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Assessment of acute and chronic toxicity of unweathered and weathered diluted bitumen to freshwater fish and invertebrates

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ABSTRACT

This paper presents the results of two different studies investigating the acute and chronic toxicity of two blends of diluted bitumens ("dilbit") and weathered dilbit on freshwater fish and invertebrates after exposure to different concentrations of physically-dispersed (water accommodated fraction; WAF) and chemically-dispersed (chemically-enhanced WAF; CEWAF). The first study evaluated the acute and chronic toxicity of weathered, unweathered and dispersed Access Western Blend (AWB) dilbit on Fathead minnow (*Pimephales promelas*). In the second study, acute and chronic toxicity of weathered and unweathered Cold Lake Blend (CLB) dilbit was assessed on Rainbow trout (*Oncorhynchus mykiss*), and two invertebrate species, Daphnia (*Daphnia magna*) and Ceriodaphnia (*Ceriodaphnia dubia*). For Fathead minnow, unweathered AWB WAF demonstrated a significantly higher acute toxicity (LC50–96 h = 0.628 g/L) compared to the weathered AWB WAF (LC50–96 h = 2.06 g/L). Chronic toxicity tests showed that Fathead minnow lethality was also higher for unweathered AWB (LC50-7 d = 0.593 g/L) compared to the weathered AWB (LC50-7 d = 1.31 g/L) whereas larval growth toxicity was lower for unweathered AWB (IC25-7 d = 0.312 g/L) compared to the weathered dilbit (IC25-7 d = 0.096 g/L). Rainbow trout exposed to unweathered CLB demonstrated a significantly higher toxicity (LC50–96 h = 5.66 g/L) compared to the weathered CLB (LC50 > 18 g/L). Lethality (LC50 = 6.43 g/L) was observed in *Ceriodaphnia* exposed to the CLB WAF while no mortality was observed with the weathered CLB. The reproductive effects on *Ceriodaphnia* were greater with the CLB (IC25 < 1.0) than with the weathered CLB (IC25 = 3.99 g/L). Volatile organic compounds (VOC), polycyclic aromatic hydrocarbons (PAH) and total petroleum hydrocarbons (TPH) increased as the dilbit CLB and AWB WAF concentrations increased. The total VOC and the total PAHs (including the alkylated PAHs) presented similar concentrations in the weathered and unweathered AWB WAF. These compounds seem therefore not be affected by the AWB WAF weathering process. However, VOC and PAH concentrations decreased significantly in the Rainbow trout and Ceriodaphnia toxicity tests using the weathered CLB WAF. VOC and PAH concentrations were also lower in the weathered AWB CEWAF used for Fathead minnow chronic study.

1. Introduction

Canada has one of the largest oil reserves in the world, as the Oil Sands region of Alberta is estimated to contain up to 50 billion cubic metres of bitumen and its extraction is over 300 million L/d (Dupuis and Ucan-Marin, 2015; Environment Canada, Fisheries and Oceans Canada, Natural Resources Canada, 2013; National Energy Board, 2006). Because of the increasing domestic and international demand, the petroleum products must be moved from the production sites to the markets (Lee et al., 2015), over long distances, increasing the risks of a spill in a wide variety of freshwater habitats (Dew et al., 2015). By pipeline, the highly viscous bitumen must be diluted with lighter products such as natural gas condensate or synthetic crude oil, in different proportions depending on the type of bitumen and the season, thus forming the diluted bitumen or dilbit (Dew et al., 2015).

Dilbit can enter into a freshwater ecosystem overland or via a leak in a pipeline (Environment Canada, Fisheries and Oceans Canada, Natural Resources Canada, 2013; Dew et al., 2015. Environmental incidents have generated public concern about the effects of potential spills of oil products. In 2010, 3.2 million L of dilbit (mostly Cold Lake Blend) entered the Talmadge Creek and the Kalamazoo River (near Michigan, USA) after a pipeline rupture (Crosby et al., 2013), where

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part of the spilled product sank at the bottom of the river (Dupuis and Ucan-Marin, 2015; US EPA, 2013). More recently, approximately 225,000 L of heavy crude oil blended with condensate were released after a pipeline rupture: about 40% of the spilled volume entered the North Saskatchewan River Government of Saskatchewan, 2016).

