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Toxicity of Cold Lake Blend and Western Canadian Select dilbits to standard aquatic test species

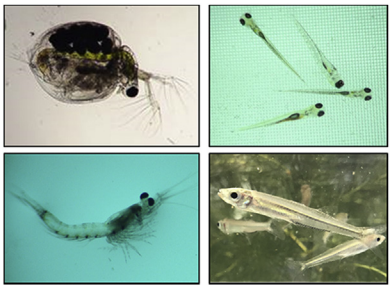
Mace G. Barron [a, *], Robyn N. Conmy [b], Edith L. Holder [c], Peter Meyer [d], Gregory J. Wilson [e], Vanessa E. Principe [e], Morgan M. Willming [a]

[a] Gulf Ecology Division, U.S. EPA, Gulf Breeze, FL 32561, USA
[b] Land Remediation and Pollution Control Division, U.S. EPA, Cincinnati, OH 45268, USA
[c] Pegasus Technical Services, Cincinnati, OH 45268, USA
[d] Hydrosphere Research, Alachua, FL 32615, USA
[e] Office of Emergency Management, U.S. EPA, Washington, DC 20460, USA

HIGHLIGHTS

- The toxicity of two dilbits was determined in standard aquatic test species.
- Acute toxicity of unweathered and weathered dilbits were similar in four species.
- Weathered dilbits were sublethally toxic at 6 to 16 μg/L total petroleum hydrocarbons.
- Dilbits can have similar acute and short term toxicity as other oils.

GRAPHICAL ABSTRACT



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ABSTRACT

Dilbits are blends of bitumen and natural gas condensates or crude oils with only limited toxicity data. Two dilbits, Cold Lake Blend and Western Canadian Select, were tested as either unweathered or weathered oils for acute and chronic toxicity to standard freshwater and estuarine organisms. Water accommodated fractions of the dilbits were characterized for total petroleum hydrocarbons (TPH), polycyclic aromatic hydrocarbons (PAHs), and monoaromatics (BTEX). Acute toxicity of unweathered and weathered dilbits ranged from 4 to 16 mg/L TPH, 8 to 40 μg/L total PAHs, and 0.7 to 16 mg/L BTEX in *Ceriodaphnia dubia*, *Pimephales promelas*, *Americamysis bahia*, and *Menidia beryllina*. Concentrations of weathered dilbits causing impaired growth (*A. bahia*) and reproduction (*C. dubia*) ranged from 0.8 to 3.5 mg/L TPH and 6 to 16 μg/L PAHs. The two dilbits had generally similar acute and short term chronic toxicity expressed as TPH or total PAHs as other crude oils and other petroleum products.
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1. Introduction

Diluted bitumens (dilbits) are blends of bitumen and lower-density hydrocarbon mixtures (e.g., natural gas condensates, light crude oils) that are a growing concern because of increasing transport in North America, recent spills, unique properties, and

* Corresponding author. Gulf Ecology Division, 1 Sabine Island Drive, Gulf Breeze, FL 32561, USA.
E-mail address: barron.mace@epa.gov (M.G. Barron).

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only limited information on environmental fate and behavior in aquatic environments. Dilbits differ from conventional crude oils because of greater density, viscosity and adhesion properties, and higher levels of asphaltenes. Dilbits can also exhibit rapid environmental weathering with the loss of the diluent components (Environment Canada, 2013; Polaris, 2013). Hazard and risk assessment remains complex because of the diversity of dilbit products that can vary seasonally in composition (Environment Canada, 2013; Polaris, 2013).

Aquatic toxicity data are extremely limited for dilbits, with only four published studies to date (Madison et al., 2015, 2017; Alderman et al., 2016; Philibert et al., 2016). The need for dilbit toxicity data has been highlighted in a number of comprehensive reviews, including Dupuis and Ucan-Marin (2015), NAS (2016) and Lee et al. (2015). Dew et al. (2015) noted the need for baseline toxicity data for a range of dilbits for application in hazard assessment and comparison to other oil products. Of the published dilbit toxicity studies, none have reported data for standard aquatic species determined with conventional test methods. Madison et al. (2015) reported the first study of dilbit toxicity in fish. Access Western Blend dilbit caused developmental toxicity and oxidative stress in medaka (*Oryzias latipes*) at total petrogenic PAH concentrations of 10–100 μg/L (40–60 analytes and homolog groups). Alderman et al. (2016) reported biomarker responses and impaired swimming performance in salmon (*Oncorhynchus nerka*) early life stages exposed to treatments of 4–67 μg/L total PAHs from Cold Lake blend (CLB) dilbit. Philibert et al. (2016) reported similar developmental toxicity, impaired avoidance behavior, and reduced swimming performance in zebrafish (*Danio rerio*) exposed to a dilbit and two crude oils. Adverse effects appeared to be more strongly associated with monoaromatics (15–20 mg/L) rather than PAHs (50–200 μg/L) during the short term exposures (Philibert et al., 2016). In the most recent report of dilbit toxicity, physically and chemically dispersed CLB caused developmental toxicity in medaka at concentrations of 3 and 0.1 μg/L total PAHs, respectively (Madison et al., 2017).

