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Toxicity in Marine Water and Freshwater

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Contact Information

Publications Coordinator
Environmental Assessment Program
Washington State Department of Ecology
P.O. Box 47600
Olympia, WA 98504-7600
Phone: 360-407-6764

Washington State Department of Ecology – https://ecology.wa.gov

- Headquarters, Olympia 360-407-6000
- Northwest Regional Office, Bellevue 425-649-7000
- Southwest Regional Office, Olympia 360-407-6300
- Central Regional Office, Union Gap 509-575-2490
- Eastern Regional Office, Spokane 509-329-3400

Environmental Effects-Based Concentrations for Weathered Diesel-Range Organics

Toxicity in Marine Water and Freshwater

by

William Hobbs
Environmental Assessment Program
Washington State Department of Ecology
Olympia, Washington 98504-7710

and

Curtis Eickhoff and Karen Lee
Nautilus Environmental Company Inc.
Burnaby, British Columbia, Canada V5N 4N7

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Acknowledgments

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Nautilus Environmental

Mimi Tran
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Dolores Montgomery
Leon Weiks
Karin Bailey
Cherlyn Milne
Kelly Donegan
Jerod Romine
Dean Momohara
Jeff Westerlund

Other Department of Ecology staff:

Arthur Buchan, TCP (HQ)
Peter Adolphson, TCP (HQ)
Russ McMillan, TCP (HQ)
Chance Asher, TCP (HQ)
Kim Wooten, TCP (HQ)
Charles San Juan, TCP (HQ)
Connie Groven, TCP (SWRO)
Joyce Mercuri, TCP (SWRO)
Stephen Nelson, EAP (HQ)
Priscilla Tomlinson, TCP (NWRO)
Brendan Dowling, TCP (SWRO)
Bill Fees, TCP (SWRO)
Fu-Shin Lee, TCP (HQ)
Chris Dudenhoeffer, WQP (HQ) - peer review
Bryson Finch, WQP (HQ) - peer review
Pam Marti, EAP (HQ)
Brian Gallagher, EAP (ERO)

EAP: Environmental Assessment Program
ERO: Eastern Regional Office
WQP: Water Quality Program
HQ: Headquarters
NWRO: Northwest Regional Office
SWRO: Southwest Regional Office
TCP: Toxics Cleanup Program

Abstract

In 2018 the Washington State Department of Ecology (Ecology) Toxics Cleanup Program (TCP) commissioned a study to establish concentration-response relationships or effects-based concentrations for petroleum contaminants that are protective of aquatic life in both the marine water and freshwater environment. This previous study established protective concentrations of total hydrocarbons in the gasoline and diesel range using fresh (unweathered) mixtures. The current follow-up study (2019), establishes protective concentrations for aquatic life in both the marine water and freshwater using weathered diesel-range organics (DRO), as defined by the *Northwest TPH* or *NWTPH* lab method.

Consistent species of test organisms and laboratories (toxicity and hydrocarbon chemistry) were used between the 2018 and 2019 studies. The test organisms included in marine studies: topsmelt (*Atherinops affinis*) and purple sea urchin (*Strongylocentrotus purpuratus*); and freshwater studies: fathead minnow (*Pimephales promelas*) and cladoceran (*Ceriodaphnia dubia*). Contaminated groundwater, impacted almost exclusively by DRO, was used in all the toxicity tests. All toxicty tests were compared with a laboratory negative control water. To confirm that toxicity effects were not due to natural characteristics of the groundwater, an on-site "background" groundwater well was used for comparison to the contaminated groundwater. The total DRO concentrations of the stock test water were 12.3 mg/L during screening, 5.95 ± 0.31 mg/L during the range-finding tests and 4.78 ± 0.25 mg/L during the final tests. Silica gel cleanup on select samples suggested that the DRO were composed largely of petroleum metabolites or polar compounds.

No measurable response was observed for either the marine or freshwater invertebrate species exposed to weathered DRO at the concentrations tested. Final estimates of the no-observable effects concentration (NOEC) threshold in marine waters, based on growth endpoints in topsmelt, was established at a concentration of 2.12 mg/L DRO. A NOEC was established for freshwater, based on the growth endpoint of fathead minnows, at a concentration of 3.04 mg/L DRO.

Introduction

The Washington State Department of Ecology (Ecology) Toxics Cleanup Program (TCP) is responsible for identifying and remediating sites impacted by hazardous substances. In 2018 TCP commissioned a study that defined environmental effects–based concentrations for aquatic organisms exposed to fresh or unweathered total petroleum hydrocarbons (referred to as *Northwest TPH* or *NWTPH* after the lab method) (Hobbs et al. 2018). Using the effects-based concentrations, the TCP's Policy and Technical Support Unit then wrote an implementation memorandum, recommending protective values under WAC 173-340-730(3)(b)(ii) (Environmental effects) – Surface Water Cleanup Standards. This memorandum is currently under review.

The previous 2018 Ecology study defined clear lethal and sublethal effects concentrations (Table 1). A laboratory-based toxicity test using NWTPH-Diesel (Dx) and NWTPH- Gasoline (Gx) was used to determine the no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) for two marine and two freshwater organisms. Certified reference standards for diesel fuel and gasoline were used to create the dilution series. The use of a reference standard created a precise dilution series of concentrations and eliminates other contaminants that would be present in field collections of contaminated waters. Hobbs et al. (2018) recommended a follow-up companion field study to establish effects-based concentrations using contaminated groundwater containing weathered diesel-range organics (DRO) as defined by NWTPH-Dx. Weathered DRO is more commonly found on contaminated sites being managed by TCP.

Table 1: Toxicity point estimates and effects concentrations for fresh, unweathered NWTPH in marine water and freshwater (Hobbs et al., 2018).

		Point Estimates (mg/L)		LOEC (mg/L)	NOEC (mg/L)
		LC50	IC25		
NWTPH-Gx	Marine water	1.7^{Aaff}	$1.7^{G\text{-}Aaff}$	$>1.7^{S\text{-}Aaff}$	$1.7^{S\text{-}Aaff}$
	Freshwater	2.5^{Ppro}	$1.5^{G\text{-}Ppro}$	$2.1^{S\text{-}Ppro}$	$1.0^{S\text{-}Ppro}$
NWTPH-Dx	Marine water	0.68^{Aaff}	$0.19^{F\text{-}Spur}$	$0.05^{F\text{-}Spur}$	$<0.05^{F\text{-}Spur}$
	Freshwater	0.23^{Cdub}	$0.17^{R\text{-}Cdub}$	$0.22^{R\text{-}Cdub}$	$0.15^{R\text{-}Cdub}$

LC = Lethal Concentration; IC = Inhibition Concentration; NOEC = No Observed Effect Concentration; LOEC = Lowest Observed Effect Concentration; superscript "S" = survival endpoint, "G" = growth endpoint, "R" = reproduction, "F" = fertilization endpoint; organism superscript: "Ppro" = *Pimephales promelas*, "Aaff" = *Atherinops affinis*, "Spur" = *Strongylocentrotus purpuratus*, "Cdub" = *Ceriodaphnia dubia*

Weathered Diesel Range Organics

Weathered DRO includes weathered diesel fuel- and oil-range petroleum hydrocarbons. Diesel-contaminated groundwater becomes weathered through microbial degradation, sorption to soils, and dissolution (Lang et al. 2009). Weathering of diesel-contaminated surface waters can also occur through photooxidation and volatilization. Aged diesel fuels in groundwater will contain concentrations of dissolved petroleum-derived chemicals. These degradation products are derived from weathering of the hydrocarbons and can be referred to as polar compounds, petroleum metabolites, or degradates.

Generally, petroleum metabolites contain alcohols, ketones, esters, phenols, aldehydes and organic acids (Lang et al., 2009; Zemo et al., 2017). Some recent non-targeted analysis of weathered DRO at multiple sites in California found the presence of ~760 tentatively identified polar compounds (Mohler et al., 2013).

The ability to identify petroleum metabolites using gas chromatography has improved over time, but many of the compounds are still referred to as an “unresolved complex mixture” (Gough and Rowland 1990). Generally, as the products oxidize and carbon chains are broken and transformed there is a shift towards heavier compounds and longer elution times during analysis (Figure 1). Guidance by TCP states that petroleum metabolites should be considered part of the NWTPH-Dx result for the purposes of site characterization and compliance (Ecology 2016). The use of silica gel cleanup as an analytical preparation method to remove polar petroleum metabolites is permitted only when the groundwater is naturally high in organic matter that would interfere with the quantification of NWTPH.

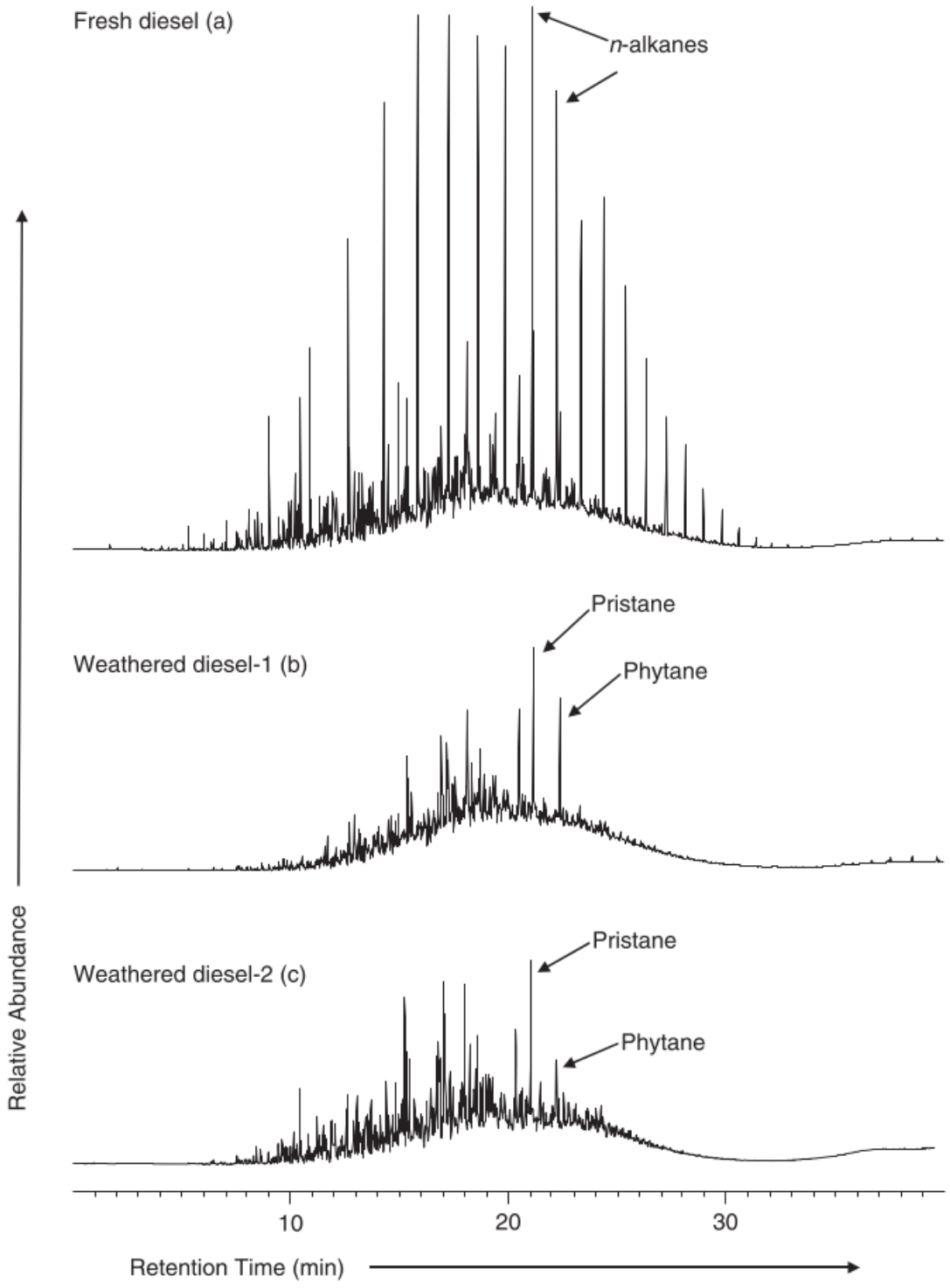

Figure 1: Gas chromatogram of fresh (a) and weathered (b and c) diesel fuel (Lang et al. 2009).

The highlighted compound peaks on the chromatograms describe the degradation of n-alkanes relative to the resistant compounds of pristane and phytane.

The ecological toxicity of petroleum metabolites is not clearly defined. Some research suggests a measurable toxicity of certain petroleum metabolites (Barron et al. 1999; Scarlett et al. 2012; Hellmann-Blumberg et al. 2016), while other researchers have made the case that toxicity is low for most petroleum metabolites (Zemo et al. 2013; O'Reilly et al. 2015; Zemo et al., 2017). This follow-up project did not explicitly address the presence or potential toxicity of petroleum metabolites.

Objectives

The goal of this follow-up study was to establish effects-based concentrations (NOEC and LOEC) for aquatic organisms in freshwater and marine waters exposed to weathered DRO. Contaminated groundwater was used as the source of weathered DRO, with upgradient uncontaminated groundwater used for comparison to the toxicity testing.

The study design for toxicity testing and observed effects–based concentrations followed the original Quality Assurance Project Plan (QAPP) (Hobbs 2017) and an addendum (Hobbs, 2019). For consistency between the 2018 study (Hobbs et al., 2018) and this study, the same species of test organisms were used. Furthermore, the same toxicity laboratory (Nautilus Environmental Company Inc.) with the same brood stock of freshwater cladoceran, *Ceriodaphnia dubia*, was used in this study.

Methods

Detailed descriptions of the methods used and associated quality objectives can be found in Hobbs (2017; 2018) and Marshall (2016). The toxicity tests were carried out by Nautilus Environmental Company Inc. (Nautilus; Burnaby, BC). All water chemistry sub-samples were taken by Nautilus and shipped to Ecology's Manchester Environmental Laboratory (MEL) for analysis. Further details on the toxicity tests by Nautilus can be found in dedicated reports (Appendix A and B).

Test Organisms

Washington's WAC 173-205, section 050, states that effluent samples must be tested using multiple species, including at a minimum one fish and one invertebrate. The toxicity tests in this study were conducted using the same marine and freshwater fish and invertebrates as the previous study examining fresh NWTPH (Hobbs et al., 2018). The organisms included:

Marine water

- Topsmelt (*Atherinops affinis*) – EPA/600/R-95/136, method 1006.0
- Sea urchin (*Strongylocentrotus purpuratus*) – EPA/600/R-95/136

Freshwater

- Fathead minnow (*Pimephales promelas*) – EPA-821-R-02-013, method 1000.0
- Cladoceran (*Ceriodaphnia dubia*) – EPA-821-R-02-013, method 1002.0

Study Sites

The selection of *one* appropriate study site required the screening of several contaminated sites that were sufficiently characterized under TCP's Voluntary Cleanup Program. There were a number of criteria the site needed to meet, based on the previous toxicity study (Hobbs et al., 2018) and willingness to participate, including:

- the groundwater should have concentrations of NWTPH-Dx > 1.0 mg/L
- the groundwater should have concentrations of NWTPH-Gx <1.0 mg/L
- the site must have an upgradient or background groundwater well with Dx and Gx concentrations at or below NWTPH method detection limits
- selection of the site could not interrupt any ongoing cleanup efforts or agreements

In addition, we looked for a site where the primary source of the contamination appeared to be diesel fuel and the spill or release was sufficiently old that in situ weathering of the organics was plausible. Four sites were identified and sampled to screen the water chemistry; two sites in western Washington and two sites in eastern Washington. At each site, the contaminated well and background well were sampled for a suite of parameters. All groundwater wells were assessed for yield, to ensure a sufficient volume of water was available to conduct all toxicity tests. Following the screening of the four sites, one site was selected as the final study site to provide the groundwater for all the toxicity testing.

Field Methods

Sampling of the groundwater wells at the potential study sites followed Ecology Standard Operating Procedures (Marti, 2016a; 2016b). Static water levels were measured at all the monitoring wells upon arriving at the site. Water levels were also measured during the purging process to ensure that the wells were not being over pumped. Wells were purged using industry standard low-flow sampling techniques at a rate of less than 0.5 L/minute using clean dedicated HDPE tubing at each well.

For optimal sampling, the drawdown should not exceed 0.3 ft; this occurred at all wells with the exception of the background well at one site. Due to slow groundwater recharge at this site, the background well was emptied and allowed to recover before measuring field parameters. All wells were purged through a continuous flow cell until field parameters stabilized (pH, temperature, specific conductance, dissolved oxygen, and oxidation reduction potential), signifying that groundwater is being drawn from the aquifer (Marti 2016b).

Wells were sampled from the lowest contaminant concentration to the highest, based on previous site investigation data under TCP's Volunteer Cleanup Program. Samples were collected from the monitoring wells directly from the pump discharge line after they were fully purged. Samples were stored on ice and transported to the lab within analytical holding times.

Once one contaminated and one background well were identified, as per the site selection criteria, for the supply of water for the toxicity testing, 100 L of water was pumped from each well using the low-flow pump. Water was collected into 20 L HDPE carboys and shipped in individual coolers to Nautilus Environmental.

Laboratory Methods

Water Chemistry

The screening samples collected from four candidate sites were analyzed for the suite of parameters listed in Table 2. The NWTPH-Dx method includes the diesel range organic (DRO) and the heavier residual range organic (RRO) fractions. The majority of the analyses were conducted at Ecology's Manchester Environmental Laboratory, with the exception of volatile / extractable petroleum hydrocarbons (VPH/EPH) and sulfides, which were analyzed at Analytical Resources Inc. in Tukwila, WA.

Samples were analyzed from each of the four screening sites for petroleum hydrocarbons using VPH/EPH methods in addition to the NWTPH because there is greater resolution of the carbon fractions within the sample using VPH/EPH methods (WA-EPH), but there is a difference in how the samples are cleaned up (Table 3). The WA-EPH method calls for silica gel cleanup with an additional sulfuric acid step, while the NWTPH-Dx method uses silica gel cleanup only if there are known biogenic interferences and the sulfuric acid step is not routine.

Table 2: Laboratory measurement methods and analytes.