The effects of petroleum products, have been studied for years and several reviews have been published on the toxicity of major constituents of dilbit, specifically naphthenic acids (Headley and McMartin, 2004; Brown and Ulrich, 2015), PAHs (Manzetti, 2012; Ball and Truskewycz, 2013), metals and their mixtures (Dew et al., 2015; Gauthier et al., 2014). However, despite several spills and the large volume transported in Canada and North America, there was little information on the effects of dilbit spills on freshwater ecosystems prior to 2015 (NRC, 2005; Dupuis and Ucan-Marin, 2015; Lee et al., 2015; Madison et al., 2015). Effects of dilbit have been observed on fish exposed to natural oil sands collected at sites along the Athabasca River (AB, Canada) (Colavecchia et al., 2006).

More recently, Madison et al. (2017) observed that Japanese medaka (*Oryzias latipes*) embryos exposed to Access Western Blend did not result in lethality y, but caused an increase in the prevalence of blue sac disease, impaired development and abnormal un-inflated swim bladders upon hatching. The same author reported that Japanese medaka embryo were affected when exposed to Cold Lake Blend using similar nominal dilutions of WAF (0.32–32%, v/v) and CEWAF (0.000–1% v/v) (Madison et al., 2017) as the prevalence of malformations and cyp1a mRNA synthesis in hatched fish increased with treatments concentrations. Baron et al. (2018) observed acute toxicity to fish and invertebrates with CLB and Western Canadian Select dilbits. Alderman et al. (2016) reported toxicity of Cold Lake Blend (CLB) exposure on juvenile Sockeye salmon (*Oncorhynchus nerka*), demonstrating cardiac sensitivity that could directly impact sockeye migration success. Studies on the effects of diluted bitumen on invertebrates are scarce. Toxicity testing using *Chironomus dilutus* and *Hyalella azteca* exposed to sediment collected from affected areas of the Kalamazoo River post-spill demonstrated some sediment samples caused a significant increase in mortality (Fitzpatrick and Dollhopf, 2016).

The present article aims at presenting the results of two different studies investigating the toxicological effects of two different blends of dilbit on freshwater organisms. These laboratory studies were carried out to improve the knowledge on the effects of different dilbit blends on freshwater organisms. In the first study, the acute and chronic effects of unweathered, weathered and dispersed weathered Access Western Blend (AWB) were measured on Fathead minnows (*P. promelas*). In the second study, acute and chronic effects of weathered and unweathered Cold Lake Blend (CLB) were assessed on Daphnia (*Daphnia magna*), Ceriodaphnia (*Ceriodaphnia dubia*) and Rainbow trout (*O. mykiss*).

2. Materials and methods

2.1. Experimental organisms

All experimental organisms were cultured and tested at the AGAT Laboratories, in St-Laurent (QC, Canada). Fathead minnows (*P. promelas*) are cultured at 25 ± 2 °C and larvae, hatched for ≤ 24 h and presenting evidence of inflated swim bladder were used for the 7-d exposures and larvae aged from 4 to 6 d were used for the 96 h exposures. Rainbow trout fingerlings (*O. mykiss*) purchased from the hatchery *Les Arpents Verts* (Sainte-Edwidge, QC) were acclimated at 15 ± 2 °C in laboratory at least two weeks before the toxicity tests. The fingerlings' average wet weight ranged from 0.3 to 0.8 g (50–100 days old). *Daphnia magna were* obtained from C*entre d'expertise en analyse environnementale du Québec* (Québec, QC) and were cultured at 20 ± 2 °C in dechlorinated tap water (hardness adjusted between 160 and 180 mg/L $CaCO_3$). Neonates (≤ 24 h old) were used as test organisms. *Ceriodaphnia dubia* obtained from the A*quatic Research Organisms* (NH; USA) are cultured at 25 ± 1 °C. Neonates (≤ 24 h old) and within 12 h of the same age were used as test organisms.

