IL-1β, CSF-1, and CSFR1 at 100 mg/kg/week for 1 week; less IL-1β at 100 mg/kg/week for 2 weeks); pro-inflammatory cytokines gene expression in spleen (less IFN γ, IL-1β, at 100 mg/kg/week for 1 week; NSD for 2 weeks); pro-inflammatory cytokines gene expression in MLN (more CCL3 and CCL4 at 100 mg/kg/week for 1 week; more CCL3 at 50 and 100 mg/kg/week for 2 weeks); peritoneal macrophage phagocytosis (NSD)	(Garcia-Garcia et al., 2012)
OSPW-OF (WIP)	Fresh OSPW	Mice	4 and 8 weeks	Body weight (NSD); pro-inflammatory cytokines gene expression in liver (NSD for 4 weeks; less TNFRFS1A, IL-1β, and CSF-1 at 100 mg/kg/week for 8 weeks); pro-inflammatory cytokines gene expression in spleen (less TNF α, TNFRFS1A, IFN γ, IL-1β, CSF-1, CSFR1, CCL3, CCL4, and CCL5 at 50 mg/kg/week for 4 weeks; less CCL4 at 100 mg/kg/week for 4 weeks; less TNF-α, TNFRFS1A, IFN γ, IL-1β, CSF-1, CSFR1, CCL2, CCL3, and CCL4 at 50 mg/kg/week for 8 weeks); pro-inflammatory cytokines gene expression in MLN (NSD for 4 weeks; more CSF-1 and CSFR1 at 100 mg/kg/week for 8 weeks); peritoneal macrophage phagocytosis (enhanced at 50 and/or 100 mg/kg/week for 4 weeks; NSD for zymosan phagocytosis at 50 and 100 mg/kg/week for 8 weeks; inhibited phagocytosis for zymosan + complement at 50 and 100 mg/kg/week for 8 weeks)	(Garcia-Garcia et al., 2012)
OSPW-OF (WIP)	Fresh OSPW	Mice	8 weeks	Pro-inflammatory cytokines gene expression in spleen (less IFN γ, IL-1β and CSF-1 at 100 mg/kg/week)	(Garcia-Garcia et al., 2011b)

Note: (NSD) no significant difference relative to control; MLSB: Mildred Lake Settling Basin built in 1989, an active settling basin on Syncrude's site; WIP: West-In-Pit, an active settling basin established in 1995, on Syncrude's site; OSPW-NAEs: OSPW naphthenic acids extracts; OSPW-OF: OSPW organic fraction.

present in the water. While the contribution of other OSPW-derived compounds (*e.g.*, PAHs, dissolved ions, and heavy metals), has not received much attention, studies on other industrial wastewaters have shown a range of biological dysfunctions caused by these compounds. Therefore, elucidation of the biological effects of individual compounds and/or additive or synergistic effects of a group of compounds should be a high priority for future research on OSPW toxicity.

In vitro studies have provided information regarding the mechanisms of OSPW toxicity. OSPW has inhibitory effects on bioluminescence production by marine bacteria *V. fischeri*, estrogenic and antiandrogenic effects on hormone-responsive cells lines, immunotoxicity in mouse primary immune cells, as well as impacts on mammalian development using mouse embryonic stem cells. However, *in vitro* tests only expose single cell types to the contaminants. The results are not necessarily related to the outcomes in complex biological systems (living tissue), due to tissue-specific differences in mechanisms of action, biotransformation, or tissue-specific bioaccumulation (Garcia-Garcia et al., 2011a; Rosengren et al., 2005; Schlenk, 2008). Therefore, the risk assessment for OSPW exposure cannot be easily made without further knowledge of its effects under more physiologically relevant conditions (integrated system of an intact organism), and the combination of biological data generated in both *in vitro* and *in vivo* assays are required to better understand OSPW toxicity. A variety of organisms have been used for OSPW toxicology research, and they have exhibited different sensitivities; for instance, OSPW was more toxic to *D. magna*, than fish (trout), and least toxic to *V. fischeri* (MacKinnon and Boerger, 1986). These findings suggest that the toxic effects of OSPW are species-specific, and that the responses observed in prokaryotic organisms may not be applicable to eukayotic organisms. To date, the majority of *in vivo* toxicological studies on OSPW have used aquatic organisms. In general, OSPW exposure has induced multiple toxic effects including compromised immunological function, impaired reproduction and development, disrupted endocrine system, and pathological changes in fish, that appear to be dose-, species-, life stage-, and duration of exposure- dependent. However, the extrapolation of data from fish to higher organisms requires careful consideration. For example, differences in routes of OSPW exposure (*e.g.*, skin for fish *vs.* oral administration for rodents), and potential differences in kinetics and dynamics of xenobiotics between fish and rodents or other mammals could affect their biological responses. So far, results of mammalian toxicity of OSPW have only been reported using rodents. The adverse effects observed were following assessment of the organic fraction of OSPW (the NAs containing fraction) that may not represent the full toxic effects induced by the whole OSPW (organic and inorganic fractions). Additionally, the laboratory-derived toxicity thresholds for NAs could differ from the possible toxic effects on animals in the wild, due to the uncertainty factors such as weather, diet, health status of wild animals and possible exposure to other contaminants and pathogens.

In summary, the results of the present review suggest that future studies should provide detailed information on OSPW sources and

types (*i.e.*, fresh OSPW, CT water, seepage, and water from reclaimed wetlands, *etc.*), and conduct the chemical analysis using standard analytical methods. It is also likely that, in addition to NAs, other organic and inorganic compounds present in OSPW may contribute to the water toxicity. We suggest that the research examining the effects of OSPW exposure on aquatic and terrestrial organisms should continue in three main directions: (1) identification of the most toxic components (priority pollutants) of OSPW to enable targeted treatment regimens; (2) investigation of the overall contribution to toxicity of OSPW using parallel assessments of organic and inorganic fractions and whole OSPW to determine potential additive and/or synergistic effects of different toxic components present in OSPW; and (3) comprehensive side-by-side comparisons of the toxic effects induced by different OSPW waters. Furthermore, the cross-species interpretation and extrapolation of the toxicological effects will be required, to enable appropriate risk assessment of OSPW exposure on living organisms.

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