Analyte	Expected Reporting limit	Actual Reporting limit	Sample prep method	Analytical (instrumental) method
NWTPH-Dx – diesel range organics (DRO)	500 µg/L	150 µg/L	SW3535 and SGC*	NWTPH-Dx
NWTPH-Dx – residual range organics (RRO)	500 µg/L	350 µg/L	SW3535 and SGC*	NWTPH-Dx
NWTPH-Gx	250 µg/L	70 µg/L	SW5030B	NWTPH-Gx
BETX‡	1.0–2.0 µg/L	1.0–2.0 µg/L	SW5030B	SW8021B
Polycyclic aromatic hydrocarbons‡	0.05 µg/L	0.05 µg/L	SW3510C	SW8270DSIM
Volatile petroleum hydrocarbons	50 µg/L	50 µg/L	SW5030B	WA VPH
Extractable petroleum hydrocarbons	40 µg/L	40 µg/L	SW3510C	WA EPH
Metals (excl. Hg)‡	0.02–1.00 µg/L‡	0.02–1.00 µg/L‡	NA	EPA 200.8
Mercury	0.05 µg/L	0.05 µg/L	MEL Hg Prep	EPA 245.1
Hardness	0.3 mg/L	0.3 mg/L	NA	SM2340B
Total dissolved solids	0.95 mg/L	27-32 mg/L	NA	SM2540C
Major cations	0.025 µg/L	0.025 µg/L	EPA 200.7	EPA 200.7
Major anions‡	0.025–0.3 µg/L	0.025–6.0 µg/L	NA	EPA 300.0
Nitrate-nitrite	0.01 µg/L	0.01-0.12 µg/L	NA	SM4500NO3I
Ammonia	0.01 mg/L	0.01	NA	SM4500 NH3H
Sulfides	0.05 mg/L	0.05	NA	SM4500-S2
Dissolved organic carbon	0.5 mg/L	0.5 – 5.0 mg/L	NA	SM5310B

‡reporting limits are compound-specific.
*If estimated results below the reporting limit are needed, lab may need to extract using SW3510C.
SGC = silica gel cleanup.

Table 3: Comparison of NWTPH-Dx and WA-EPH methods.

	NWTPH-Dx	WA-EPH
Sample container	1L amber glass	1L amber glass
Sample preservation	1:1 HCl; cool to 4°C	1:1 HCl; cool to 4°C
Extraction solvent	methylene chloride	methylene chloride
Extraction apparatus	separatory funnel (SW3510C) Solid Phase (SW3535A)	separatory funnel (EPA 3510C)
Solvent exchange	NA	hexane
Cleanup	silica gel (TPH-D; add free flowing to sample)	silica gel (EPA 3630)
	centrifugation	centrifugation
	sulfuric acid (not routine)	sulfuric acid (aromatic fraction)
Instrument	gas chromatography - flame ionization detector (GCFID)	gas chromatography - flame ionization detector (GCFID)

During toxicity testing, cleanup steps were not used on the samples collected for NWPTH-Dx analysis. After toxicity testing was completed, a subset of extracts were selected for free-flowing silica gel cleanup and a silica gel cleanup with a sulfuric acid cleanup. The two gel cleanup methods were compared to the original result to detail the loss of polar metabolites, and understand the degree to which the DRO was weathered. These cleanup steps are detailed in the method and intended for use on samples that contain naturally occurring non-petroleum organics to reduce the interference of these compounds on analytical test results for hydrocarbons (Ecology, 1997).

Toxicity Testing

During screening of the groundwater wells, samples were also collected from the background wells to evaluate any possible effects caused by the on-site conditions of the groundwater. *Ceriodaphnia dubia* can be sensitive to dissolved solids (Mount et al. 2016 and 2019) and therefore a 7-day survival and reproduction test was used to evaluate the suitability of the groundwater based on background wells. Results were compared with laboratory negative controls (Appendix A).

Following the screening of four potential study sites, a single site was chosen for the study toxicity testing. Four chronic toxicity tests (Table 4) two in marine water and two in freshwater were performed for the project. The tests were based on a dilution series using a stock solution mixed from groundwater contaminated with weathered DRO and laboratory control water. All test results were compared to the negative laboratory control waters. The site background groundwater well was used to evaluate the potential effects of agents not related to weathered diesel on the test organisms. However, in some cases the site control had measurable levels of petroleum hydrocarbons and therefore it was not used as a control for the statistical comparison of concentration-response results. The background groundwater well does allow for a comparison to groundwater collected and handled similarly to the test water.

All groundwater used for the toxicity testing was shipped to Nautilus Environmental within 24 hours of collection. The water was combined and stored at 4°C in teflon-lined drums for the freshwater and marine toxicity tests. Prior to beginning the toxicity tests, the mixed groundwater was sampled to confirm the exposure concentrations of NWPTH-Dx.

The range-finding tests were conducted at a dilution of 100, 25, 6.3, 1.6, 0.39 and 0.10 (% v/v) beginning with the mean concentration of 5.07 mg/L DRO for the topsmelt, 6.09 mg/L for the echinoderm tests, 6.23 mg/L for the fathead minnow test and 5.53 mg/L for the *Ceriodaphnia* test. Following the range-finding tests for NWTPH-Dx, it was decided that the dilution series mixed for the definitive chronic tests would be 100, 75, 50, 25, and 12.5 (% v/v).

Table 4: Description of chronic toxicity test methods.

Test Organism and EPA Method	Test Type	Chamber Size	Solution Volume	# Organisms Per Chamber	# Replicates (Minimum)	Age	Temperature	Aeration	Feeding	Endpoints
Ceriodaphnia dubia EPA-821-R-02-013, method 1002.0	7-day static renewal (80% renewal daily)	20 mL	15 mL	1 from a female with ≥ 8 neonates in the 3rd or subsequent broods	10	< 24 hrs and within an 8-hr age range	25° ± 1°C	if DO < 2.0 mg/L	0.1 mL YCT and 0.1 mL algal suspension daily	Number of survivors at 7 days and number of neonates per female at 3 broods.
Pimephales promelas EPA-821-R-02-013, method 1000.0	7-day static renewal (80% renewal daily)	375 mL	250 mL	minimum 10	4	< 24 hrs (< 48 hrs if shipped)	25° ± 1°C	if DO < 4.0 mg/L	0.1 g wet weight per container 3 times daily at 4-hour intervals or 0.15 g wet weight per container twice daily at 6-hour intervals: no food in final 12 hours	Survival rate; Total weight of survivors divided by the initial count (biomass); Total weight of survivors divided by the final count (weight).
Atherinops affinis EPA/600/R-95/136, method 1006.0	7-day static renewal (80% renewal daily)	1000 mL	500 mL	minimum 5	5	9 - 15 days post-hatch	20° ± 1°C	if DO < 4.0 mg/L	Twice daily (40 Artemia nauplii/fish at each feeding) morning and afternoon; no food on day 7	Survival rate; Total weight of survivors divided by the initial count (biomass); Total weight of survivors divided by the final count (weight).
Strongylocentrotus purpuratus EPA/600/R-95/136	24-hr static	30 mL	5 mL	about 5 X 10^7 sperm/mL and about 2000 eggs/mL	4	< 4 hrs after collection of gametes	20° ± 1°C	if DO < 4.0 mg/L	NA	Fertilization of eggs.

DO: dissolved oxygen; YCT: yeast-cerophyl-trout mixture

Additional groundwater was needed from the study site following the range-finding tests and this resulted in a lower starting mean DRO concentration for the final toxicity tests: 3.14 mg/L for the topsmelt, 2.71 mg/L for the echinoderm, 4.33 mg/L for the fathead minnow and 4.12 mg/L for the *Ceriodaphnia* test. The mortality of the organisms was recorded as both “ecological” mortality, where the anesthetizing properties of the contaminants incapacitate the organism, and absolute mortality of the organism.

All toxicity tests were carried out in dedicated climate-controlled rooms. All tests were conducted under full-spectrum lighting, with the exception of the echinoderm tests due to the short test duration. Water quality monitoring for temperature, pH, dissolved oxygen, salinity (if applicable), and conductivity were conducted for each toxicity test. The EPA methods describe the optimal conditions for these parameters, which were documented by Nautilus along with any deviations (Appendices A and B).

The general conditions of the bioassays met the following (as per Marshall, 2016):

- The approved standard method for chronic testing using EPA-821-R-02-012 (USEPA, 2002).
- Dual endpoint tests for chronic toxicity testing.
- Illumination for 16 hours at 10 - 20 μE/m^2/s (50 - 100 ft-c) followed by 8 hours of darkness.
- The performance criteria (survival, growth, and reproduction) for control samples in each bioassay.

Statistical Methods

Chronic toxicity data from each test was used to develop concentration-response relationships based on the biological endpoints of survival, growth, reproduction, and fertilization. Point estimates calculated in this study included the lethal concentration where 50% mortality is observed (LC50), inhibitory concentration where the growth of 50% of the organisms is impeded (IC50), and inhibitory concentration where the growth of 25% of the organisms is impeded (IC25). Regression analysis was used to establish LC50, IC50, and IC25 point estimates using tests described in Table 5.

In all cases, the laboratory control prepared with laboratory dilution water, was used as the negative control for statistical comparison. Hypothesis testing was used to determine the NOECs and LOECs. Tests for significance are used to establish independence or a difference between the “effect” or unacceptable concentration and a control or acceptable concentration. Homogeneity of variances and normality was tested to determine the appropriate statistical test. Typically a Shipro-Wilks test for normality is used and a Bartlett’s test is used for homogeneity. The type of test used depended on data variability and independence of the test concentrations in the dilution series.

The fundamental difference between the NOEC and LOEC and the point estimates is the effects concentration is established directly from biological responses at the individual test concentrations, whereas the point estimates are estimated from the modeled concentration-response relationships for the biological endpoints (survival and growth). The point estimate should always be presented with the 95% confidence limits to show the uncertainty in the estimate. When considering sublethal inhibitory effects (e.g. IC25) at the extremes of the concentration-response curve, the confidence limits may have a greater range (less certainty) than the LC50 endpoints. It is possible to have low toxicity point estimates that overlap with measured NOECs.

All statistical calculations were made using the software Comprehensive Environmental Toxicity Information System (CETIS™), Tidepool Scientific Software and additional concentration-response curves were fitted and graphed using the drc package in R (Ritz et al., 2015; R Core Team, 2019). The decisions on the appropriate statistical tests are built into the CETIS software and largely follow USEPA guidance (2002). Each of the tests used in this study are described in Table 5.

Table 5: Statistical tests used during this study and the appropriate use.

Statistical test	Use
Point estimates (modeling data)	
Nonlinear regression	An interpolation method from a nonlinear regression (e.g., exponential function).
Logistic regression	A regression model based on binary (e.g. dilution series) results. Also called a logit regression.
Spearman-Kärber	A nonparametric method for estimating the LC50.
Linear interpolation	Simple linear interpolation from a linear regression.
NOEC/LOEC (significance tests of independence between test populations)	
Steel Many-one Rank Sum test	Used when the data have heterogeneous variance and an equal number of replicates.
Dunnett multiple comparison	Used when the data has homogenous variance and an equal number of replicates.
Fisher Exact/Bonferroni-Holm test	Used when the data has homogenous variance and an unequal number of replicates. The Bonferroni-Holm correction is used when making multiple comparisons and reduces the chances of a Type 1 error (false positive).

Data Quality

Blanks

All laboratory method blanks analyzed as part of MEL's QC were below the method detection limit (Appendix C, Table C-1). Clean test waters from Nautilus were submitted as blanks during the mixing, range-finding, and final chronic testing phases of the project. In three samples the blank from Nautilus contained trace amounts of detectable DRO, however concentrations were near the method reporting limit (MRL) and no further action was taken.

During the screening of the study sites, a filter blank for dissolved metals contained detectable concentrations of lead (sample 1812024-10). This result highlights background concentrations of lead in filter media and HDPE bottles and has been noted during other studies. Sample results were not further qualified, but interpreted accordingly.

Precision

Precision is a measure of variability between results of replicate measurements that is due to random error. Precision is measured using the relative percent difference (RPD) between replicate samples. The samples collected for screening the study sites generally had excellent precision for laboratory duplicates and met the project measurement quality objectives (MQOs).

For all samples submitted during toxicity testing, laboratory replicate precision for the lab control standard and duplicates met the project MQOs (<40% RPD) for all but one NWTPH-Dx QC sample (Appendix C, Table C-2). This was due to low recoveries of the spikes and attributed to a problem with the extraction of the sample. As a result, the blank sample associated with this batch was qualified as "UJ" – the analyte not detected at or above the estimated detection limit. No further corrective action was necessary.

Replicate samples collected during the toxicity tests for NWTPH-Dx were generally well below the project MQO (<40%). One replicate sample (1904072-24) had an RPD of 47% for DRO and 57% for RRO. Both results were used in the calculation of the test DRO concentrations, which directly accounts for this variability.

Precision for the toxicity tests is measured and controlled through the use of reference toxicants. In comparison to an inter-laboratory study by the United States Environmental Protection Agency (USEPA, 2000), the coefficient of variations (CV) for precision around the toxicity tests were at or lower than the 25^{th} percentile (Table 6). The echinoderm (sea urchin) fertilization test had the lowest precision and was at the 25^{th} percentile CV for the EPA study. All tests met the internal historical performance (% CV) of the reference toxicant test performed at the laboratory, summarized as the mean ± 2 standard deviations (Appendix B).

Table 6: Percentiles of the coefficient of variation (CV) for the reference toxicants (USEPA, 2000).

Test Organism	Method	EPA Percentiles			NWTPH-Dx	
		25th	50th (median)	75th	Range-finding CV	Final test CV
Fathead minnow larval survival	1000.0	0.26	0.39	0.48	0.12	0.13
Fathead minnow larval growth	1000.0	0.22	0.37	0.53	0.15	0.15
Ceriodaphnia survival	1002.0	0.21	0.30	0.43	0.05	0.04
Ceriodaphnia reproduction	1002.0	0.25	0.33	0.49	0.18	0.20
Topsmelt larval survival*	1010.0	0.42	0.42	0.42	0.21	0.17
Topsmelt larval growth*	1010.0	0.31	0.31	0.31	0.20	0.16
Echinoderm fertilization	EPA/600/ R-95/136	0.40	0.50	0.69	0.41	0.36

* One lab participated using this method in the EPA study.
CVs are calculated based on the most recent 20 tests by Nautilus.

All toxicity tests required daily renewal of solutions and fresh mixtures, with the exception of the echinoderm fertilization test. It is desirable to have the concentrations of the stock solutions remain consistent during the tests. The CV among the daily stock solutions for each test can be viewed as a measure of precision, which affects the ability to dilute the water and measure the concentration in the lab. The CV for 100% nominal concentration of DRO in the final chronic tests was 17% for the topsmelt and fathead minnow and 5% for the *Ceriodaphnia dubia*. The CV for the 100% nominal concentration of RRO in the final tests were, 22% for the topsmelt, 9% for the fathead minnow and 5% for the *Ceriodaphnia dubia*. There is no defined threshold for assessing the CV among daily test solutions, however the tests can be summarized as having a variability of around 20% or less in starting stock water concentrations which is less than the variability for the laboratory replicates.

Bias

Bias is the difference between the sample mean and the true value. Laboratory bias was addressed by analyzing lab control samples, matrix spikes, and/or standard reference materials. Carbazole was not recovered from the laboratory matrix spike and the result for the sample 1812024-01 was rejected. The sample was not rerun because a replicate sample (1812024-02) had adequate spike recovery and a reliable result. Fluorene had a surrogate recovery below the MQOs for samples 1903038-1, -2 and -3. These sample results should be viewed as biased low, despite no laboratory qualifiers being manually added. All other samples for the screening parameters met the laboratory MQOs for bias.

Table 7: Laboratory recovery of sample surrogates and control samples.

Project stage	Sample surrogate recovery (%)					
	n	median	mean	sd	min	max
Screening	12	97	96	11	77	117
Mixing	19	107	104	12	82	124
Range-finding	101	99	103	16	66	145
Final toxicity tests	86	101	98	11	70	120
QC type	**Laboratory control sample (LCS) recovery (%)**					
LCS	18	83	85	10	75	114
LCS duplicate	18	86.5	85	16	34	112

Sensitivity

Sensitivity is a measure of the capability of a method to detect a substance. For each parameter, the laboratory was able to achieve the desired method detection limit (MDL) and reporting limit (RL) set by the QAPP (Hobbs, 2018).

The sensitivity of the toxicity tests is dependent on the number of replicates per concentration. The sensitivity is assessed by comparing the treatment results against the control tests that are run concurrently. There is a recommended minimum significant difference (MSD) for each method (USEPA, 2000). The MSD is the smallest difference between the control and another test treatment that can be determined as statistically significant. The MSD is often expressed as the %MSD of the mean control value. In Washington State, WAC 173-205 defines a “Chronic statistical power standard” that represents the maximum %MSD of the test control. The chronic statistical power standard is 39%, meaning the percent difference in a statistically significant response (i.e., %MSD) must be less than or equal to 39% to be acceptable. Negative laboratory controls were used to assess all toxicity testing. All final toxicity tests had an MSD below 39% (Table 8).

Table 8: The minimum significant difference (MSD) between the toxicity tests and the control.
In accordance with WAC-173-205 the MSD should be below 39%.

Organism	Lab Control : Background well			Lab Control : DRO		
	Survival	**Growth (biomass)**	**Growth (weight)**	**Survival**	**Growth (biomass)**	**Growth (weight)**
Fathead minnow	6.1	9.5	7.4	14.9	19.1	12.4
Topsmelt	10.8	20.1	19.7	16.0	30.1	26.4
	Survival	**Reproduction**		**Survival**	**Reproduction**	
Ceriodaphnia dubia	NA	12.8		NA	19.8	
	Fertilization			**Fertilization**		
Echinoderm (purple sea urchin)	8.9			9.1		

NA=not applicable due to no adverse effect on survival.

Results and Discussion

Screening Study Sites

Four sites were screened to determine if they were suitable for the supply of test water for this study (Appendix D, Table D-1). Each potentially contaminated well was sampled in duplicate, along with a paired background well that was situated upgradient in the predominant groundwater flow direction. At Site 1, an upgradient well was not identified. Subsequently a well that is at a similar groundwater elevation to the contaminated well but located outside the impacted area was sampled. During sampling, water from the contaminated wells, with the exception of Site 2, had a hydrocarbon odor and sheen.