The objective of this study was to develop toxicity data for unweathered and weathered CLB and Western Canadian Select (WCS) dilbits to standard aquatic test organisms determined with conventional test methods. Acute toxicity was assessed in four species: the freshwater invertebrate *Ceriodaphnia dubia*, the freshwater fish *Pimephales promelas* (fathead minnow), the saltwater invertebrate *Americamysis bahia* (mysid), and the saltwater fish *Menidia beryllina* (inland silverside). Sublethal toxicity of weathered CLB and WCS was determined in mysids and silversides in short term chronic tests. Additional tests determined the toxicity of an Alaska North Slope crude oil (ANSCO) for comparison to dilbit toxicity determined using identical methods. Water accommodated fractions (WAF) were prepared using conventional slow-stir methods (e.g., Barron and Ka'aihue, 2003) and analytically characterized to quantify different petroleum hydrocarbon exposure measures for evaluating concentration-response relationships and expressing toxicity endpoints. Increasing the knowledge base of dilbit toxicities will have application in the risk assessments of spills, and allow comparison of the relative hazards of dilbits to other oil products.

2. Materials and methods

2.1. Test organisms and test conditions

Test organisms were from in house cultures that met specified quality control requirements for sensitivity, and were reared in either moderately hard (80–100 mg $CaCO_3$/L) reconstituted synthetic fresh water (*C. dubia, P. promelas*) or 20 parts per thousand (ppt) synthetic sea water (*A. bahia*) following standard methods (USEPA, 2002a; 2002b, 2002c). *M. beryllina* were obtained from a commercial supplier (Aquatic Indicators, St. Augustine, FL USA) and acclimated to 20 ppt culture water (USEPA, 2002a). Neonatal *C. dubia* less than 24-h old were used in acute and chronic tests. Additionally, for the chronic tests, they were also isolated within an eight-hour period (USEPA, 2002b, method 1002.0). Larval *A. bahia* were used at 3 to 4 day old in acute tests (USEPA, 2002a, method 2007.0), and when seven days old in chronic tests (USEPA, 2002c, method 1007.0). Larval *P. promelas* were used in acute tests when 7 to 12 days old (USEPA, 2002a, method 2000.0), and *M. beryllina* were used in acute tests as 10 to 14 day old larvae (USEPA, 2002a, method 2006.0).

Test methods were species specific and generally followed U.S. EPA effluent test guidelines as modified for tests with petroleum (Table 1). Chambers were covered to minimize loss of volatiles hydrocarbons, but were not sealed to allow gas exchange. Static (no test solution renewal) acute tests were either 48 h (*C. dubia, A. bahia*) or 96 h (*P. promelas, M. beryllina*) that measured mortality and morbidity (no response to gentle prodding). Chronic tests were 7 day exposures with daily test solution renewals that measured mortality and morbidity, and either reproduction (*C. dubia*) or growth (*A. bahia*). Test chambers were covered, but not sealed, to allow gas exchange and minimize hydrocarbon loss.

2.2. Test oils, weathering, and WAF preparation

The CLB and WCS dilbits were obtained from Crude Quality (Edmonton, Alberta, Canada), and subsamples were artificially weathered by nitrogen gas stripping to no change in volume (~20% volume reduction). ANSCO was a laboratory stock originally obtained in 2010 and was tested without weathering. All oil samples were stored sealed in the dark at approximately 20 °C, and subsampled by removing aliquots to minimize loss of volatiles. WAFs were prepared in species-specific fresh or 20 ppt salinity culture water (3 L water) in 4 L sealed and covered glass jars following the standard slow-stir method (e.g., Barron and Ka'aihue, 2003). The objective of the slow-stir method is standardization following general guidelines (Chemical Response to Oil Spills: Ecological Research Forum), rather than modeling a particularly oil spill scenario. Oil was added at either 25 or 50 g/L, stirred to achieve an approximately 20% vortex within the fluid for 18 h, then settled for 6 h. The aqueous phase was removed via slow siphon, then serially diluted and used in toxicity tests.

2.3. Analytical chemistry

WAF samples were collected immediately after preparation and analyzed for BTEX, PAHs including alkyl homologs, and TPH as total extracted hydrocarbons. BTEX samples were collected in 40 mL glass head space vials and samples for analysis of PAHs and TPH were collected in 1 L glass jars. Samples were extracted with dichloromethane and analyzed for oil components following SW-846 Method 3500C (USEPA, 2007a). Alkane and PAH concentrations were quantified using an Agilent 6890N Gas Chromatograph (GC) with an Agilent 5975 mass selective detector (MSD) and an Agilent 7683 series autosampler, equipped with a DB-5 capillary column by J&W Scientific (30 m, 0.25 mm I.D., and 0.25 μm film thickness) and a splitless injection port following EPA Method 8270D (USEPA, 2014). Alkanes consisted of normal aliphatics ranging in carbon number from 10 to 35 as well as branched alkanes (pristine and phytane). PAHs consisted of forty-five 2, 3 and 4 ring PAH compounds and their alkylated homologs (i.e. $C_{0\text{-}4}$ – naphthalenes, $C_{0\text{-}4}$ -phenanthrenes, $C_{0\text{-}3}$ – fluorenes, $C_{0\text{-}4}$ dibenzothiophenes, $C_{0\text{-}3}$ – napthobenzothiophenes, $C_{0\text{-}2}$ -pyrenes, $C_{0\text{-}4}$ –