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

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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 ug/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 ug/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.

Keywords

dilbit; bitumen; toxicity; water accommodated fraction

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 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

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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-Marín (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 to 100 µg/L (40 to 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 to 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 (ANSKO) for comparison to dilbit toxicity determined using identical methods. Water accommodated fractions (WAF) were prepared using conventional slow-stir methods (e.g., Barron et al. 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 to 100 mg CaCO₃/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-hour 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 hours (*C. dubia*, *A. bahia*) or 96 hours (*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 liter sealed and covered glass jars following the standard slow-stir method (e.g., Barron et al. 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 hours, then settled for 6 hours. 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. C₀₋₄ – naphthalenes, C₀₋₄ -

phenanthrenes, C₀₋₃ – fluorenes, C₀₋₄ dibenzothiophenes, C₀₋₃ – naphthobenzothiophenes, C₀₋₂ -pyrenes, C₀₋₄ – chrysenes). TPH in solvent extracts were determined with an Agilent 7890B GC equipped with a flame ionization detector (FID) and 7693 autosampler following EPA Method 8015C (USEPA 2007b). Concentrations of all the detected individual alkanes and PAHs were summed to compute total alkane and PAH concentrations. BTEX were measured using an Agilent 7890A GC with a 5975C MSD with Triple Axis Detector and CombiPal autosampler (CTC Analytics) following EPA Method 524.3 modified to perform head space analysis instead of purge and trap (USEPA 2009). All reported hydrocarbon measurements met EPA method quality assurance and quality control requirements, including precision, reproducibility and required detection limits.

2.4 Statistical Analyses

Median and 20% lethal concentrations for acute tests (LC50/LC20) were calculated from a log-logistic regression using the drc package in R Statistical Software (Ritz and Streibig 2005) as a percent WAF value. Single toxicity values were computed from the combined replicate data for each test where there were sufficient responses, rather than replicate-specific values. Percent WAF values (as the corresponding fraction) were then multiplied by initial test stock solution concentrations for BTEX, total PAH (sum of detected analytes and homolog groups), and TPH to determine lethal concentrations for each test species, dilbit, and WAF loading level. The average TPH concentration measured in control samples was subtracted from stock solution measurements before determining effective concentrations; no corrections were made to PAH or BTEX values. For 7 day chronic tests, no observed effect concentrations (NOEC) and 25% inhibition (IC25) values were calculated using the geometric mean concentration of BTEX, PAH, and TPH in two samples (day 0 and day 4) of test specific stock solution WAF. Relationships between species-specific dilbit toxicity and concentrations of TPH, PAH, or BTEX were determined by linear regression analysis using r-squared and p-value.

3. Results

3.1 Oil and WAF chemistry

Both total PAH and alkane concentrations were similar in unweathered and weathered CLB and WCS, ranging from 5.8 to 9.1 mg/g oil (Table S1; Fig. S1). Total PAH (17.7 mg/g) and alkane (50.5 mg/g) concentrations in the ANSCO reference oil were 2 to 10 fold higher than in the dilbits. Mean TPH concentrations in 100% WAF were about 1.5 to 3 fold higher in CLB and WCS (5.9 to 16.2 mg/L) compared to the weathered dilbits (3.9 to 5.6 mg/L) (Table S1). Mean PAH concentrations in 100% WAF were similar in fresh or weathered CLB and WCS, and ranged from 13.8 to 40 ug/L (Table S1). Mean BTEX concentrations in 100% WAF were about tenfold higher in CLB and WCS (4.6 to 11.4 mg/L) than in the weathered dilbits (0.53 to 0.94 mg/L) (Table S1).

3.2 Acute and chronic toxicity

All acute and chronic tests met quality control and quality assurance requirements, including control survival and water quality conditions (Table 1). The toxicity of WAF prepared from unweathered and weathered CLB and WCS was generally similar across all four test

species. LC20 and LC50 values determined at loading rates of 25 or 50 g oil/L ranged from 3.6 to >16 mg/L TPH and 7.4 to >40 ug/L PAH (Table 2, 3). Acute toxicity values based on measured BTEX in WAF were lower in weathered CLB and WCS and were substantially more variable (>0.7 to >15.9 mg/L) because of depleted BTEX levels (Table 2, 3). TPH concentrations were highly correlated ($r^2 = 0.72\text{--}0.91$) and a significant predictor of acute dilbit toxicity to three of the four test species (excluding *C. dubia*) (Fig S2, Table S2). In contrast, BTEX and total PAHs were generally not significantly correlated with dilbit toxicity except for mysids (Fig S1, Table S2). Weathered CLB and WCS impaired growth (*A. bahia*) and reproduction (*C. dubia*) at concentrations of 0.41 to 3.5 mg/L TPH, 5.7 to 16 ug/L PAH, and 0.0023 to 1.1 mg/L BTEX (Table 4). Acute toxicity values of ANSCO in the four test species ranged from 2.1 to >5 mg/L TPH, 75 to >107 ug/L PAH, and >4.3 to >5.6 mg/L BTEX (Table S3).