The only site that met all of the selection criteria was Site 1 (Table 9). The DRO concentrations in replicate samples from the contaminated well was 12.4 and 12.1 mg/L, with RRO of 9.1 and 9.3 mg/L. Additional analysis of groundwater from the contaminated well for VPH/EPH showed detectable concentrations of aliphatic and aromatic hydrocarbons in the C16-C21 range. However, concentrations were quite low, suggesting that EPH cleanup steps for the analysis had removed the weathered DRO compounds. To confirm the original DRO results, the VPH/EPH contract lab ran the remaining sample for NWTPH-Dx; these samples were qualified as estimates due to an exceedance of the hold time. The DRO concentrations were similar, but had lower RRO concentrations than the original MEL analysis (Appendix D, Table D-1).

In addition to the confirmatory analysis by a contract lab, MEL also re-ran the original extracts with silica gel cleanup and silica gel cleanup with sulfuric acid; the latter cleanup more closely emulates the more aggressive cleanup of the WA-EPH method. The DRO concentrations were still detectable above 1.00 mg/L (the site selection criteria), but were less than half the original concentration (Table 9). The background well on Site 1 did contain detectable concentrations of DRO when analyzed without any cleanup steps (0.52 mg/L).

Table 9: NWTPH-Gx, Diesel Range Organics (DRO), and Residual Range Organics (RRO) results from groundwater at all four screening sites.

All results are in mg/L.

		Sample Date	MEL ID	DRO	RRO		DRO - SGC		RRO - SGC		DRO - SGC+ H_2SO_4	RRO - SGC + H_2SO_4	NWTPH-Gx	
Site 1	Contaminated	12/13/2018	1812024-01	12.1	9.08		9.43		6.55	J	4.83	2.44	0.07	U
		12/13/2018	1812024-02	12.4	9.31		9.49		6.55		6.03	3.36	0.07	U
	Background	12/13/2018	1812024-03	0.52	0.39	U	0.15	U	0.39	U	NA	NA	0.07	U
Site 2	Contaminated	12/17/2018	1812024-04	0.43	0.37	U	0.26	U	0.37	U	NA	NA	0.07	U
		12/17/2018	1812024-05	0.38	0.38	U	0.2	U	0.38	U	NA	NA	0.07	U
	Background	12/17/2018	1812024-06	0.23	0.38	U	0.15	U	0.38	U	NA	NA	0.07	U
Site 3	Contaminated	12/18/2018	1812024-07	0.88	0.38	U	0.6		0.38	U	NA	NA	0.65	
		12/18/2018	1812024-08	0.92	0.36	U	0.63		0.36	U	NA	NA	0.678	
	Background	12/18/2018	1812024-09	0.35	0.38	U	NA		NA		NA	NA	0.203	
Site 4	Contaminated	3/18/2019	1903038-01	0.59	0.41	U	0.44		0.38	U	NA	NA	0.102	
		3/18/2019	1903038-02	0.58	0.39	U	0.39		0.38	U	NA	NA	0.095	
	Background	3/18/2019	1903038-03	0.34	0.39	U	0.16		0.39	U	NA	NA	0.07	

SGC = silica gel cleanup; H_2SO_4 = sulfuric acid cleanup; U = result is non-detectable;
NWTPH-Gx = NWTPH gasoline range.

Analysis of additional parameters from the groundwater wells on Site 1 showed detections of several PAHs near the analytical detection limit (Appendix D, Table D-1). Dissolved metals (cadmium, copper, lead and zinc) were detected in samples from the contaminated well at Site 1. These results were evaluated against the chronic aquatic life criteria for Washington State (WAC 173-201A) and found to be well below concentrations that would suggest an impact to aquatic organisms. Conventional parameters were similar between the contaminated well and the on-site background well, with the exception of dissolved organic carbon (DOC). The higher DOC results in the contaminated well likely represented weathered hydrocarbons and not naturally occurring organics, which would also be present in the on-site background well.

Screening of the on-site background wells by Nautilus Environmental was carried out to determine if the *Ceriodaphnia* brood would be sensitive to this water. A complete 7-day, three brood exposure of the *Ceriodaphnia* was run for each site and survival and growth were assessed against the laboratory negative control (Appendix A). Sites 1-3 passed the screening *Ceriodaphnia* tests, meaning the upgradient groundwater collected at these sites would act as a suitable on-site background.

Based on the screening portion of this study, Site 1 was selected to be used as a supply of source water for the toxicity testing. The contaminated well on Site 1 is situated in the vicinity of underground diesel fuel tanks and waste oil tanks. Contamination at the site was first identified in 1990, however the specific age of the DRO in groundwater and level of biodegradation is not clear.

Water Chemistry

Mixing and Range-Finding Tests

Groundwater was collected from the study site and shipped directly to Nautilus Environmental for the toxicity testing. The water was mixed into larger drums, lined with Teflon, for storage over the period of testing. The holding time for the water does not meet the analytical method for NWTPH-Dx. However, the DRO and RRO content of the test water was sampled and characterized prior to beginning the range-finding toxicity testing (Table 10). The stock water had concentrations (mean ± SD) of 5.95 ± 0.31 mg DRO/L and 4.51 ± 0.23 mg RRO/L. Residues of DRO were also measurable in the background water, 0.28 ± 0.02 mg L, but RRO was undetectable. The relative standard deviations among triplicate samples of the 100% stock solution and a 50% dilution were low (≤ 10%) providing confidence that there was a relatively homogenous stock mixture for testing. Furthermore, the 50% dilution concentration was 2.7 ± 0.29 mg DRO/L which matches the nominal concentration (i.e. 50% of the stock concentration), giving us confidence that the stock solution could be reliably diluted.

Table 10: DRO and RRO results for the stock test water. Sample surrogate recovery for each analysis is also included.

Relative standard deviation describes the variability among triplicate samples for 100% stock solution and 50% dilution.

Sample ID	sample date	MEL ID	analysis date	DRO (mg/L)		% relative standard deviation	RRO (mg/L)		% spike rec.
mix-stk-100-0-1	2/5/2019	1902025-01	2/13/2019	6.29	J		4.75	J	124
mix-stk-100-0-2	2/5/2019	1902025-02	2/13/2019	5.67	J	5.3	4.29	J	118
mix-stk-100-0-3	2/5/2019	1902025-03	2/13/2019	5.9	J		4.5	J	109
mix-stk-0.5-0-1	2/5/2019	1902025-04	2/13/2019	2.77	J		2.05	J	113
mix-stk-0.5-0-2	2/5/2019	1902025-05	2/13/2019	2.38	J	10.8	1.71	J	101
mix-stk-0.5-0-3	2/5/2019	1902025-06	2/13/2019	2.95	J		2.13	J	117
mix-control-0-1	2/5/2019	1902025-07	2/14/2019	0.3	J		0.38	UJ	103
mix-control-0-2	2/5/2019	1902025-08	2/14/2019	0.29	J	5.3	0.38	UJ	109
mix-control-0-3	2/5/2019	1902025-09	2/14/2019	0.27	J		0.38	UJ	96
mix-Ppro-BLNK-0-1	2/5/2019	1902025-10	2/14/2019	0.15	UJ	NA	0.38	UJ	109
mix-Cdub-BLNK-0-1	2/5/2019	1902025-11	2/14/2019	0.15	UJ	NA	0.38	UJ	103
mix-MARINE-BLNK-0-1	2/5/2019	1902025-12	2/14/2019	0.17	J	NA	0.37	UJ	109
RF-Ppro-100-7-1	2/26/2019	1902033-41	3/8/2019	6.69			8.2		107
RF-Ppro-100-7-2	2/26/2019	1902033-42	3/8/2019	6.85		1.3	8.71		107
RF-Ppro-100-7-3	2/26/2019	1902033-43	3/8/2019	6.72			8.69		107

Some results were qualified as estimates (“J”) due to arrival at the lab at a temperature slightly higher than 4°C.

The NWTPH-Dx chromatograms of the contaminated groundwater showed a similar “unresolved complex mixture” to the screening samples and this composition remained consistent throughout the mixing and range-finding tests (Appendix D, Figure D-2). The range-finding test followed a 100, 25, 6.3, 1.6, 0.4, and 0.1 dilution series (%v/v). Samples were collected each day during renewal of the test water for the 100, 6.3 and 0.4 dilutions. The waters from the 0.4 dilution were at or below detection for all test organisms. As described previously in the *Data Quality* section, there was an acceptable amount of variability among the daily test waters; this resulted in a reliable relationship between measured DRO concentrations and the dilution series concentrations (Figure 2). Based on these relationships mean DRO water concentrations were assigned to all dilutions of the test (Table 11).

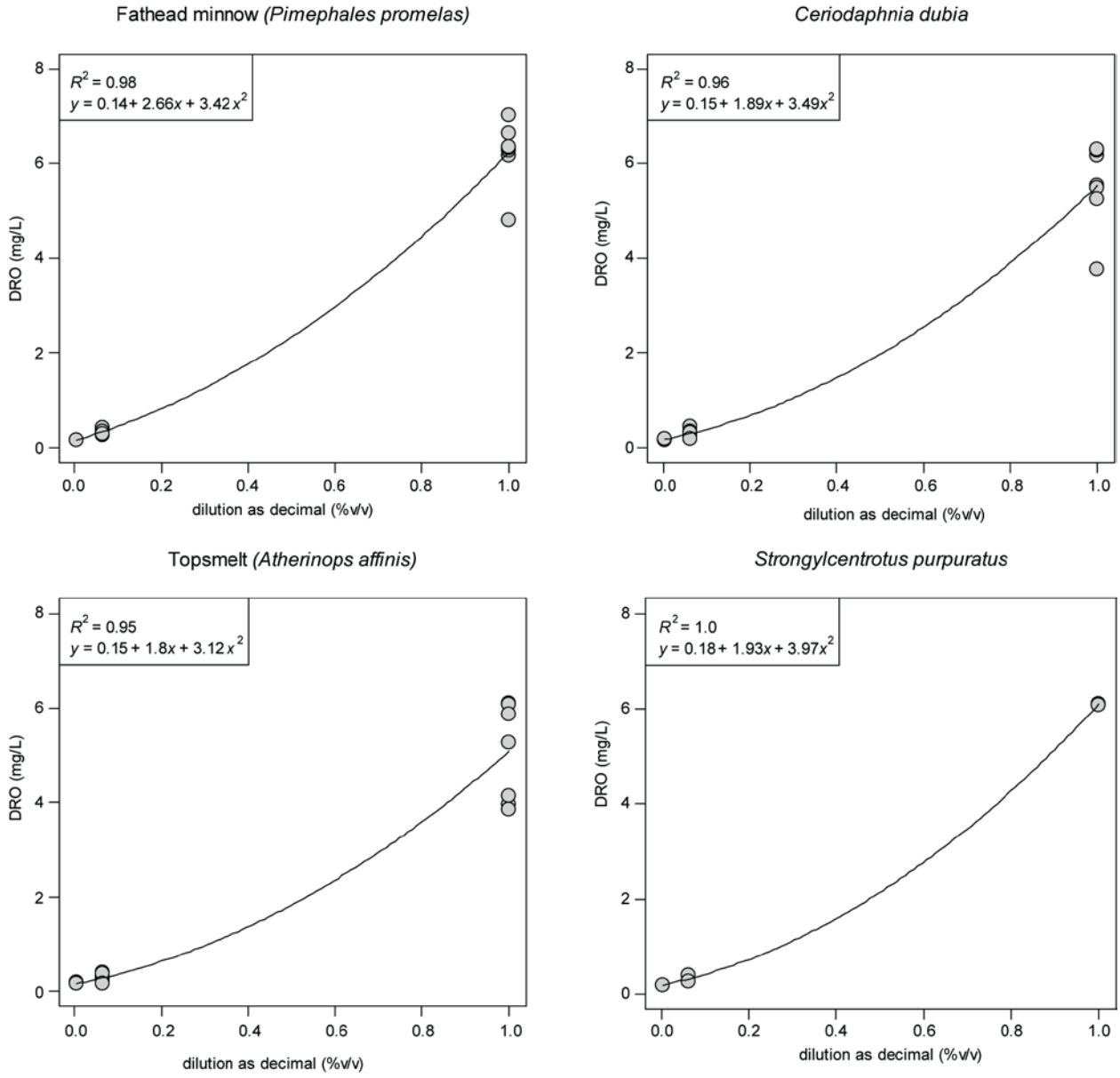

Figure 2: Polynomial relationships between measured DRO concentrations and dilution series concentrations (as % volume) for all organisms during the range-finding tests.

Table 11: Mean concentrations of the DRO used in each range-finding chronic toxicity test.
Median concentrations of the DRO measured throughout the tests are in parentheses.

Dilution	Topsmelt	Purple sea urchin	Fathead Minnow	Ceriodaphnia
100	5.07 (5.26)	6.09	6.23 (6.33)	5.53 (5.53)
25	*0.79*	*0.91*	*1.00*	*0.89*
6.3	0.27 (0.28)	0.32	0.32 (0.3)	0.29 (0.28)
1.6	*0.18*	*0.21*	*0.19*	*0.18*
0.4	0.16 (0.15) U	0.19	0.15 (0.15) U	0.16 (0.16) U
0.1	*0.15 U*	*0.18*	*0.15 U*	*0.15 U*
On-site background	0.29 (0.29)	0.31	0.2 (0.2)	0.26 (0.26)
Lab control	0.15 (0.15) U	0.15 U	0.16 (0.16) U	0.19 (0.19) U

Italicized values are interpolated from the relationships in Figure 2 based on mean concentrations.
U qualified results are non-detect

During the range-finding tests, test waters from the fish bioassay chambers were collected prior to renewal. These samples were termed “stale” and analyzed for both DRO and RRO to look at loss of hydrocarbons/polar compounds over the daily exposure (Appendix D, Table D-2). For all of the samples except one (RF-Aaff-100-7-1-S) there was about a 10-20% loss of DRO and RRO. Organismal uptake may have been the main mechanism of loss from the test chambers. Loss could also be due to binding of the metabolites/hydrocarbons to the chamber containers or organics within the chambers. The difference between fresh and stale test waters was not explicitly incorporated into the results of the toxicity tests.

Final Chronic Tests

Additional groundwater was collected from the study site prior to the final toxicity testing to ensure there was a sufficient volume to complete all of the tests. The new water was combined with remaining water at Nautilus, mixed and subsamples were taken to confirm the DRO concentration and ensure a homogenous mixture (Table 12). The starting concentration (mean ± SD) was 4.78 ± 0.25 mg DRO/L and 4.42 ± 0.39 mg RRO/L. Similar to the stock water prior to the range-finding tests, there was a low relative standard deviation among triplicate samples (5%), giving us confidence that the water was a homogenous mixture. In addition to verifying the starting DRO and RRO concentration of the stock water mixture, the chromatograph of the unresolved complex mixture of DRO and RRO was consistent among the testing stages (screening, range-finding and final chronic tests) (Appendix D, Figure D-2).

Table 12: DRO and RRO results for the stock test water prior to final testing. Sample surrogate recovery for each analysis is also included.

Sample ID	Sample date	MEL ID	Analysis date	DRO (mg/L)		% relative standard deviation	RRO (mg/L)		% spike rec.
Mix-stk-100-1	3/20/2019	1903061-01	3/26/2019	4.5		5.2	3.99		86
Mix-stk-100-2	3/20/2019	1903061-02	3/26/2019	4.84			4.51		86
Mix-stk-100-3	3/20/2019	1903061-03	3/26/2019	4.99			4.75		90
Mix-stk-0-1	3/20/2019	1903061-04	3/26/2019	0.31		NA	0.45		82

Based on the results of the range-finding tests, the decision was made to carry out the final toxicity tests with a dilution series of 100, 75, 50, 25, and 12.5. Subsamples were collected throughout the test during the daily renewal of the test water from the 100, 50 and 25 dilutions (Figure 3). The relationships between measured DRO and the nominal dilution series concentrations (%v/v) were strong, enabling us to accurately interpolate the DRO concentrations for the 75 and 12.5 dilutions (Table 13). Based on the findings from the range-finding tests, both invertebrate tests were carried out using only the 100% stock water with concentrations analyzed at the time of testing, 2.71 mg/L for the echinoderm test (purple sea urchin fertilization) and 4.12 mg/L for the *Ceriodaphnia* test.

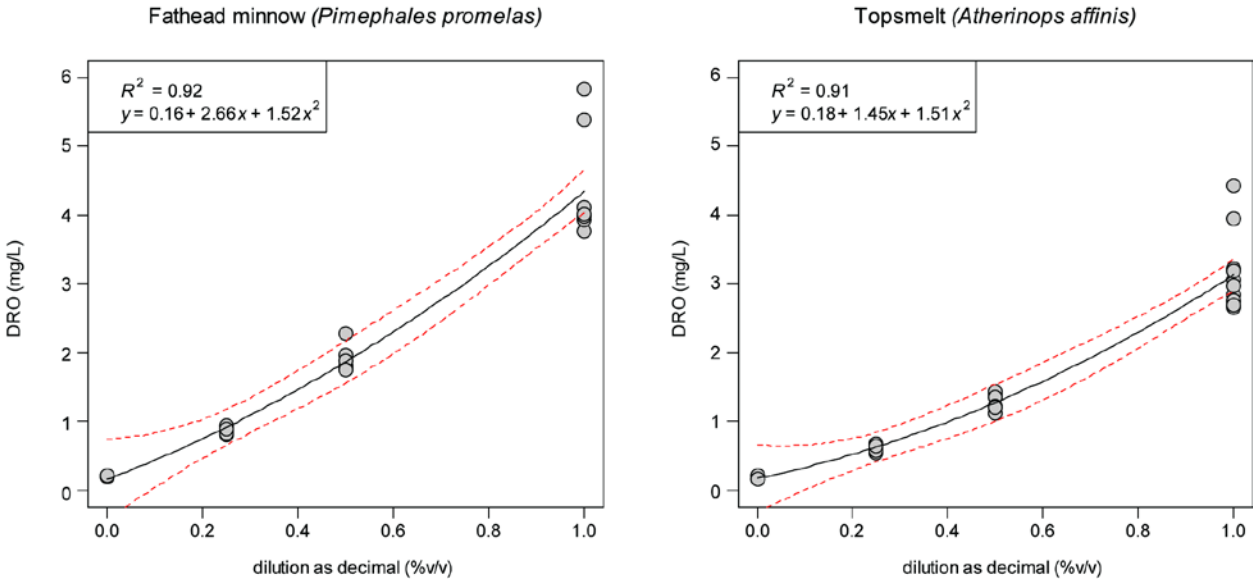

Figure 3: Polynomial relationships between measured DRO (mg/L) and the nominal dilution series (%v/v) for the final toxicity tests.

Table 13: Final mean concentrations of the DRO used in each chronic toxicity test.
Median concentrations of the DRO measured throughout the tests are in parentheses.