2.2. Tested products

Unweathered and weathered Access Western Blend (AWB; winter blend 2015) dilbits were used for the toxicity tests on Fathead minnow. SPC 1000 was used to chemically dispersed the AWB. Unweathered and weathered Cold Lake Blend (CLB; winter blend 2015) dilbits were used for the toxicity tests on Rainbow trouts, Daphnia, and Ceriodaphnia. The dilbits were artificially weathered by a laboratory process to mimic the changes which occur in the environment due to the effect of waves and wind.

2.3. Preparation of dilbit exposure solutions

The Water Accommodated Fraction (WAF) was prepared following the CROSERF method (Chemical Response to Oil Spills: Ecological Research Forum; Singer et al., 2000; Baron and Ka'aihue, 2003; Baron et al., 2018). The water used to prepare the WAF is the same as the one used for breeding or holding organisms in the laboratory. Municipal water was dechlorinated with activated carbon, passed through UV lamps and tempered at 15 ± 1 °C (trout), 25 ± 1 °C (minnow) and 20 ± 2 °C for Daphnia. In addition, the hardness was adjusted between 160 and 180 mg/L of $CaCO_3$ for the Daphnia toxicity tests. For the Ceriodaphnia bioassays, spring water bottles (Amaro, St-Cuthbert, QC) water were used. In all cases, the water used for the WAF was filtered on a polyethersulfone (PES) membrane with a porosity of 0.22 μm. Table 1 summarizes parameters and conditions for the preparation of the WAF using the two dilbits and the selected toxicity tests.

For the Fathead minnow toxicity experiments, WAF concentrations were prepared based on oil loading in grams of AWB (unweathered or weathered)/ liter of water (Table 1). An Hamilton-type gas-tight syringes were filled with AWB and weighed before and after the distribution to obtain the selected nominal concentration. Each concentration was prepared individually by performing a WAF for the corresponding AWB charge. Briefly, a volume of 3.5 L of filtered water was measured and transferred to six aspirator glass bottles. Then this water was stirred individually in each of the bottles at 200 rpm with digital stirring plates and magnetic bars. This speed allows a slight agitation, just before an apparent vortex is obtained. Once stirring had started and stabilized, the weighed unweathered AWB or weathered AWB was deposited on the surface of the water for each of the test concentrations. The agitation continued to darkness (in order to reduce the degradation) at room temperature (20–24 °C) for a period of 24 h, after which no settling time was allowed before the WAF recovery for chemical analysis and organism exposures.

For the trout, Daphnia and Ceriodaphnia toxicity experiments using the CLB, the different concentrations tested were obtained by diluting the prepared WAF with a pre-established oil charge (g/L; Table 1). Thus, the WAF becomes 100% (v/v) concentration and the subsequent concentrations were dilutions (%, v/v) from the 100% concentration. This approach is different compared to the previous experiments with the Fathead minnow exposed to AWB and was favored due to volume constraints (16 L per concentration for Rainbow trout tests) and large number of repetitions (up to 8 daily renewals of the solutions for the Ceriodaphnia toxicity tests). To prepare the WAF, the initial oil concentration of CLB was determined based on previous studies or preliminary assays. The oil quantity was calculated based on the volume of water solution used for each toxicity test. The amount of unweathered and weathered CLB required to prepare the WAF solution for each test was measured by weight difference. The preparation of the WAF for trouts and invertebrates was similar to the one used Fathead minnow described above. However, the WAF was prepared and diluted with water to obtain the determined concentrations (%, v/v).

A Chemically-Enhanced Water Accommodated Fraction (CEWAF) was used for experiments using the AWB and was prepared according to