4. Discussion

Compared to conventional crudes, middle distillates and heavy fuel oils, very little is known about the toxicity of dilbits and synthetic crudes derived from bitumen (Dew et al. 2015). Previous studies have reported the toxicity of bitumen, bitumen extracts, or process water from the Alberta tar sands region of Canada (e.g., Colavecchia et al. 2004; Alharbi et al. 2016; Bauer et al. 2017). The toxicity of a few dilbits has only recently been reported in three species of fish, and there has been no published studies on the toxicity to freshwater or saltwater invertebrates or estuarine fish species (Madison et al. 2015, 2017; Alderman et al. 2016; Philibert et al. 2016).

The current study determined the toxicity of two dilbits, CLB and WCS, that represent a range of the diluted bitumens shipped extensively through pipelines in North America. Toxicity was determined in four North American standard aquatic test species using conventional slow-stir WAF and static or static renewal methods. Serial dilutions were used, rather than variable loading, as recommended by Barron and Kaaihue (2003) to allow extrapolation of chemistry results in 100% WAF to lower dilution levels. Standard larval life stages and endpoints of survival, growth and reproduction were used, in contrast to the developmental and embryonic exposures used in most previous dilbit studies (Madison et al. 2015, 2017; Philibert et al. 2016). The standardized approaches were used in the current study to allow comparison to the broader literature on the toxicity of oil products. In addition to median lethal levels, LC20s were also computed to allow comparison across species and dilbits in the absence of 50% mortality. The toxicity of CLB and WCS determined in standard aquatic toxicity tests were generally similar to other oil products based on both TPH and PAH-based measures of effect, including ANSCO tested in the current study. For example, TPH-based LC50s for unweathered CLB and WCS determined in mysids (5.6–7.0 mg/L) and menidia (5.9–8.3 mg/L) were consistent with average acute toxicity values for mysids (2.7 mg/L) and menidia (7.5 mg/L) using conventional WAF preparation and toxicity test methods across a range of crude oils (Barron et al. 2013). PAH concentrations impairing survival, growth or reproduction in short term chronic studies with the four North American species (6 to >16 ug/L) were consistent with PAH effect concentrations (3 to 200 ug/L) determined in developmental toxicity studies of dilbits with zebrafish and medaka (Madison et al. 2015, 2017; Alderman et al. 2016; Pilibert et al. 2016). PAH-based LC50 values of

CLB and WCS (9.8 to >40 ug/L) were also within the range of acute toxicity values of crude oils for a diversity of aquatic species (30–150 ug PAH/L) (Bejarano et al. 2017). There were general trends of greater dilbit acute toxicity at lower concentrations in the estuarine species compared to the freshwater species, but additional research is needed to quantitatively evaluate species or habitat differences.

Toxicity values were also reported as as total BTEX concentrations, with the recognition that petroleum exposures are mixtures of a group of diverse hydrocarbons that can contribute to toxicity. Monoaromatics and naphthalenes have long been considered the drivers of acute toxicity of unweathered petroleum, including in a recent study of two crude oils and a dilbit (Philibert et al. 2016). However, BTEX and other monoaromatics are rapidly depleted during oil weathering, and these compounds are generally not considered to be toxicity drivers of weathered oils and longer term exposures (Redman and Parkerton 2015). The weathered dilbits were chronically toxic, but showed only limited acute toxicity, likely due to observed depletion of BTEX. Consistent with the general literature, effect levels of the dilbits were based on measured concentrations in 100% WAF and did not reflect loss of hydrocarbons over time caused by degradation, adhesion and volatilization (Redman and Parkerton 2015). Thus actual effect levels could be lower than reported here.

5. Conclusions

Dilbits present an increasing environmental concern because of extensive transport in North America, recent spills into aquatic habitats, and limited understanding of environmental fate and toxicity (Dupuis and Ucan-Marin 2015, NAS 2016; Lee et al. 2015). Dilbits are blends of highly weathered bitumen and lighter diluent oils that contain higher concentrations of asphaltenes (>10%) and lower levels of saturates (~40%) (NAS 2016). Dilbits possess some unique combinations of properties, including high adhesion, and the potential for rapid weathering, sinking and associating with sediments. The available aquatic effects data, including this study, indicate that dilbits have similar short-term toxicity as crude oils and other products, but information is still extremely limited. Additional research on the toxicity and environmental fate of unweathered and weathered dilbits is needed to allow for more informed hazard assessments in case of dilbit spills in aquatic and terrestrial environments. Dilbit toxicity to a greater diversity of both native and standard test species is needed, as well as understanding the chemical and physical effects of dilbits in sediments. Additionally, the unique hydrocarbon composition of dilbits consisting of both heavy and light components may necessitate including additional analytes such as an asphaltenes, C5-C9 alkanes, and additional monoaromatics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Summary of acute and chronic test conditions for four aquatic species: Ceriodaphnia (Ceriodaphnia dubia), myid (Americamysis bahia), fathead minnow (Pimephales promelas), and inland silverside (Menidia beryllina).