Dilution (%v/v)	Topsmelt	Purple sea urchin	Fathead minnow	*Ceriodaphnia dubia*
100	3.14 (2.97)	2.71	4.33 (4.00)	4.12 (4.13)
75	*2.12*	NA	*3.04*	NA
50	1.29 (1.29)	NA	1.90 (1.82)	NA
25	0.62 (0.62)	NA	0.89 (0.89)	NA
12.5	*0.37*	NA	*0.51*	NA
On-site background	0.20 (0.20)	0.22	0.21 (0.21)	0.25 (0.25)
Lab control	0.16 (0.16) U	0.16 U	0.17 (0.17) U	0.17 (0.17) U

Italicized values are interpolated from the relationships in Figure 3 based on mean concentrations.
U qualified results are non-detect; NA = not applicable

Silica Gel Cleanup

As per the TCP guidance on contaminated sites assessment (Ecology, 2016), when analyzing for NWTPH-Dx it is permissible to use silica gel cleanup methods if the waters contain a significant amount of naturally occurring non-petroleum organics which may contribute to biogenic interferences. However, the issue is that weathered DRO contains a number of unresolved metabolite compounds that may be removed during the cleanup steps. During the current project a subset of sample extracts from the final fish toxicity tests were re-analyzed following two separate silica gel cleanups using: (1) free-flowing silica and (2) free-flowing silica with a sulfuric acid cleanup. The latter method is prescribed in the Ecology TPH methods document (Ecology, 1997); however, it is our understanding that some analytical labs typically use only the free-flowing silica.

This additional analysis was conducted to provide an example of the range in final DRO and RRO concentrations following cleanup, not to provide guidance on whether and how to use cleanup methods. The DRO results were reduced following both cleanup steps (Figure 4). In the case of the more aggressive silica gel and sulfuric acid cleanup, the DRO content of the extracts was reduced to below or near the practical quantitation limit of 0.25 mg/L as provided in Table 7.3 of *Guidance for Remediation of Petroleum Contaminated Sites* (Ecology 2016).

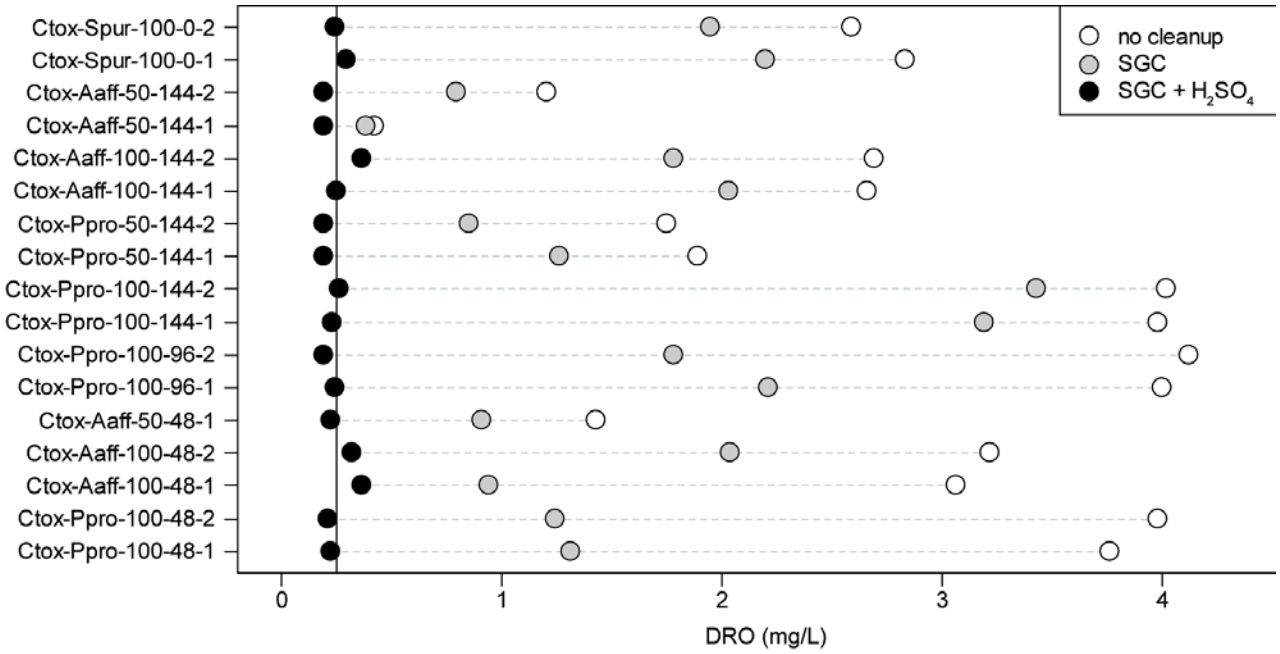

Figure 4: DRO concentrations of replicate samples with no cleanup and silica gel cleanup (SGC). *SGC with H_2SO_4 includes the additional sulfuric acid cleanup as per the NWTPH-Dx method. Vertical line represents the 0.25 mg/L practical quantitation limit (Ecology, 2016).*

The results for the heavier RRO fractions were also reduced under both cleanup methods (Figure 5). The silica gel and sulfuric acid cleanup reduced the RRO in all the samples to undetectable (below the reporting limit).

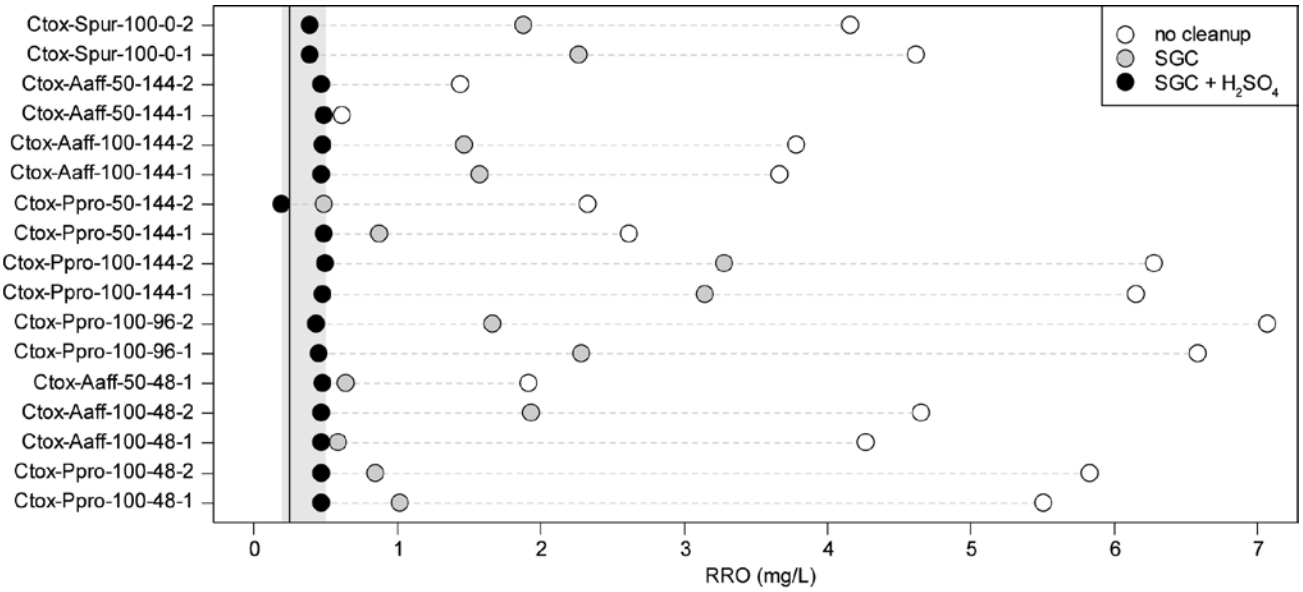

Figure 5: RRO concentrations of replicate samples with no cleanup and silica gel cleanup (SGC). *SGC with H_2SO_4 includes the additional sulfuric acid cleanup as per the NWTPH-Dx method. Vertical line represents the 0.25 mg/L practical quantitation limit (Ecology, 2016). Shaded area represents the range of detection limits during the tests.*

The reduction in DRO and RRO concentrations is due to the loss of petroleum or polar metabolites and/or additional dissolved organic compounds (Lang et al., 2009; Zemo et al., 2017). With the analytical instruments and methods used in this study it is not possible to decipher which specific compounds were lost. However, the significant reduction in DRO and RRO concentrations suggests that there were likely very few primary hydrocarbons present in the test water.

Volatile and Additional Semi-volatile Hydrocarbons

To confirm that toxicity of the final stock solutions was not attributable to volatile hydrocarbons in the NWTPH-Gx range (GRO) or benzene- toluene-ethylbenzene- xylenes (BTEX), split samples from the final tests of the fish bioassays were analyzed. There was no detectable GRO or BETX in any of the samples (Appendix D, Table D-5). Furthermore, additional samples were analyzed using the VPH method at a contract laboratory and found no evidence of volatile hydrocarbons in the stock water. Samples from the same fish toxicity tests were also analyzed for EPH and no detectable hydrocarbon fractions were measured. The lack of EPH fractions is similar to findings of the DRO samples once they had gone through silica gel and acid cleanup. Lastly, one sample of the stock water was analyzed for polycyclic aromatic hydrocarbons (Appendix D, Table D-5). No detectable PAHs were measured in the stock water.

Toxicity Testing

Freshwater

Range-Finding Tests

Fathead minnow

The range-finding tests on fathead minnows began with a maximum concentration of 6.23 mg/L DRO. Survival and growth endpoints were assessed using the laboratory (negative) control waters. On-site background waters for the test contained minor amounts of DRO (0.2 ± 0.01 mg/L), however there was no significant difference between fathead minnow response in the lab control waters and the on-site background waters. The only response measured for the fathead minnows was at the maximum concentration for the growth endpoints (Figure 6). Using concentration-response curves, the calculated IC25 was 2.60 with 95% confidence limits (0 – 5.4) mg/L using linear interpolation (Appendix B). This suggests an inhibitory effect near the upper range of the test concentrations. The maximum concentration resulted in a lower mean survival but the differences were not statistically significant.

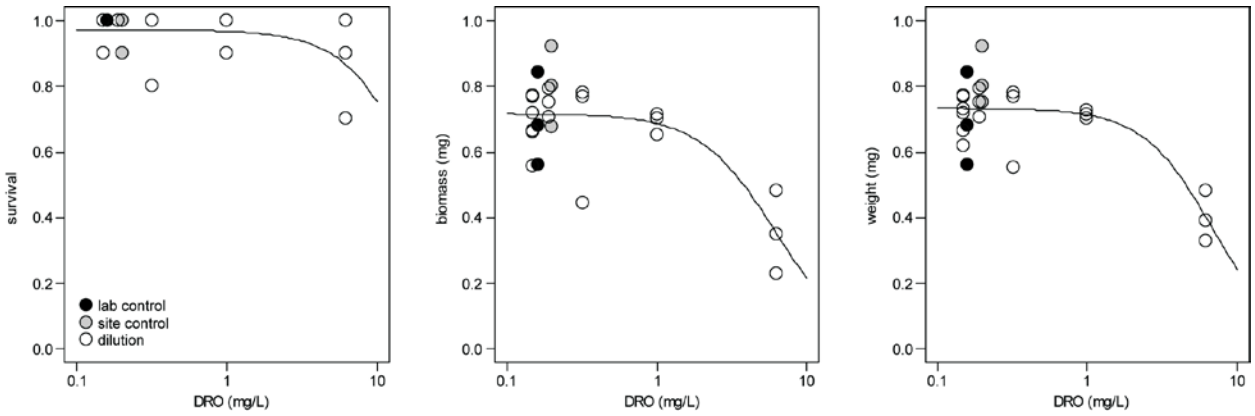

Figure 6: Concentration – Response curve for the fathead minnow (*Pimephales promelas*) range-finding tests.
Laboratory (black dot) and on-site (grey dot) background waters included.

Cladoceran

The range-finding tests on the cladoceran *Ceriodaphnia dubia* had a maximum concentration of 5.53 mg/L DRO. On-site background waters had minor amounts of DRO (0.26 mg/L). The on-site background waters resulted in a significantly lower reproduction in the *Ceriodaphnia*, which was unexpected given that there was not a significant difference between the laboratory control waters and the on-site background during screening tests. The chemistry of the water from the on-site background well did not differ from the screening tests. It is not clear what affected the reproductive endpoint during the test. No measurable effect was detected for the dilution series compared to the laboratory control during the range-finding tests for either the survival or reproduction endpoints (Figure 7).

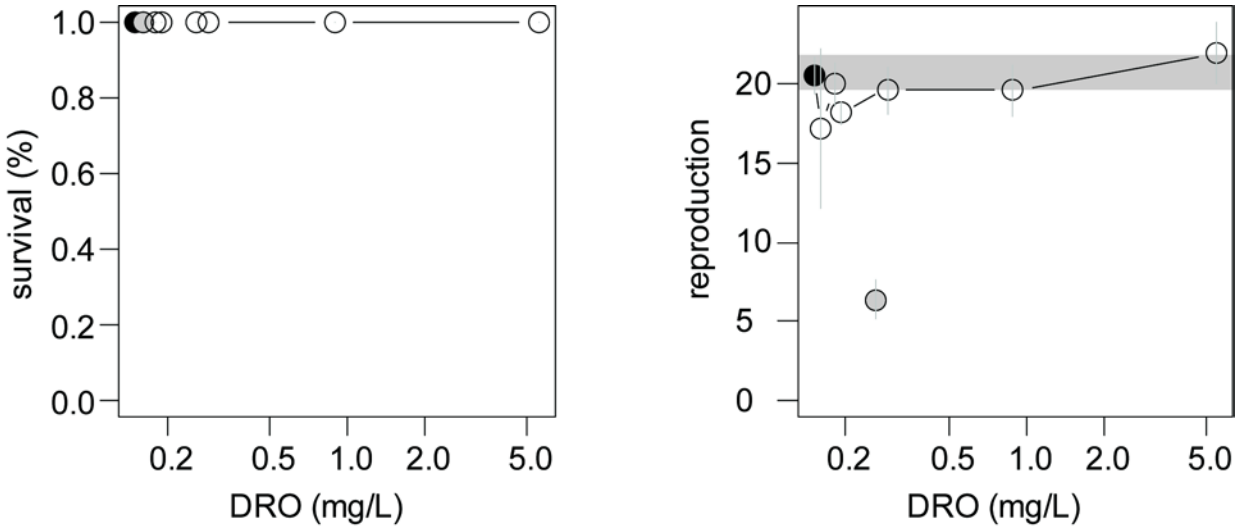

Figure 7: Mean (± 95% confidence interval) response for survival and reproduction from the *Ceriodaphnia* range-finding tests.

Grey shaded area represents the 95% confidence interval for the laboratory control samples; Laboratory (black dot) and on-site (grey dot) background waters included

Final toxicity testing

Fathead minnow

During the final toxicity testing, effects concentrations were refined with a different dilution series which began with a maximum concentration of 4.33 mg/L DRO. No significant effect was measured when comparing the lab control waters and the on-site background waters. The concentration-response curves for the tests were shallow and toxicity point estimates (LC50) were not calculated based on survival data. During the final test, measurable effects were quantifiable for the growth endpoints at the maximum concentration (Figure 8). The IC25 based on the biomass endpoint was 4.28 with 95% confidence limits (3.8 – 4.6) mg/L. The IC25 based on the dry weight endpoint was > 4.33 mg/L. Accordingly, the NOEC for the test was 3.04 mg/L, while the LOEC was 4.33 mg/L (Table 14).

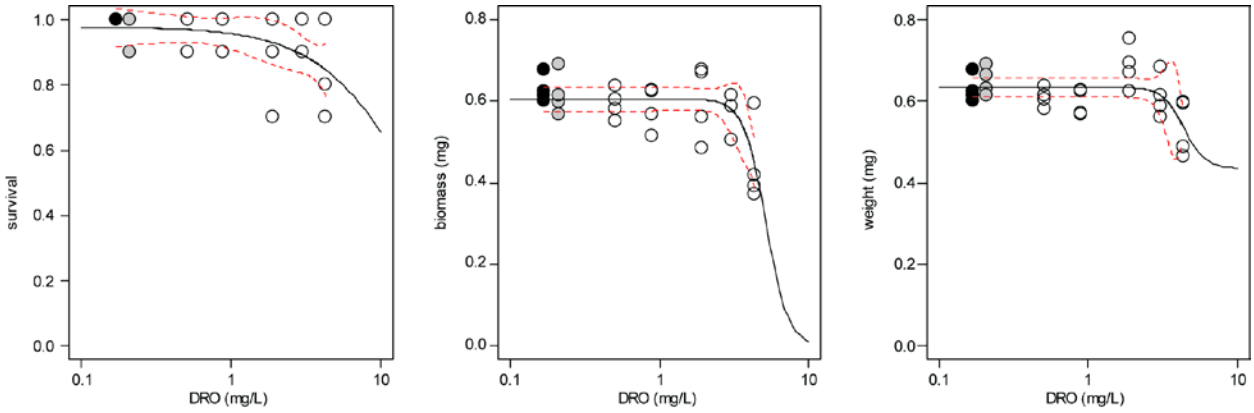

Figure 8: Concentration – Response curve for the fathead minnows (*Pimephales promelas*) final toxicity tests.

Laboratory (black dot) and on-site (grey dot) control waters included; red dashed lines are 95% confidence interval.

Cladoceran

Based on the range-finding results, only the 100% stock solution was analyzed during the final chronic tests, which were carried out using water with a DRO of 4.12 mg/L (Appendix B). On-site background waters used in the final test did not elicit a measurable effect on the *Ceriodaphnia* relative to the laboratory control water. No point estimates of toxicity were calculated. No measurable effect was found for the *Ceriodaphnia dubia* at a concentration of 4.12 mg/L DRO (Table 14).

Table 14: Summary of the effects thresholds and toxicity point estimates for freshwater organisms.

Endpoint	Point Estimates (95% CL) (mg/L)		Statistical Test	NOEC (mg/L)	LOEC (mg/L)	Statistical Test
Fathead minnow						
survival	LC50	> 4.33 (> *6.28**)	Linear interpolation	3.04	4.33	Dunnett multiple comparison
biomass	IC25	4.28 (3.8 – 4.6)		3.04	4.33	
biomass	IC50	> 4.33 (>*6.28**)				
Ceriodaphnia dubia						
survival	LC50	(>*5.53**)	Linear interpolation	>4.12	>4.12 (>*5.53**)	Fisher Exact/Bonferroni-Holm test
reproduction	IC25	(>*5.53**)		>4.12	>4.12 (>*5.53**)	Equal variance two sample t-test
reproduction	IC50	(>*5.53**)				

LC = Lethal Concentration, IC = Inhibition Concentration, NOEC = No Observed Effect Concentration, LOEC = Lowest Observed Effect Concentration.

* *Italicized* results in parentheses are from the range-finding tests, and are included as additional estimates.

As discussed earlier in this report, the groundwater supplied from the study site contained a fraction of weathered DRO and a fraction of residual (heavier) range organics (RRO). Thresholds or toxicity point estimates for the RRO were not explicitly calculated, but the RRO concentrations that were present at the thresholds found for DRO were summarized (Table 15). This is relevant because the current guidance states that petroleum metabolites (which includes RRO) should be considered part of the NWTPH-Dx result for the purposes of site characterization and compliance (Ecology, 2016).

Table 15: Summary of both DRO and RRO at the no observed effect level (NOEC) for freshwater.

Test species	DRO (mg/L)	RRO (mg/L)
Fathead minnow	3.04	4.14
Ceriodaphnia dubia	>5.53*	>5.63*

DRO: diesel-range organics; RRO: residual-range organics
* Result of the range-finding test endpoint

There is very little research available that describes the toxicity testing of weathered diesel-range organics in freshwater. Generally, the available results are for tests that have been carried out using unrefined mixtures or weathered crude oil. In a study on slimy sculpin, dolly varden, and threespine stickleback using crude oil, Moles et al. (1979) found an acute toxicity (LC50) ranging from 1.25 to 6.89 mg/L (2.75 to 10.45 mg/L total aromatic hydrocarbons by GC). The LC50 from the fathead minnow tests were not calculated, however it was likely greater than the upper concentration of 6.28 mg/L for the range-finding tests (Table 14).

Calfee et al. (1999) tested the phototoxicity of weathered oil collected from a groundwater well on *Ceriodaphnia dubia* under different UV regimes. At a concentration of 1.6 mg TPH/L there was a significant decrease in neonate reproduction, and a further significant decrease as exposure to UV increased. The TPH concentrations tested by Calfee et al. (1999) were much lower than the current study, however the presence of PAHs likely played a key role in the toxicity to the *Ceriodaphnia* – PAHs were absent from stock waters. This study only analyzed parent PAHs and not the alkylated homologs which have been implicated in the cumulative mode of action for toxicity in other studies (Colavecchia et al., 2004; Barron, 2017).

Overall, the findings from the toxicity tests on two freshwater organisms using water contaminated with weathered DRO established an effects threshold that is much higher than fresh diesel (Hobbs et al., 2018).

Marine

Range-Finding Tests

Topsmelt

The range-finding tests on the topsmelt stock solution was 5.07 mg/L DRO. On-site background waters contained minor amounts of DRO (0.29 ± 0.04 mg/L), but did not exhibit significantly different effects relative to the lab control waters. Topsmelt demonstrated a measurable effect at the highest concentration (Figure 9). Using the concentration-response curves, the calculated LC50 was 2.4 (1.7 – 3.3) mg/L (Appendix B). The results suggest a clear lethal toxicity threshold near the upper range of the test concentrations, thus a dilution series was used for the final toxicity tests to better understand effects near the highest concentration.

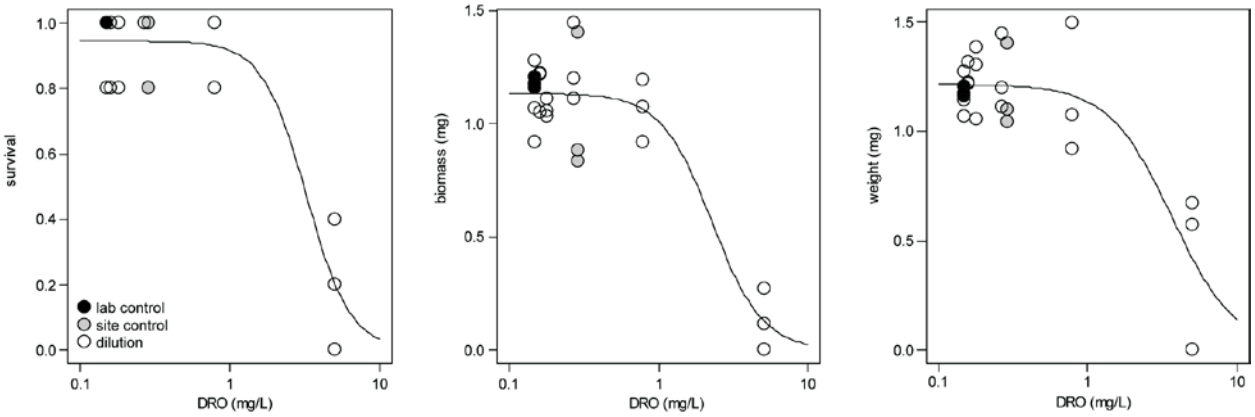

Figure 9: Concentration – Response curve for the topsmelt (*Atherinops affinis*) range-finding tests.

Laboratory (black dot) and on-site (grey dot) background waters included.

Echinoderm

The echinoderm fertilization test was run using the same waters and dilution series as the topsmelt. During the range-finding test, the NOEC for fertilization was the maximum exposure concentration of 6.09 mg/L DRO (Figure 10). In the subsequent final toxicity test on the echinoderm, only the maximum stock solution concentration was tested – 2.71 mg/L DRO. No measurable effects were observed on the purple sea urchin fertilization at a concentration of 2.71 mg/L DRO (Table 16).

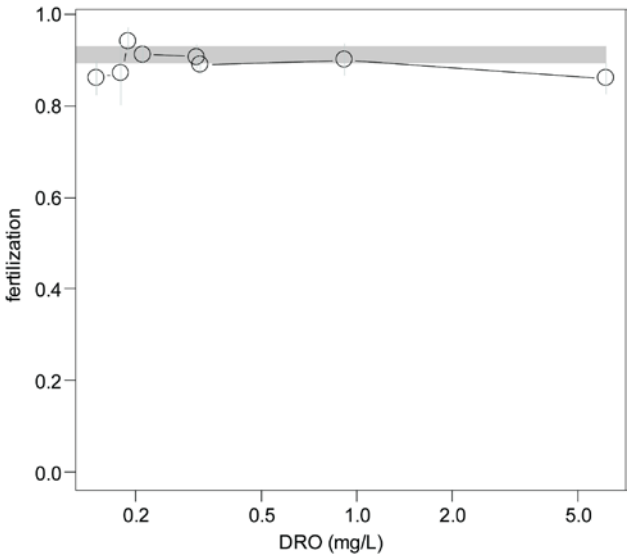

Figure 10: Mean (± 95% confidence interval) response for echinoderm (purple sea urchin) fertilization range-finding tests.

Final Tests

Topsmelt

Final testing with topsmelt began with a stock concentration of 3.14 mg/L DRO. Quantifiable effects to the growth endpoints were observed during the final test (Figure 11). The concentration-response curve for the survival endpoint is shallow and yields an LC50 above the maximum concentration tested. A NOEC of 3.14 mg/L and a LOEC of >3.14 mg/L DRO was observed for the survival endpoint. The concentration-response curves detail an IC25 for growth at a concentration of 2.0 (1.4 – 2.6) mg/L for biomass and 2.4 (1.7 – 3.0) mg/L for weight (Appendix B). The growth endpoints exhibited effects concentrations of 2.12 mg/L for the NOEC and 3.14 mg/L DRO for the LOEC.

The concentration range of the IC25 with 95% confidence (2.0 ± 0.6 mg/L) overlaps with the measured NOEC (2.12 mg/L). As discussed in the *Methods - Statistical Methods* section, this suggests that the low inhibitory toxicity effects are compatible with the estimated no-effects concentration. A NOEC does not necessarily mean absolutely zero biological effects were present, but that the effects were statistically indistinguishable from the lab control. The estimated NOEC is derived from the measured test concentrations used in the study.

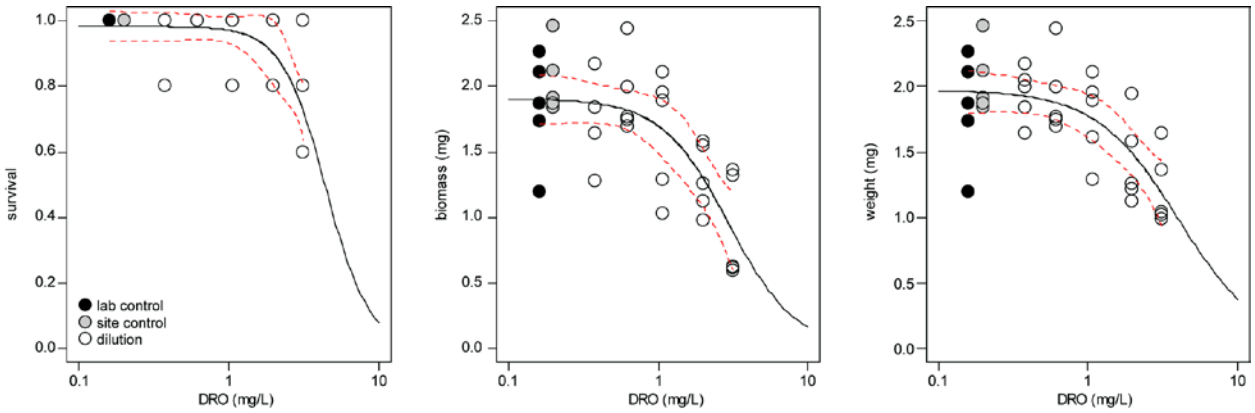

Figure 11: Concentration – Response curve for the topsmelt (*Atherinops affinis*) final toxicity tests.

Laboratory (black dot) and on-site (grey dot) background waters included; red dashed lines are 95% confidence interval

Echinoderm

The final toxicity test on the echinoderm was completed using only the maximum stock solution concentration – 2.71 mg/L DRO. No point estimates of toxicity were calculated. No measurable effects were observed on echinoderm fertilization at a concentration of 2.71 mg/L DRO (Table 16).

Table 16: Summary of the effects thresholds and toxicity point estimates for marine organisms.

Endpoint	Point Estimates (95% CL) (mg/L)		Statistical Test	NOEC (mg/L)	LOEC (mg/L)	Statistical Test
Topsmelt						
survival	LC50	>3.14 (*2.37**)	Trimmed Spearman-Kärber	3.14	>3.14 (*5.07**)	Steel Many-One Rank Sum Test
biomass	IC25	2.0 (1.4 – 2.6)	Log-Logistic	2.12	3.14	Dunnett multiple comparison
biomass	IC50	3.1 (2.4 – 4.0)				
Echinoderm						
fertilization	IC25	(*>6.09**)	Linear interpolation	>2.71 (*>6.09**)	>2.71 (*>6.09**)	Dunnett multiple comparison
fertilization	IC50	(*>6.09**)				

LC = Lethal Concentration, IC = Inhibition Concentration, NOEC = No Observed Effect Concentration, LOEC = Lowest Observed Effect Concentration.
* *Italicized* results in parentheses are from the range-finding tests, and are included as additional estimates.

The corresponding residual oil (RRO) concentrations based on the DRO effects concentrations for the marine organisms are presented in Table 17.

Table 17: Summary of both DRO and RRO at the no observed effect level (NOEC) for marine water.

Test species	DRO (mg/L)	RRO (mg/L)
Topsmelt	2.12	2.68
Purple sea urchin	>6.09*	>5.73*

DRO: diesel-range organics; RRO: residual-range organics
* Result of the range-finding test endpoint

In a study by Little et al. (2000) inland silverside (*Menidia beryllina*), which is similar to topsmelt based on life history and habitat, was exposed to weathered middle distillate petroleum under different UV intensities. They established a NOEC of 0.700 mg/L TPH and a LOEC of 1.50 mg/L TPH for mortality. These findings are lower than thresholds for survival in the current study, however the water being used for the testing in the Little et al. (2000) study had measurable amounts of parent and alkylated PAHs, suggesting these compounds may have been involved in the mechanism of toxicity.

Previous studies on marine invertebrates using a petroleum mixture have described some effects thresholds. Taban et al. (2004) established a LOEC for general damage to an urchin at 0.06 mg/L. In a study with effects endpoints similar to the current study, O'Clair and Rice (1985) found an LOEC of 0.20 mg/L for a sea star and a NOEC of 0.12 mg/L for growth effects. These previous results are significantly lower than the 2.71 and 6.09 mg/L DRO tested in the current study that resulted in no effect.

Similar to the results on the freshwater organisms, the marine water tests resulted in much higher effects concentrations than the previous study using fresh diesel (Hobbs et al., 2018).

Hydrocarbon Toxicity and Petroleum Metabolites

The toxicity of polar compounds or metabolites in diesel-range organics is difficult to assess because of the complexity of the mixture and accurate identification of the compounds, which prevents identification of a mode of action in toxicity. Zemo et al. (2017) proposed some expected toxicity levels to humans based on USEPA reference concentrations for the dominant families of polar compounds present in weathered DRO. Scarlett et al. (2012) predicted lethal effects for several aquatic receptors from naphthenic acids, which are carboxylic acids and a potential metabolite. Tollefsen et al. (2008) tested the cytotoxic effect of alkylphenolics, a possible metabolite, on rainbow trout liver cells and found evidence of toxic effects for a range of compounds and that toxicity increased with solubility of the compound. The possible impacts of a mixture of petroleum metabolites to aquatic organisms have not been explicitly tested. The DRO tested in this study is likely dominated by polar metabolites based on the lack of measurable primary hydrocarbons and loss of compounds during silica gel cleanup. The findings of this study suggest that there is an effect threshold for aquatic organisms in marine water and freshwater attributable to petroleum (polar) metabolites.

Ecology has completed two studies on the potential effects of hydrocarbon releases on aquatic organisms (Table 18): (1) establishing the effect concentrations for fresh diesel and gasoline (Hobbs et al., 2018), and (2) this study, on weathered DRO. These two studies cover a gradient of hydrocarbon/metabolite exposure and weathering, and it should be acknowledged that all sites will likely have different levels of weathering and DRO composition. The effects thresholds for DRO at most contaminated sites with hydrocarbon releases should fall somewhere along this gradient.

Table 18: Comparison of effects-concentrations and LC50 for fresh and weathered DRO in marine water and freshwater.

	NOEC		LOEC		LC50	
	fresh	**weathered**	**fresh**	**weathered**	**fresh**	**weathered**
Freshwater						
Fathead minnow	0.65	3.04	1.30	4.33	1.87 (1.43 – 2.45)	>4.33 (>*6.28**)
Ceriodaphnia dubia	0.15	4.12	0.22	>4.12 (>*5.53**)	0.23 (0.20 – 0.26)	NA (>*5.53**)
Marine water						
Topsmelt	0.26	2.12	0.57	3.14	0.68 (0.55 – 0.83)	>3.14 *(2.37 ± 0.68*)*
Strongylocentrotus purpuratus	<0.05	>2.71 (>*6.09**)	0.05	>2.71 (>*6.09**)	0.34 (0.29 – 0.38)†	NA (>*6.09**)

† IC50 used for *S. purpuratus* test.; LC50 estimates and (95% confidence interval)
NA is no applicable – no point estimates could be calculated for the final invertebrate toxicity tests.
DRO: diesel-range organics; RRO: residual-range organics.
* *Italicized* results in parentheses are from the range-finding tests, and are included as additional estimates.

Conclusions

Results of this 2019 study support the following conclusions:

- Contaminated groundwater used for the toxicity testing contained weathered diesel range organics (DRO) and residual (heavy) range organics (RRO) almost exclusively. Based on the NWTPH-Dx methods, the chromatography of the hydrocarbons resembled an "unresolved complex mixture".

- Silica gel cleanup on select samples reduced the total DRO and RRO concentrations to below or near the 0.25 mg/L practical quantitation limit (Ecology, 2016). The significant reduction in DRO and RRO concentrations suggests that primary hydrocarbons were a minor part of the test water. This was further confirmed by no detections of volatile petroleum hydrocarbons, extractable petroleum hydrocarbons and primary polycyclic aromatic hydrocarbons.

- Concentrations of weathered DRO during the 7-day static renewal toxicity tests were measured each day. Low variability in the exposure concentration over the test period was observed at multiple concentrations across the tests (< 20% relative standard deviation).

- Both invertebrates, *Strongylocentrotus purpuratus* (marine) and *Ceriodaphnia dubia* (freshwater), did not exhibit an adverse response to the maximum exposure concentrations in either the range-finding tests or the final toxicity tests.

- During the range-finding tests topsmelt (marine) exhibited a lethal response to DRO at the highest concentration of the dilution. An LC50 was calculated at 2.4 (1.7 – 3.3) mg/L. Fathead minnows exhibited a growth response at the highest concentration of the dilution. An IC25 of 2.60 (0 – 5.4) mg/L was calculated.

- During the final tests, point estimates of lethality for the fish tests (topsmelt and fathead minnows) were difficult to establish from the shallow concentration-response curves. Point estimates defining growth inhibition endpoints could be calculated from both freshwater and marine tests. Based on fish growth endpoints, the no-effects threshold (NOEC) for freshwater was 3.04 mg/L DRO and 2.12 mg/L DRO for marine waters.

- Residual (heavier) range organics (RRO) were present in the test waters at the no-effects thresholds: 4.14 mg/L for freshwater and 2.68 mg/L for marine waters.

Recommendations

Based on the goals and findings from this study, the following recommendations can be made:

- NOECs for weathered DRO (as defined by the NWTPH-Dx method) in marine water (2.12 mg/L) and freshwater (3.04 mg/L) have been derived at a contaminated site. These values represent the estimated "no-effects" levels for weathered NWTPH-Dx in surface waters. Use these values to inform appropriate guidance under WAC-173-340-730(3)(b)(ii) (Environmental effects) – Surface Water Cleanup Standards.

- The use of silica gel cleanup methods in the assessment and cleanup of contaminated sites can dramatically affect the reported NWTPH-Dx results. TCP should undertake a follow-up study to clarify the method (i.e. use of sulfuric acid or not) and provide further guidance on when and how to use these cleanup methods.

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Glossary, Acronyms, and Abbreviations

Glossary

Dissolved oxygen (DO): A measure of the amount of oxygen dissolved in water.

Inhibitory concentration (IC): The toxicant concentration that would cause a given percent reduction in a nonquantal biological measurement for the test population. For example, the IC25 is the concentration of toxicant that would cause a 25% reduction in mean young per female or in growth for the test population.