Test Parameter	Acute <i>C. dubia</i>	Acute <i>A. bahia</i>	Acute <i>P. promelas</i>	Acute <i>M. beryllina</i>	Chronic <i>C. dubia</i>	Chronic <i>A. bahia</i>
Test method	USEPA (2002a) Method 2002	USEPA (2002a) Method 2007	USEPA (2002a) Method 2000	USEPA (2002a) Method 2006	USEPA (2002b) Method 1002	USEPA (2002c) Method 1007
Test type	Static	Static	Static	Static	Static renewal	Static renewal
Test duration	48 hours	48 hours	96 hours	96 hours	7 days	7 days
Salinity	NA	20 ± 2‰	NA	20 ± 2‰	NA	20 ± 2‰
Renewal	NA	NA	NA	NA	Daily	Daily
Temperature	25 ± 1°C. Test temperatures must not deviate (maximum minus minimum temperature) by more than 3°C during the test.					
Light quality	Ambient laboratory illumination					
Light intensity	10–20 (E/m ² /s)					
Photoperiod	16 h light, 8 h darkness, with phase in/out period recommended					
Test chamber size	30 mL	500 mL	1 L	1 L	30 mL	500 mL
Test solution volume	20 mL	200 mL	200 mL	200 mL	20 mL	200 mL
Age of test organism	<24 hours	1–5 days	1–14 days	9–14 days	<24 hours ¹	7 days
Organisms per test chamber	5	10	10	10	1	5
Replicate chambers per concentration	4	3	3	3	10	8
Feeding regime	Refer to specific feeding procedures provided in each test method					
Aeration	None, unless DO falls below 4.0 mg/L, then aerate all chambers. Rate: <100 bubbles/minute					

Test Parameter	Acute <i>C. dubia</i>	Acute <i>A. bahia</i>	Acute <i>P. promelas</i>	Acute <i>M. beryllina</i>	Chronic <i>C. dubia</i>	Chronic <i>A. bahia</i>
Physical/Chemical Measurements	Daily temperatures were measured in one replicate for each test concentration. Exposure test solutions were analyzed daily for pH, dissolved oxygen, and either conductivity or salinity.					
Test concentrations	5 exposure concentrations and a control					
Test acceptability	90% control survival	90% control survival	90% control survival	90% control survival	80% control survival ²	80% control survival ³

¹ . Released within an 8 hour period;

² . Minimum 15 young per surviving female;

³ . Minimum weight of 0.2 mg per adult in surviving controls.

Acute toxicity of fresh and weathered Cold Lake Blend dilbit to four species as three measures of hydrocarbon exposure.

Table 2

Test Species	Weathering State	Test Endpoint	Oil Loading (g/L)	% WAF (v/v)	BTEX ^I (mg/L)	tPAH ^I (ug/L)	TPH ^I (mg/L)
<i>Cladoceran (Ceriodaphnia dubia)</i>	Fresh	48 hr LC20	50	94.2	6.82	25.4	15.2
			25	68.2	7.74	14.0	7.54
	Fresh	48 hr LC50	50	>100	>7.24	>27.0	>16.2
			25	70.7	8.02	14.5	7.82
<i>Mysid (Americamysis bahia)</i>	Weathered	48 hr LC20	50	>100	>0.76	>26.8	>5.57
		48 hr LC50	50	>100	>0.76	>26.8	>5.57
	Fresh	48 hr LC20	50	67.8	3.84	14.1	6.7
			25	55.2	2.81	7.63	4.61
<i>Fathead minnow (Pimephales promelas)</i>	Weathered	48 hr LC20	50	70.5	3.99	14.6	6.97
			25	70.9	3.61	9.80	5.93
	Fresh	48 hr LC20	50	91.1	0.48	20.1	3.57
		48 hr LC50	50	>100	>0.53	>22.1	>3.92
<i>Inland silverside (Menidia beryllina)</i>	Weathered	96 hr LC20	50	96.9	7.02	26.2	15.7
			25	97.2	11.03	19.9	10.8
	Fresh	96 hr LC50	50	>100	>7.24	>27.0	>16.3
			25	>100	>11.4	>20.5	>11.1
<i>Inland silverside (Menidia beryllina)</i>	Weathered	96 hr LC20	50	>100	>0.76	>26.8	>5.57
		96 hr LC50	50	>100	>0.76	>26.8	>5.57
	Fresh	96 hr LC20	50	61.3	3.47	12.7	6.06
			25	89.9	4.58	12.4	7.52