Lowest-observed effect concentration (LOEC): The lowest concentration of toxicant to which organisms are exposed in a life-cycle or partial life-cycle (short-term) test, which causes adverse effects on the test organisms (i.e., where the values for the observed responses are statistically significantly different from the controls).

Lethal concentration (LC): The toxicant concentration that would cause death in a given percent of the test population. Identical to EC when the observable adverse effect is death. For example, the LC50 is the concentration of toxicant that would cause death in 50% of the test population.

Method Detection Limit (MDL): The minimum concentration of an analyte that, in a given matrix and with a specific method, has a 99% probability of being identified, and reported to be greater than zero.

No-observed effects concentration (NOEC): The highest concentration of toxicant to which organisms are exposed in a full life-cycle or partial life-cycle (short-term) test, that causes no observable adverse effects on the test organisms (i.e., the highest concentration of toxicant in which the values for the observed responses are not statistically significantly different from the controls). This value is used, along with other factors, to determine toxicity limits in permits..

Practical Quantitation Limit (PQL): The analyte concentration selected as the lowest non-zero standard in the instrument calibration curve, adjusted for sample specific conditions (e.g.: sample size, percent solids, dilutions, cleanup procedures, etc.). Results below the PQL are considered less accurate and are qualified as estimates.

Water accommodated fraction (WAF): A laboratory-prepared media from the low-energy mixing of a low solubility liquid (e.g. diesel fuel) into water. It is essentially the dissolved portion of the test material which is free of particles.

Whole effluent toxicity (WET) testing: Refers to the aggregate toxicity of pollutants contained in wastewater effluent. It represents the total exposure of aquatic life to pollutants in a controlled lab environment. It is conducted by a qualified lab using EPA methods on test organisms.

Acronyms and Abbreviations

DRO	Diesel-range organics
Ecology	Washington State Department of Ecology
EIM	Environmental Information Management database
EPA	U.S. Environmental Protection Agency
LC50	*see above*
LOEC	Lowest-observed effect concentration
MDL	Method detection limit
MEL	Manchester Environmental Laboratory
NOEC	No-observed effects concentration
NWTPH-Gx	Northwest Total Petroleum Hydrocarbons – Gasoline fraction
NWTPH-Dx	Northwest Total Petroleum Hydrocarbons – Diesel fraction
PQL	Practical quantitation limit
RPD	Relative percent difference
RRO	Residual range organics
RSD	Relative standard deviation
SRM	Standard reference materials
TCP	Toxics Cleanup Program (Department of Ecology
WAC	Washington Administrative Code

Units of Measurement

°C	degrees centigrade
dw	dry weight
ft	feet
ft-c	foot-candle (measurement of illumination)
g	gram, a unit of mass
mg	milligram
mg/L	milligrams per liter (parts per million)
µE/m^2/s	microeinsteins per meter squared per second (measurement of illumination)
µg/L	micrograms per liter (parts per billion)

Appendices

Appendix A. Screening Toxicity Tests (Nautilus)

Appendices A and B are available only on the internet, linked to this report at:
https://fortress.wa.gov/ecy/publications/SummaryPages/2003008.html

Appendix B. Final Report for Toxicity Tests (Nautilus)

Appendices A and B are available only on the internet, linked to this report at:
https://fortress.wa.gov/ecy/publications/SummaryPages/2003008.html

Appendix C. Project Data Quality Results

Table C-1: Project quality control samples – laboratory blanks, laboratory control samples and laboratory duplicates.

MEL batch	Sample ID	analysis date	DRO (mg/L)		RRO (mg/L)		% spike rec.	Lab Duplicate ID
1902025	B19B033-BLK1	2/13/2019	0.15	UJ	0.38	U	76	
1902025	B19B033-BS1	2/13/2019	77				106	
1902025	B19B033-BSD1	2/13/2019	**34**				**56**	
1902025	B19B033-DUP1	2/13/2019	5.67		3.94		113	1902025-01
1902032	B19B093-BLK1	3/1/2019	0.15	U	0.38	U	83	
1902032	B19B093-BS1	3/1/2019	76				97	
1902032	B19B093-BSD1	3/1/2019	77				93	
1902032	B19B093-DUP1	3/1/2019	6.29	J	5.78	J	108	1902032-02
1902032	B19B093-DUP2	3/1/2019	1.87		1.71		73	1902032-09
1902032	B19B094-BLK1	2/28/2019	0.15	U	0.38	U	91	
1902032	B19B094-BS1	2/28/2019	83				102	
1902032	B19B094-BSD1	2/28/2019	85				106	
1902033	B19C011-BLK1	3/6/2019	0.15	U	0.38	U	96	
1902033	B19C011-BS1	3/6/2019	76				114	
1902033	B19C011-BSD1	3/6/2019	74				112	
1902033	B19C012-BLK1	3/7/2019	0.15	U	0.38	U	89	
1902033	B19C012-BS1	3/7/2019	87				99	
1902033	B19C012-BSD1	3/7/2019	89				102	
1902033	B19C013-BLK1	3/8/2019	0.15	U	0.38	U	97	
1902033	B19C013-BS1	3/8/2019	75				90	
1902033	B19C013-BSD1	3/8/2019	91				102	
1903061	B19C136-BLK1	3/26/2019	0.15	U	0.38	U	86	
1903061	B19C136-BLK2	3/28/2019	0.15	U	0.38	U	95	
1903061	B19C136-BS1	3/26/2019	81				84	
1903061	B19C136-BS2	3/28/2019	114				87	
1903061	B19C136-BSD1	3/26/2019	80				87	
1903061	B19C136-BSD2	3/28/2019	112				92	
1903026	B19C110-BLK1	4/4/2019	0.15	U	0.38	U	106	
1903026	B19C110-BS1	4/4/2019	82				94	
1903026	B19C110-BSD1	4/4/2019	97				105	
1903026	B19C110-DUP1	4/4/2019	5.16		4.73		92	1903026-02
1903027	B19C132-BLK1	4/5/2019	0.15	U	0.38	U	93	
1903027	B19C132-BS1	4/5/2019	82				97	
1903027	B19C132-BSD1	4/5/2019	82				98	
1904046	B19E028-BLK1	5/7/2019	0.15	U	0.38	U	90	

MEL batch	Sample ID	analysis date	DRO (mg/L)		RRO (mg/L)		% spike rec.	Lab Duplicate ID
1904046	B19E028-BS1	5/7/2019	87				91	
1904046	B19E028-BSD1	5/7/2019	84				91	
1905034	B19E031-BLK1	5/22/2019	0.15	U	0.38	U	99	
1905034	B19E031-BLK2	6/12/2019	0.15	U	0.38	U	108	
1905034	B19E031-BLK3	6/12/2019	0.15	U	0.38	U	106	
1905034	B19E031-BS1	5/22/2019	92				98	
1905034	B19E031-BS2	6/12/2019	100				110	
1905034	B19E031-BS3	6/12/2019	83				109	
1905034	B19E031-BSD1	5/22/2019	90				98	
1905034	B19E031-BSD2	6/12/2019	94				105	
1905034	B19E031-BSD3	6/12/2019	81				107	
1905034	B19E047-BLK1	5/23/2019	0.15	U	0.38	U	105	
1905034	B19E047-BLK2	6/11/2019	0.15	U	0.38	U	149	
1905034	B19E047-BLK3	6/11/2019	0.15	U	0.38	U	120	
1905034	B19E047-BS1	5/23/2019	80				99	
1905034	B19E047-BS2	6/11/2019	91				108	
1905034	B19E047-BS3	6/11/2019	84				106	
1905034	B19E047-BSD1	5/23/2019	89				106	
1905034	B19E047-BSD2	6/11/2019	100				116	
1905034	B19E047-BSD3	6/11/2019	88				112	
1905056	B19F001-BLK1	6/13/2019	0.15	U	0.38	U	111	
1905056	B19F001-BS1	6/13/2019	84				114	
1905056	B19F001-BSD1	6/13/2019	81				99	
1905056	B19F001-DUP1	6/13/2019	3.76	J	4.33	J	113	1905056-11

Bold results were considered results that should be evaluated by the project officer.

Table C-2: Project quality control samples – analytical duplicates.

Sample ID	sample date	MEL ID	analysis date	DRO (mg/L)		RPD	RRO (mg/L)		RPD	% spike rec.
RF-Aaff-100-0-1	2/19/2019	1902032-01	3/1/2019	6.1	J	0.003	5.71	J	0.005	101
RF-Aaff-100-0-2	2/19/2019	1902032-02	3/1/2019	6.08	J		5.74	J		91
RF-Aaff-6.3-0-1	2/19/2019	1902032-03	3/1/2019	0.38	J	0.375	0.38	UJ	0	85
RF-Aaff-6.3-0-2	2/19/2019	1902032-04	3/1/2019	0.26	J		0.38	UJ		94
RF-Ppro-100-0-1	2/19/2019	1902032-08	3/1/2019	6.16		0.018	5.88		0.048	86
RF-Ppro-100-0-2	2/19/2019	1902032-09	3/1/2019	6.27	J		6.17	J		94
RF-Ppro-6.3-0-1	2/19/2019	1902032-10	3/1/2019	0.39		0.294	0.38	U	0	86
RF-Ppro-6.3-0-2	2/19/2019	1902032-11	3/1/2019	0.29			0.38	U		90
RF-Aaff-6.3-1-1-F	2/20/2019	1902032-18	3/1/2019	0.3	J	0.182	0.38	UJ	0.000	91
RF-Aaff-6.3-1-2-F	2/20/2019	1902032-19	3/1/2019	0.25	J		0.38	UJ		91
RF-Ppro-6.3-1-1-F	2/20/2019	1902032-25	2/28/2019	0.29		0.034	0.38	U	0.000	103
RF-Ppro-6.3-1-2-F	2/20/2019	1902032-26	3/1/2019	0.3			0.38	U		110
RF-Aaff-6.3-3-1-F	2/22/2019	1902033-02	3/6/2019	0.22		0.047	0.38	U	0.000	131
RF-Aaff-6.3-3-2-F	2/22/2019	1902033-03	3/6/2019	0.21			0.38	U		128
RF-Ppro-6.3-3-1-F	2/22/2019	1902033-06	3/6/2019	0.26		0.424	0.39	U	0.02597	136
RF-Ppro-6.3-3-2-F	2/22/2019	1902033-07	3/6/2019	0.4			0.38	U		129
RF-Ppro-6.3-4-1-F	2/23/2019	1902033-14	3/7/2019	0.15	U	0.000	0.38	U	0.000	113
RF-Ppro-6.3-4-2-F	2/23/2019	1902033-15	3/7/2019	0.15	U		0.38	U		116
RF-Ppro-6.3-6-1-F	2/25/2019	1902033-30	3/7/2019	0.28		0.000	0.38	U	0.051	95
RF-Ppro-6.3-6-2-F	2/25/2019	1902033-31	3/7/2019	0.28			0.4	U		100
RF-Ppro-100-7-1	2/26/2019	1902033-41	3/8/2019	6.69		0.024	8.2		0.060	107
RF-Ppro-100-7-2	2/26/2019	1902033-42	3/8/2019	6.85			8.71			107
RF-Cdub-100-0-1	3/11/2019	1903026-01	4/4/2019	6.16		0.016	6.15		0.035	88
RF-Cdub-100-0-2	3/11/2019	1903026-02	4/4/2019	6.26			6.37			97
RF-Cdub-6.3-0-1	3/11/2019	1903026-03	4/4/2019	0.32		0.270	0.41	U	0.103	95
RF-Cdub-6.3-0-2	3/11/2019	1903026-04	4/4/2019	0.42			0.37	U		91
RF-Cdub-6.3-24-1	3/12/2019	1903026-09	4/4/2019	0.34		0.194	0.41	U	0.024	97
RF-Cdub-6.3-24-2	3/12/2019	1903026-10	4/5/2019	0.28	U		0.42	U		107
RF-Cdub-6.3-72-1	3/14/2019	1903027-02	4/5/2019	0.28	UJ	0.036	0.41	UJ	0.024	102
RF-Cdub-6.3-72-2	3/14/2019	1903027-03	4/5/2019	0.27	UJ		0.42	UJ		97
RF-Cdub-6.3-96-1	3/15/2019	1903027-06	4/5/2019	0.25	UJ	0.041	0.46	UJ	0.067	104
RF-Cdub-6.3-96-2	3/15/2019	1903027-07	4/5/2019	0.24	UJ		0.43	UJ		100
Ctox-Ppro-100-0-1	4/23/2019	1904046-01	5/7/2019	5.39	J	0.078	5.14	J	0.114	83
Ctox-Ppro-100-0-2	4/23/2019	1904046-02	5/7/2019	5.83	J		5.76	J		86
Ctox-Aaff-100-0-1	4/23/2019	1904046-07	5/7/2019	3.94		0.324	3.84		0.045	88
Ctox-Aaff-100-0-2	4/23/2019	1904046-08	5/14/2019	2.84			3.67			88
Ctox-Ppro-100-48-1	4/25/2019	1904072-01	5/15/2019	3.76		0.057	5.51		0.056	90

Sample ID	sample date	MEL ID	analysis date	DRO (mg/L)		RPD	RRO (mg/L)		RPD	% spike rec.
Ctox-Ppro-100-48-2	4/25/2019	1904072-02	5/15/2019	3.98			5.83			88
Ctox-Aaff-100-48-1	4/25/2019	1904072-05	5/21/2019	3.06		0.051	4.27		0.087	99
Ctox-Aaff-100-48-2	4/25/2019	1904072-06	5/21/2019	3.22			4.66			95
Ctox-Ppro-100-96-1	4/27/2019	1904072-15	5/21/2019	4		0.030	6.59		0.070	97
Ctox-Ppro-100-96-2	4/27/2019	1904072-16	5/21/2019	4.12			7.07			98
Ctox-Ppro-50-96-1	4/27/2019	1904072-17	5/21/2019	1.82		0.034	2.82		0.055	104
Ctox-Ppro-50-96-2	4/27/2019	1904072-18	5/21/2019	1.76			2.67			97
Ctox-Aaff-100-96-1	4/27/2019	1904072-23	5/21/2019	4.43		0.465	7.03		0.570	104
Ctox-Aaff-100-96-2	4/27/2019	1904072-24	5/22/2019	2.76			3.91			99
Ctox-Ppro-100-144-1	4/29/2019	1905034-01	5/22/2019	3.98		0.010	6.16		0.019	107
Ctox-Ppro-100-144-2	4/29/2019	1905034-02	5/22/2019	4.02			6.28			105
Ctox-Ppro-50-144-1	4/29/2019	1905034-03	5/22/2019	1.89		0.077	2.62		0.117	111
Ctox-Ppro-50-144-2	4/29/2019	1905034-04	5/22/2019	1.75			2.33			106
Ctox-Aaff-100-144-1	4/29/2019	1905034-11	5/23/2019	2.66		0.011	3.67		0.032	110
Ctox-Aaff-100-144-2	4/29/2019	1905034-12	5/23/2019	2.69			3.79			107
Ctox-Aaff-50-144-1	4/29/2019	1905034-13	5/23/2019	0.42		0.963	0.62		0.796	88
Ctox-Aaff-50-144-2	4/29/2019	1905034-14	5/23/2019	1.2			1.44			107
Ctox-Spur-100-0-1	5/1/2019	1905034-31	5/23/2019	2.83		0.089	4.62		0.105	102
Ctox-Spur-100-0-2	5/1/2019	1905034-32	5/23/2019	2.59			4.16			102
Ctox-Spur-0-0-1	5/1/2019	1905034-33	5/23/2019	0.28		0.545	0.39	U	0.025	95
Ctox-Spur-0-0-2	5/1/2019	1905034-34	5/23/2019	0.16	U		0.4	U		74
Ctox-Spur-LabCont-0-1	5/1/2019	1905034-35	5/23/2019	0.16	U	0.065	0.39	U	0.026	102
Ctox-Spur-LabCont-0-2	5/1/2019	1905034-36	5/23/2019	0.15	U		0.38	U		101
Ctox-Cdub-100-48-1	5/16/2019	1905056-02	6/13/2019	4.13	J	0.026	4.6	J	0.009	103
Ctox-Cdub-100-48-2	5/16/2019	1905056-03	6/13/2019	4.24			4.56			106
Ctox-Cdub-100-96-1	5/18/2019	1905056-05	6/13/2019	4.02		0.027	4.29		0.061	110
Ctox-Cdub-100-96-2	5/18/2019	1905056-06	6/13/2019	4.13			4.56			112
Ctox-Cdub-100-0-1	5/14/2019	1905056-10	6/13/2019	3.76	J	0.152	3.97	J	0.183	112
Ctox-Cdub-100-0-2	5/14/2019	1905056-11	6/13/2019	4.38	J		4.77	J		120

Appendix D. Water Chemistry Results

Table D-1: Analytical results from the screening of potential sample sites.

		Contaminated				Background		Contaminated				Background		Contaminated								Background		
		Site 1		Site 1 duplicate		Site 1		Site 2		Site 2 duplicate		Site 2		Site 3		Site 3 duplicate		Site 3		Site 4		Site 4 duplicate		Site 4	
Sample Date		**12/13/2018**		**12/13/2018**		**12/13/2018**		**12/17/2018**		**12/17/2018**		**12/17/2018**		**12/18/2018**		**12/18/2018**		**12/18/2018**		**3/18/2019**		**3/18/2019**		**3/18/2019**	
pH		6.47		NA		7.02		7.37		NA		7.04		6.95		NA		7.02		5.53		NA		6.76	
Sample temperature.		14.04		NA		11.58		18.01		NA		17.48		14.58		NA		15.96		8.8		NA		8.3	
Conductivity (uS/cm)		320		NA		298		1014		NA		1600		892		NA		1053		203.3		NA		118.9	
Dissolved oxygen (mg/L)		0.48		NA		2.19		2		NA		0.8		0.53		NA		2.48		0.04		NA		1.53	
Oxidation/reduction potential (mV)		27		NA		-63		203		NA		219		-113		NA		-59		-12.7		NA		78.1	
MEL ID		1812024-01		1812024-02		1812024-03		1812024-04		1812024-05		1812024-06		1812024-07		1812024-08		1812024-09		1903038-01		1903038-02		1903038-03	
Conventional Parameters																									
alkalinity	mg/L	136		135		167		463		465		321		254		255		244		104		104		44.8	
hardness	mg/L	95.7		94.9		142		278		279		544		332		329		389		83.3		83.2		49.6	
total dissolved solids	mg/L	223		207		150		606		654		**1090**		523		514		611		140		148		116	
bromide	mg/L	0.046		0.041		0.047		0.911		0.904		0.935		0.283		0.282		0.298		0.059		0.06		0.075	
chloride	mg/L	13.6		13.7		4.97		50.9		50.5		87.3		80.7		82.8		113		4.15		4.26		4.06	
fluoride	mg/L	0.1	U	0.1	U	0.11		0.46		0.47		0.45		0.4		0.4		0.35		0.1	U	0.1	U	0.1	U
NH3-N	mg/L	0.129		0.131		0.17		0.01	U	0.01	U	0.01	U	0.061		0.063		0.01	U	0.016		0.016		0.01	U
NO2-NO3-N	mg/L	0.652		0.652		0.019		0.856		0.831		22.6		0.616		0.55		5.39		0.01	U	0.01	U	0.153	
sulfate	mg/L	7.51		7.62		0.3	U	59.7		59.6		**335**		74.2		72.6		93.1		2.46		2.52		6.72	
sulfide	mg/L	0.05	U	0.05	U	0.05	U	0.05	U	0.05	U	0.05	U	0.17		0.203		0.128		0.05	U	0.05	U	0.199	
calcium	mg/L	29.1		28.9		27.5		44.7		45.2		102		86.3		85.4		102		12.2		12.2		15.3	
potassium	mg/L	2.38		2.28		3.67		11.3		11.1		18		3.16		3.13		3.36		1.75		1.69		1.48	
magnesium	mg/L	5.58		5.55		18		40.4		40.3		70.3		28.4		28		32.6		12.8		12.8		2.77	
sodium	mg/L	32.5		32.2		10.4		133		131		123		39.3		39.3		46.7		10.9		10.9		4.45	
dissolved organic carbon	mg/L	**37.1**		**37.4**		2.87		5.81		5.83		6.46		1.83		1.71		1.46		3.96		4.01		2.18	
Hydrocarbons																									
NWTPH-Dx (#2 Diesel range)	mg/L	**12.1**		**12.4**		**0.52**		**0.43**		**0.38**		**0.23**		**0.88**		**0.92**		**0.35**		**0.59**		**0.58**		**0.34**	
NWTPH-Dx (Lube Oil range)	mg/L	**9.08**		**9.31**		0.39	U	0.37	U	0.38	U	0.38	U	0.38	U	0.36	U	0.38	U	0.41	U	0.39	U	0.39	U
NWTPH-Dx (#2 Diesel range) - SGC	mg/L	**9.43**		**9.49**		0.15	U	0.26	U	0.2	U	0.15	U	**0.6**		**0.63**		NA		0.44		0.39		0.16	
NWTPH-Dx (Lube Oil range) - SGC	mg/L	**6.55**	J	**6.55**		0.39	U	0.37	U	0.38	U	0.38	U	0.38	U	0.36	U	NA		0.38	U	0.38	U	0.39	U
NWTPH-Dx (#2 Diesel range) - SGC+ACU	mg/L	**4.83**		**6.03**		NA		NA		NA		NA		NA		NA		NA		NA		NA		NA	
NWTPH-Dx (Lube Oil range) - SGC+ACU	mg/L	**2.44**		**3.36**		NA		NA		NA		NA		NA		NA		NA		NA		NA		NA	
ARI - NWTPH-Dx (DRO; C12-24)	mg/L	**8.72/9.14**	J	**9.74/9.93**	J	NA		NA		NA		NA		NA		NA		NA		NA		NA		NA	
ARI - NWTPH-Dx (Motor Oil; C24-38)	mg/L	**1.5/1.4**	J	**1.9/1.41**	J	NA		NA		NA		NA		NA		NA		NA		NA		NA		NA	

		Contaminated				Background		Contaminated				Background		Contaminated				Background		Contaminated				Background		
		Site 1		Site 1 duplicate		Site 1		Site 2		Site 2 duplicate		Site 2		Site 3		Site 3 duplicate		Site 3		Site 4		Site 4 duplicate		Site 4		
Sample Date		**12/13/2018**		**12/13/2018**		**12/13/2018**		**12/17/2018**		**12/17/2018**		**12/17/2018**		**12/18/2018**		**12/18/2018**		**12/18/2018**		**3/18/2019**		**3/18/2019**		**3/18/2019**		
ARI - NWTPH-Dx ((DRO; C12-24)) - SGC+ACU	mg/L	**0.541**		**0.543**		NA		NA		NA		NA		NA		NA		NA		NA		NA		NA		
ARI - NWTPH-Dx (Motor Oil; C24-38) - SGC+ACU	mg/L	**0.2**	U	**0.2**	U	NA		NA		NA		NA		NA		NA		NA		NA		NA		NA		
NWTPH-Gx	mg/L	0.07	U	0.07	U	0.07	U	0.07	U	0.07	U	0.07	U	**0.65**		**0.678**		0.203		0.102		0.095		0.07		
Benzene	ug/L	1	U	1	U	1	U	1	U	1	U	1	U	1	U	1	U	1	U	1	U	1	U	1	U	
Ethylbenzene	ug/L	1	U	1	U	1	U	1	U	1	U	1	U	**3.07**		**3.76**		1	U	1	U	1	U	1	U	
m,p-Xylene	ug/L	2	U	2	U	2	U	2	U	2	U	2	U	**1.24**		**1.27**		2	U	2	U	2	U	2	U	
o-Xylene	ug/L	1	U	1	U	1	U	1	U	1	U	1	U	**1.12**		**0.935**		1	U	1	U	1	U	1	U	
Toluene	ug/L	1	U	1	U	1	U	1	U	1	U	1	U	1	U	1	U	1	U	1	U	1	U	1	U	
EPH, C8-C10 Aliphatics	ug/L	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	
EPH, >C10-C12 Aliphatics	ug/L	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	
EPH, >C12-C16 Aliphatics	ug/L	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	
EPH, >C16-C21 Aliphatics	ug/L	**47**		**62**		40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	
EPH, >C21-C34 Aliphatics	ug/L	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	
EPH, C8-C10 Aromatics	ug/L	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	
EPH, >C10-C12 Aromatics	ug/L	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	
EPH, >C12-C16 Aromatics	ug/L	40	U	40	U	40	U	40	U	40	U	40	U	**56**		**56**		40	U	40	U	40	U	40	U	
EPH, >C16-C21 Aromatics	ug/L	**43**		**55**		40	U	40	U	40	U	40	U	48		40	U	40	U	40	U	40	U	40	U	
EPH, >C21-C34 Aromatics	ug/L	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	40	U	
VPH, C5-C6 Aliphatics	ug/L	50	U	50	U	50	U	50	U	50	U	50	U	50	U	50	U	50	U	50	U	50	U	50	U	
VPH, >C6-C8 Aliphatics	ug/L	50	U	50	U	50	U	50	U	50	U	50	U	50	U	50	U	50	U	50	U	50	U	50	U	
VPH, >C8-C10 Aliphatics	ug/L	50	U	50	U	50	U	50	U	50	U	50	U	50	U	50	U	50	U	50	U	50	U	50	U	
VPH, >C10-C12 Aliphatics	ug/L	50	U	50	U	50	U	50	U	50	U	50	U	50	U	50	U	50	U	50	U	50	U	50	U	
VPH, >C8-C10 Aromatics	ug/L	50	U	50	U	50	U	50	U	50	U	50	U	50	U	50	U	50	U	50	U	50	U	50	U	
VPH, >C10-C12 Aromatics	ug/L	50	U	50	U	50	U	50	U	50	U	50	U	**81**		**84**		50	U	50	U	50	U	50	U	
VPH, >C12-C13 Aromatics	ug/L	50	U	50	U	50	U	50	U	50	U	50	U	**65**		**64**		50	U	50	U	50	U	50	U	
Polycyclic Aromatic Hydrocarbons																										
1-Methylnaphthalene	ug/L	**0.0108**	NJ	**0.0115**	NJ	0.0549	U	0.0505	U	0.0515	U	0.0498	U	**0.484**		**0.513**		**0.0479**	NJ	0.0515	U	0.0515	U	0.0515	U	
2-Chloronaphthalene	ug/L	0.05	U	0.0493	U	0.0549	U	0.0505	U	0.0515	U	0.0498	U	0.0515	U	0.051	U	0.051	U	0.0515	U	0.0515	U	0.0515	U	
2-Methylnaphthalene	ug/L	0.05	U	0.0493	U	0.0549	U	0.0505	U	0.0515	U	0.0498	U	0.0515	U	0.051	U	0.051	U	0.0515	U	0.0515	U	0.0515	U	
Acenaphthene	ug/L	0.05	U	0.0493	U	0.0549	U	0.0505	U	0.0515	U	0.0498	U	**0.509**	NJ	**0.511**	NJ	**0.0866**	NJ	0.0515	U	0.0515	U	0.0515	U	
Acenaphthylene	ug/L	0.05	U	0.0493	U	0.0549	U	0.0505	U	0.0515	U	0.0498	U	**0.122**	NJ	**0.118**	NJ	**0.0664**	NJ	0.0515	U	0.0515	U	0.0515	U	
Anthracene	ug/L	0.05	U	0.0493	U	0.0549	U	0.0505	U	0.0515	U	0.0498	U	**0.0984**	NJ	**0.0957**	NJ	**0.005**	NJ	0.0515	U	0.0515	U	0.0515	U	
Benzo(a)anthracene	ug/L	0.05	U	0.0493	U	0.0549	U	0.0505	U	0.0515	U	0.0498	U	0.0515	U	0.051	U	0.051	U	0.0515	U	0.0515	U	0.0515	U	
Benzo(a)pyrene	ug/L	0.05	U	0.0493	U	0.0549	U	0.0505	U	0.0515	U	0.0498	U	0.0515	U	0.051	U	0.051	U	0.0515	U	0.0515	U	0.0515	U	
Benzo(b)fluoranthene	ug/L	0.05	U	0.0493	U	0.0549	U	0.0505	U	0.0515	U	0.0498	U	0.0515	U	0.051	U	0.051	U	0.0515	U	0.0515	U	0.0515	U	
Benzo(ghi)perylene	ug/L	**0.0216**	J	**0.0216**	J	0.0549	U	0.0505	U	0.0515	U	0.0498	U	0.0515	U	0.051	U	0.051	U	0.0515	U	0.0515	U	0.0515	U	
Benzo(k)fluoranthene	ug/L	0.05	U	0.0493	U	0.0549	U	0.0505	U	0.0515	U	0.0498	U	0.0515	U	0.051	U	0.051	U	0.0515	U	0.0515	U	0.0515	U	

		Contaminated				Background		Contaminated				Background		Contaminated				Background		Contaminated				Background		
		Site 1		Site 1 duplicate		Site 1		Site 2		Site 2 duplicate		Site 2		Site 3		Site 3 duplicate		Site 3		Site 4		Site 4 duplicate		Site 4		
Sample Date		**12/13/2018**		**12/13/2018**		**12/13/2018**		**12/17/2018**		**12/17/2018**		**12/17/2018**		**12/18/2018**		**12/18/2018**		**12/18/2018**		**3/18/2019**		**3/18/2019**		**3/18/2019**		
Carbazole	ug/L		REJ	0.0493	U	0.0549	U	0.0505	U	0.0515	U	0.0498	U	**0.0487**	J	**0.049**	J	**0.0037**	J	0.0515	U	0.0515	U	0.0515	U	
Chrysene	ug/L	0.05	U	0.0493	U	0.0549	U	0.0505	U	0.0515	U	0.0498	U	**0.0042**	J	**0.0041**	J	0.051	U	0.0515	U	0.0515	U	0.0515	U	
Dibenzo(a,h)anthracene	ug/L	0.05	U	0.0493	U	0.0549	U	0.0505	U	0.0515	U	0.0498	U	0.0515	U	0.051	U	0.051	U	0.0515	U	0.0515	U	0.0515	U	
Dibenzofuran	ug/L	0.05	U	0.0493	U	0.0549	U	0.0505	U	0.0515	U	0.0498	U	**0.956**		**0.97**		**0.349**		**0.32**		**0.345**		0.0515	U	
Fluoranthene	ug/L	0.05	U	0.0493	U	0.0549	U	0.0505	U	0.0515	U	0.0498	U	**0.0153**	J	**0.0154**	J	0.051	U	0.0515	U	0.0515	U	0.0515	U	
Fluorene	ug/L	0.05	U	0.0493	U	0.0549	U	0.0505	U	0.0515	U	0.0498	U	**1.12**		**1.13**		**0.269**		**0.742**		**0.842**		0.0515	U	
Indeno(1,2,3-cd)pyrene	ug/L	**0.0379**	J	**0.0375**	J	0.0549	U	0.0505	U	0.0515	U	0.0498	U	0.0515	U	0.051	U	0.051	U	0.0515	U	0.0515	U	0.0515	U	
Naphthalene	ug/L	0.05	U	0.0493	U	0.0549	U	0.0505	U	0.0515	U	0.0498	U	**0.764**	NJ	**0.783**	NJ	**0.0923**	NJ	0.0515	U	0.0515	U	0.0515	U	
Phenanthrene	ug/L	0.05	U	0.0493	U	0.0549	U	0.0505	U	0.0515	U	**0.0041**	J	**0.0209**	NJ	**0.0202**	NJ	**0.0038**	J	**0.389**		**0.057**		0.0515	U	
Pyrene	ug/L	0.05	U	0.0493	U	0.0549	U	0.0505	U	0.0515	U	0.0498	U	**0.102**		**0.102**		**0.0037**	J	0.0515	U	0.0515	U	0.0515	U	
Retene	ug/L	0.05	U	0.0493	U	0.0549	U	0.0505	U	0.0515	U	0.0498	U	0.0515	U	0.051	U	0.051	U	0.0515	U	0.0515	U	0.0515	U	
Metals																										**field filte blank**
Silver	ug/L	0.02	U	0.02	U	0.02	U	0.02	U	**0.029**		0.02	U	0.02	U	0.02	U	0.02	U	0.02	U	0.02	U	0.02	U	0.02
Arsenic	ug/L	3.3		3.1		6.53		19.6		19.9		18.5		2.17		1.99		4.17		**1.55**		**1.52**		**0.44**		0.1
Beryllium	ug/L	0.1	U	0.1	U	0.1	U	0.1	U	0.1	U	1	U	0.1	U	0.1	U	1	U	0.1	U	0.1	U	0.1	U	0.1
Cadmium	ug/L	**0.593**		**0.491**		0.02	U	**0.047**		**0.047**		0.2	U	0.2	U	0.2	U	0.2	U	0.2	U	0.2	U	0.056		0.2
Chromium	ug/L	0.56		**0.55**		0.1	U	**0.15**		**0.11**		1	U	0.1	U	0.1	U	**1.26**		0.25		0.25		0.2		0.1
Copper	ug/L	**8.29**		**6.87**		0.49		0.8		0.8		2.62		0.1	U	0.1	U	1	U	0.44		0.41		0.56		0.1
Mercury	ug/L	0.05	U	0.05	U	0.05	U	0.05	U	0.05	U	0.05	U	0.05	U	0.05	U	0.05	U	0.05	U	0.05	U	0.05	U	0.05
Nickel	ug/L	8.52		7.91		2.25		3.28		3.43		3.41		0.27		0.28		1	U	**1.41**		**1.39**		**0.87**		0.1
Lead	ug/L	**6.32**		**5.24**		0.032		0.02	U	0.02	U	0.02	U	0.02	U	0.02	U	0.02	U	0.02	U	0.02	U	0.02	U	**2.24**
Antimony	ug/L	2.14		1.96		0.2	U	0.44		0.43		2	U	0.2	U	0.2	U	2	U	0.02	U	0.02	U	0.02	U	0.2
Selenium	ug/L	0.3		0.32		0.1	U	0.63		0.6		**13.4**		0.4		0.32		2.17		0.1	U	0.1	U	0.1	U	0.1
Thallium	ug/L	0.1	U	0.1	U	0.1	U	0.1	U	0.1	U	1	U	0.1	U	0.1	U	1	U	0.1	U	0.1	U	0.1	U	0.1
Zinc	ug/L	**11.5**		**10.2**		1.4		1	U	1	U	10	U	1.3		1	U	10	U	1	U	1	U	**4.1**		1

Bold results were considered detections that should be evaluated (comparison to state water quality criteria) for possible impacts to the test organisms.

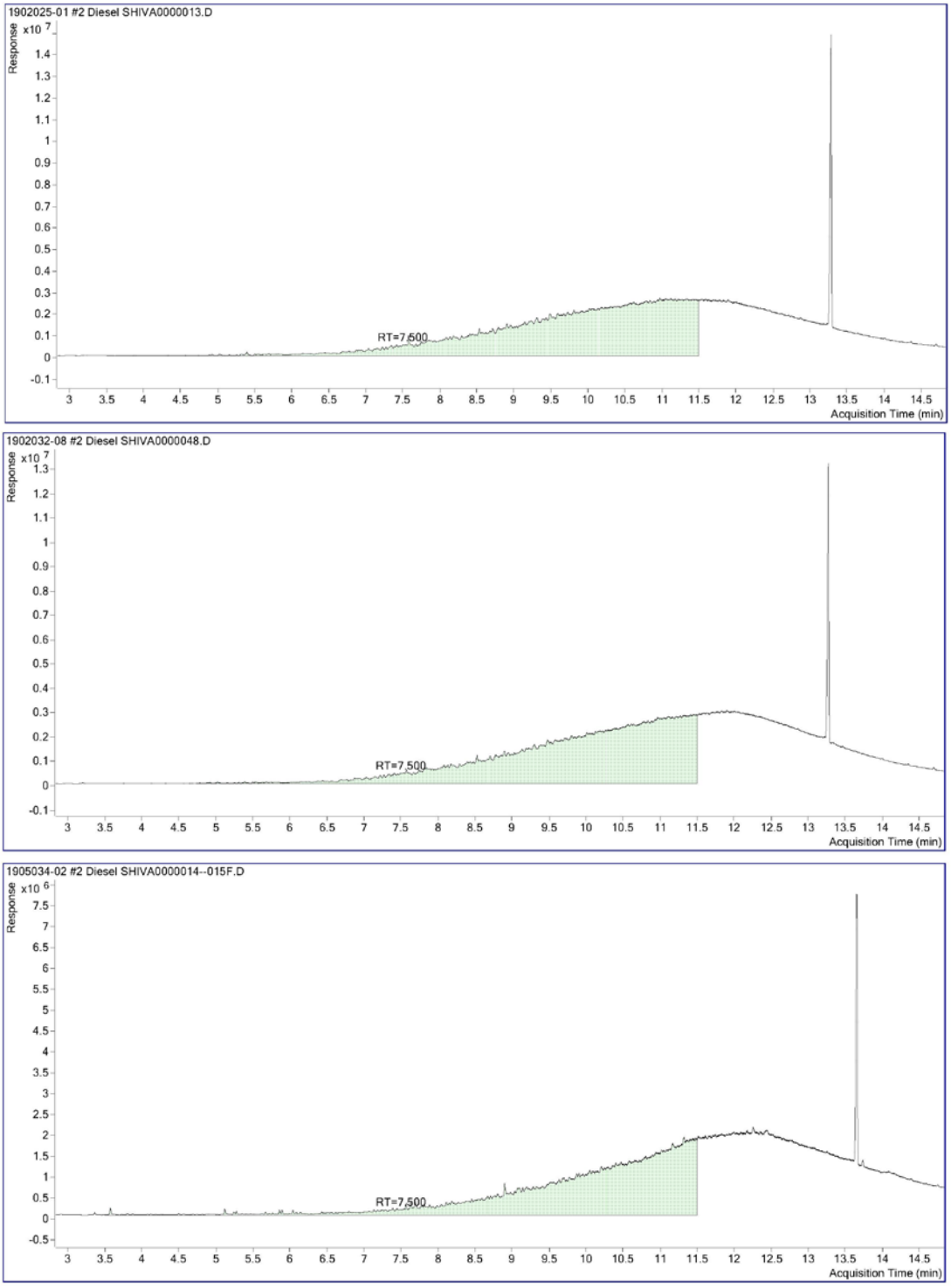

Figure D-2: Chromatograms from the diesel-range organics (DRO) in samples from the initial mixing/collection (upper), range-finding toxicity tests (middle) and final toxicity tests (lower). Shaded regions of the curve represent the area quantified for reported concentrations (mg/L).