Test Species	Weathering State	Test Endpoint	Oil Loading (g/L)	% WAF (v/v)	BTEX ^I (mg/L)	tPAH ^I (ug/L)	TPH ^I (mg/L)
		96 hr LC50	50	70.7	4.00	14.5	6.99
			25	99.1	5.04	13.7	8.28
	Weathered	96 hr LC20	50	>100	>0.53	>22.1	>3.92
		96 hr LC50	50	>100	>0.53	>22.1	>3.92

^I . BTEX: benzene, toluene, ethylbenzene, xylenes; tPAH: sum of detected polycyclic aromatic hydrocarbons and homolog groups; TPH: total petroleum hydrocarbons.

Table 3

Acute toxicity of fresh and weathered Western Canadian Select dilbit to four species as three measures of hydrocarbon exposure.¹

Test Species	Weathering State	Test Endpoint	Oil Loading (g/L)	% WAF (v/v)	BTEX ¹ (mg/L)	tPAH ¹ (ug/L)	TPH ¹ (mg/L)
Cladoceran (<i>Ceriodaphnia dubia</i>)	Fresh	48 hr LC20	50	>100	>5.86	>40.0	>10.7
			25	>100	>5.56	>29.4	>8.33
		48 hr LC50	50	>100	>5.86	>40.0	>10.7
	Weathered	48 hr LC20	50	>100	>0.94	>34.9	>4.69
			50	>100	>0.94	>34.9	>4.69
		48 hr LC50	50	>100	>0.94	>34.9	>4.69
Mysid (<i>Americamysis bahia</i>)	Fresh	48 hr LC20	50	67.8	3.33	22.1	5.37
			25	91.1	4.18	16.4	5.37
		48 hr LC50	50	70.5	3.46	23.0	5.61
	Weathered	48 hr LC20	50	>100	>4.59	>18.0	>5.90
			50	>100	>0.70	>28.7	>3.93
		48 hr LC50	50	>100	>0.70	>28.7	>3.93
Fathead minnow (<i>Pimephales promelas</i>)	Fresh	96 hr LC20	50	91.1	5.34	36.5	9.78
			25	>100	>5.56	>29.4	>8.33
		96 hr LC50	50	>100	>5.86	>40.0	>10.7
	Weathered	96 hr LC20	50	>100	>5.56	>29.4	>8.33
			50	>100	>0.94	>34.9	>4.69
		96 hr LC50	50	>100	>0.94	>34.9	>4.69
Inland silverside (<i>Menidia beryllina</i>)	Fresh	96 hr LC20	50	50.7	2.49	16.5	4.04
			25	>100	>4.59	>18.0	>5.90

Test Species	Weathering State	Test Endpoint	Oil Loading (g/L)	% WAF (v/v)	BTEX ^I (mg/L)	tPAH ^I (ug/L)	TPH ^I (mg/L)
		96 hr LC50	50	73.8	3.62	24.1	5.87
			25	>100	>4.59	>18.0	>5.90
	Weathered	96 hr LC20	50	>100	>0.7	>28.7	>3.93
		96 hr LC50	50	>100	>0.7	>28.7	>3.93

^I . BTEX: benzene, toluene, ethylbenzene, xylenes; tPAH: sum of detected polycyclic aromatic hydrocarbons and homolog groups; TPH: total petroleum hydrocarbons.

Table 4

Short-term chronic toxicity of two weathered dilbits to *C. dubia* and *A. bahia* as three measures of hydrocarbon exposure.¹

Test Species	Test Type	Dilbit	Endpoint ²	% WAF (v/v)	BTEX ¹ (mg/L)	tPAH ¹ (ug/L)	TPH ¹ (mg/L)
Cladoceran (<i>Ceriodaphnia dubia</i>)	7 day survival and reproduction	Cold Lake Blend	NOEC	25	0.088	5.99	1.67
			IC25	52	0.185	12.5	3.49
		Western Canadian Select	NOEC	6.25	0.0003	1.90	0.094
			IC25	53	0.0024	16.0	0.789
Mysid (<i>Americamysis bahia</i>)	7 day survival and growth	Cold Lake Blend	NOEC	25	0.0015	3.67	0.262
			IC25	39	0.0023	5.72	0.409
		Western Canadian Select	NOEC	25	0.865	6.15	0.561
			IC25	32	1.10	7.82	0.713

¹. BTEX: benzene, toluene, ethylbenzene, xylenes; tPAH: sum of detected polycyclic aromatic hydrocarbons and homolog groups; TPH: total petroleum hydrocarbons.

². NOEC: no observed effect concentration; IC25: concentrations causing 25% inhibition.