Table D-2: NWPTH-Dx results for the range-finding toxicity tests.

Sample ID	sample date	MEL ID	analysis date	DRO (mg/L)		RRO (mg/L)		% spike rec.	Test species	% dilution	Test stage
RF-Aaff-100-0-1	2/19/2019	1902032-01	3/1/2019	6.1	J	5.71	J	101	Aaff	100	fresh
RF-Aaff-100-0-2	2/19/2019	1902032-02	3/1/2019	6.08	J	5.74	J	91	Aaff	100	fresh
RF-Aaff-6.3-0-1	2/19/2019	1902032-03	3/1/2019	0.38	J	0.38	UJ	85	Aaff	6.3	fresh
RF-Aaff-6.3-0-2	2/19/2019	1902032-04	3/1/2019	0.26	J	0.38	UJ	94	Aaff	6.3	fresh
RF-Aaff-0.39-0-1	2/19/2019	1902032-05	3/1/2019	0.19	J	0.38	UJ	80	Aaff	0.39	fresh
RF-Aaff-0-0-1	2/19/2019	1902032-06	3/1/2019	0.31	J	0.38	UJ	83	Aaff	siteblnk	fresh
RF-Aaff-LabCont-0-1	2/19/2019	1902032-07	3/1/2019	0.15	UJ	0.38	UJ	85	Aaff	labblnk	fresh
RF-Ppro-100-0-1	2/19/2019	1902032-08	3/1/2019	6.16		5.88		86	Ppro	100	fresh
RF-Ppro-100-0-2	2/19/2019	1902032-09	3/1/2019	6.27	J	6.17	J	94	Ppro	100	fresh
RF-Ppro-6.3-0-1	2/19/2019	1902032-10	3/1/2019	0.39		0.38	U	86	Ppro	6.3	fresh
RF-Ppro-6.3-0-2	2/19/2019	1902032-11	3/1/2019	0.29		0.38	U	90	Ppro	6.3	fresh
RF-Ppro-0.39-0-1	2/19/2019	1902032-12	3/1/2019	0.15	U	0.38	U	94	Ppro	0.39	fresh
RF-Ppro-0-0-1	2/19/2019	1902032-13	3/1/2019	0.15	U	0.38	U	66	Ppro	siteblnk	fresh
RF-Ppro-LabCont-0-1	2/19/2019	1902032-14	3/1/2019	0.15	U	0.38	U	90	Ppro	labblnk	fresh
RF-Aaff-100-1-1-S	2/20/2019	1902032-15	3/1/2019	5.62	J	5.29	J	101	Aaff	100	stale
RF-Aaff-100-1-1-F	2/20/2019	1902032-16	3/1/2019	5.26	J	5.01	J	88	Aaff	100	fresh
RF-Aaff-6.3-1-1-S	2/20/2019	1902032-17	3/1/2019	0.2	J	0.38	UJ	84	Aaff	6.3	stale
RF-Aaff-6.3-1-1-F	2/20/2019	1902032-18	3/1/2019	0.3	J	0.38	UJ	91	Aaff	6.3	fresh
RF-Aaff-6.3-1-2-F	2/20/2019	1902032-19	3/1/2019	0.25	J	0.38	UJ	91	Aaff	6.3	fresh
RF-Aaff-0.39-1-1-S	2/20/2019	1902032-20	3/1/2019	0.15	UJ	0.38	UJ	87	Aaff	0.39	stale
RF-Aaff-0.39-1-1-F	2/20/2019	1902032-21	2/28/2019	0.15	UJ	0.38	UJ	99	Aaff	0.39	fresh
RF-Ppro-100-1-1-S	2/20/2019	1902032-22	2/28/2019	5.39		4.65		113	Ppro	100	stale
RF-Ppro-100-1-1-F	2/20/2019	1902032-23	2/28/2019	6.33		5.78		120	Ppro	100	fresh
RF-Ppro-6.3-1-1-S	2/20/2019	1902032-24	2/28/2019	0.35	J	0.38	UJ	111	Ppro	6.3	stale
RF-Ppro-6.3-1-1-F	2/20/2019	1902032-25	2/28/2019	0.29		0.38	U	103	Ppro	6.3	fresh
RF-Ppro-6.3-1-2-F	2/20/2019	1902032-26	3/1/2019	0.3		0.38	U	110	Ppro	6.3	fresh
RF-Ppro-0.39-1-1-S	2/20/2019	1902032-27	3/1/2019	0.15	UJ	0.38	UJ	107	Ppro	0.39	stale
RF-Ppro-0.39-1-1-F	2/20/2019	1902032-28	3/1/2019	0.15	UJ	0.38	UJ	108	Ppro	0.39	fresh
RF-Aaff-100-2-1-F	2/21/2019	1902032-29	3/1/2019	5.88	J	5.11	J	124	Aaff	100	fresh
RF-Aaff-6.3-2-1-F	2/21/2019	1902032-30	3/1/2019	0.31	J	0.4	UJ	107	Aaff	6.3	fresh
RF-Aaff-0.39-2-1-F	2/21/2019	1902032-31	3/1/2019	0.15	UJ	0.38	UJ	106	Aaff	0.39	fresh
RF-Ppro-100-2-1-F	2/21/2019	1902032-32	3/1/2019	6.35	J	6.23	J	119	Ppro	100	fresh
RF-Ppro-6.3-2-1-F	2/21/2019	1902032-33	3/1/2019	0.39	J	0.41	UJ	110	Ppro	6.3	fresh
RF-Ppro-0.39-2-1-F	2/21/2019	1902032-34	3/1/2019	0.15	UJ	0.38	UJ	110	Ppro	0.39	fresh
RF-Aaff-100-3-1-F	2/22/2019	1902033-01	3/6/2019	3.96		1.72		123	Aaff	100	fresh
RF-Aaff-6.3-3-1-F	2/22/2019	1902033-02	3/6/2019	0.22		0.38	U	131	Aaff	6.3	fresh
RF-Aaff-6.3-3-2-F	2/22/2019	1902033-03	3/6/2019	0.21		0.38	U	128	Aaff	6.3	fresh

Sample ID	sample date	MEL ID	analysis date	DRO (mg/L)		RRO (mg/L)		% spike rec.	Test species	% dilution	Test stage
RF-Aaff-0.39-3-1-F	2/22/2019	1902033-04	3/6/2019	0.15	U	0.38	U	133	Aaff	0.39	fresh
RF-Ppro-100-3-1-F	2/22/2019	1902033-05	3/7/2019	7.04		8.33		122	Ppro	100	fresh
RF-Ppro-6.3-3-1-F	2/22/2019	1902033-06	3/6/2019	0.26		0.39	U	136	Ppro	6.3	fresh
RF-Ppro-6.3-3-2-F	2/22/2019	1902033-07	3/6/2019	0.4		0.38	U	129	Ppro	6.3	fresh
RF-Ppro-0.39-3-1-F	2/22/2019	1902033-08	3/6/2019	0.15	U	0.38	U	131	Ppro	0.39	fresh
RF-Aaff-100-4-1-F	2/23/2019	1902033-09	3/7/2019	5.26		2.33		145	Aaff	100	fresh
RF-Aaff-6.3-4-1-F	2/23/2019	1902033-10	3/7/2019	0.34		0.39	U	131	Aaff	6.3	fresh
RF-Aaff-6.3-4-2-F	2/23/2019	1902033-11	3/7/2019	0.35		0.38	U	136	Aaff	6.3	fresh
RF-Aaff-0.39-4-1-F	2/23/2019	1902033-12	3/7/2019	0.15	U	0.38	U	135	Aaff	0.39	fresh
RF-Ppro-100-4-1-F*	2/23/2019	1902033-13	3/7/2019	0.32		0.42	U	121	Ppro	100	fresh
RF-Ppro-6.3-4-1-F*	2/23/2019	1902033-14	3/7/2019	0.15	U	0.38	U	113	Ppro	6.3	fresh
RF-Ppro-6.3-4-2-F	2/23/2019	1902033-15	3/7/2019	0.15*	U	0.38*	U	116	Ppro	6.3	fresh
RF-Ppro-0.39-4-1-F	2/23/2019	1902033-16	3/7/2019	0.15	U	0.38	U	118	Ppro	0.39	fresh
RF-Aaff-100-5-1-F	2/24/2019	1902033-17	3/7/2019	4.15		4.2		143	Aaff	100	fresh
RF-Aaff-6.3-5-1-F	2/24/2019	1902033-18	3/7/2019	0.36		0.38	U	136	Aaff	6.3	fresh
RF-Aaff-0.39-5-1-F	2/24/2019	1902033-19	3/7/2019	0.15	U	0.38	U	121	Aaff	0.39	fresh
RF-Ppro-100-5-1-F	2/24/2019	1902033-20	3/7/2019	4.8		5.53		114	Ppro	100	fresh
RF-Ppro-6.3-5-1-F	2/24/2019	1902033-21	3/7/2019	0.33		0.38	U	98	Ppro	6.3	fresh
RF-Ppro-0.39-5-1-F	2/24/2019	1902033-22	3/7/2019	0.15	U	0.7		98	Ppro	0.39	fresh
RF-Aaff-100-6-1-F	2/25/2019	1902033-23	3/7/2019	3.86		4.02		104	Aaff	100	fresh
RF-Aaff-6.3-6-1-F	2/25/2019	1902033-24	3/7/2019	0.16	U	0.39	U	94	Aaff	6.3	fresh
RF-Aaff-6.3-6-2-F	2/25/2019	1902033-25	3/7/2019	0.15	U	0.39	U	93	Aaff	6.3	fresh
RF-Aaff-0.39-6-1-F	2/25/2019	1902033-26	3/7/2019	0.15	U	0.38	U	98	Aaff	0.39	fresh
RF-Aaff-0-6-1	2/25/2019	1902033-27	3/7/2019	0.26		0.39	U	95	Aaff	siteblnk	fresh
RF-Aaff-LabCont-6-1	2/25/2019	1902033-28	3/7/2019	0.15	U	0.38	U	95	Aaff	labblnk	fresh
RF-Ppro-100-6-1-F	2/25/2019	1902033-29	3/7/2019	6.63		8.47		116	Ppro	100	fresh
RF-Ppro-6.3-6-1-F	2/25/2019	1902033-30	3/7/2019	0.28		0.38	U	95	Ppro	6.3	fresh
RF-Ppro-6.3-6-2-F	2/25/2019	1902033-31	3/7/2019	0.28		0.4	U	100	Ppro	6.3	fresh
RF-Ppro-0.39-6-1-F	2/25/2019	1902033-32	3/7/2019	0.15	U	0.39	U	96	Ppro	0.39	fresh
RF-Ppro-0-6-1	2/25/2019	1902033-33	3/7/2019	0.25		0.4	U	91	Ppro	siteblnk	fresh
RF-Ppro-LabCont-6-1	2/25/2019	1902033-34	3/7/2019	0.16	U	0.4	U	99	Ppro	labblnk	fresh
RF-Aaff-100-7-1-S	2/26/2019	1902033-35	3/7/2019	4.4		5.12		107	Aaff	100	stale
RF-Aaff-6.3-7-1-S	2/26/2019	1902033-36	3/7/2019	0.25		0.36	U	91	Aaff	6.3	stale
RF-Aaff-0.39-7-1-S	2/26/2019	1902033-37	3/7/2019	0.14	U	0.36	U	95	Aaff	0.39	stale
RF-Ppro-100-7-1-S	2/26/2019	1902033-38	3/7/2019	5.7		7.9		111	Ppro	100	stale
RF-Ppro-6.3-7-1-S	2/26/2019	1902033-39	3/8/2019	0.3		0.36	U	97	Ppro	6.3	stale
RF-Ppro-0.39-7-1-S	2/26/2019	1902033-40	3/8/2019	0.15		0.41		96	Ppro	0.39	stale
RF-Cdub-100-0-1	3/11/2019	1903026-01	4/4/2019	6.16		6.15		88	Cdub	100	fresh

Sample ID	sample date	MEL ID	analysis date	DRO (mg/L)		RRO (mg/L)		% spike rec.	Test species	% dilution	Test stage
RF-Cdub-100-0-2	3/11/2019	1903026-02	4/4/2019	6.26		6.37		97	Cdub	100	fresh
RF-Cdub-6.3-0-1	3/11/2019	1903026-03	4/4/2019	0.32		0.41	U	95	Cdub	6.3	fresh
RF-Cdub-6.3-0-2	3/11/2019	1903026-04	4/4/2019	0.42		0.37	U	91	Cdub	6.3	fresh
RF-Cdub-0.39-0-1	3/11/2019	1903026-05	4/4/2019	0.15	U	0.38	U	108	Cdub	0.39	fresh
RF-Cdub-0-0-1	3/11/2019	1903026-06	4/4/2019	0.28	U	0.38	U	99	Cdub	siteblnk	fresh
RF-Cdub-LabCont-0-1	3/11/2019	1903026-07	4/4/2019	0.22	U	0.45	U	92	Cdub	labblnk	fresh
RF-Cdub-100-24-1	3/12/2019	1903026-08	4/4/2019	6.29		6.4		88	Cdub	100	fresh
RF-Cdub-6.3-24-1	3/12/2019	1903026-09	4/4/2019	0.34		0.41	U	97	Cdub	6.3	fresh
RF-Cdub-6.3-24-2	3/12/2019	1903026-10	4/5/2019	0.28	U	0.42	U	107	Cdub	6.3	fresh
RF-Cdub-0.39-24-1	3/12/2019	1903026-11	4/5/2019	0.16	U	0.48	U	100	Cdub	0.39	fresh
RF-Cdub-100-48-1	3/13/2019	1903026-12	4/5/2019	5.53		5.69		84	Cdub	100	fresh
RF-Cdub-6.3-48-1	3/13/2019	1903026-13	4/5/2019	0.27	U	0.37	U	97	Cdub	6.3	fresh
RF-Cdub-0.39-48-1	3/13/2019	1903026-14	4/5/2019	0.15	U	0.44	U	98	Cdub	0.39	fresh
RF-Cdub-100-72-1	3/14/2019	1903027-01	4/5/2019	5.48	J	5.71	J	81	Cdub	100	fresh
RF-Cdub-6.3-72-1	3/14/2019	1903027-02	4/5/2019	0.28	UJ	0.41	UJ	102	Cdub	6.3	fresh
RF-Cdub-6.3-72-2	3/14/2019	1903027-03	4/5/2019	0.27	UJ	0.42	UJ	97	Cdub	6.3	fresh
RF-Cdub-0.39-72-1	3/14/2019	1903027-04	4/5/2019	0.17	UJ	0.43	UJ	103	Cdub	0.39	fresh
RF-Cdub-100-96-1	3/15/2019	1903027-05	4/5/2019	3.75	J	3.71	J	85	Cdub	100	fresh
RF-Cdub-6.3-96-1	3/15/2019	1903027-06	4/5/2019	0.25	UJ	0.46	UJ	104	Cdub	6.3	fresh
RF-Cdub-6.3-96-2	3/15/2019	1903027-07	4/5/2019	0.24	UJ	0.43	UJ	100	Cdub	6.3	fresh
RF-Cdub-0.39-96-1	3/15/2019	1903027-08	4/5/2019	0.17	UJ	0.42	UJ	98	Cdub	0.39	fresh
RF-Cdub-100-120-1	3/16/2019	1903027-09	4/5/2019	5.24	J	5.38	J	90	Cdub	100	fresh
RF-Cdub-6.3-120-1	3/16/2019	1903027-10	4/5/2019	0.3	UJ	0.4	UJ	102	Cdub	6.3	fresh
RF-Cdub-0.39-120-1	3/16/2019	1903027-11	4/5/2019	0.17	UJ	0.42	UJ	95	Cdub	6.3	fresh
RF-Cdub-0-144-1	3/17/2019	1903027-12	4/5/2019	0.23	UJ	0.42	UJ	97	Cdub	siteblnk	fresh
RF-Cdub-LabCont-144-1	3/17/2019	1903027-13	4/5/2019	0.16	UJ	0.4	UJ	101	Cdub	labblnk	fresh

Test species include: Aaff = *Atherinops affinis*; Spur = *Strongylocentrotus purpuratus;*
Ppro = *Pimephales promelas*; Cdub = *Ceriodaphnia dubia*

*sample result was considered an outlier and not included in the calculation of the mean test DRO concentration.

Table D-3: NWPTH-Dx results for the final toxicity tests.

Sample ID	sample date	MEL ID	analysis date	DRO (mg/L)		RRO (mg/L)		% spike rec.	species
Ctox-Ppro-100-0-1	4/23/2019	1904046-01	5/7/2019	5.39	J	5.14	J	83	Ppro
Ctox-Ppro-100-0-2	4/23/2019	1904046-02	5/7/2019	5.83	J	5.76	J	86	Ppro
Ctox-Ppro-50-0-1	4/23/2019	1904046-03	5/7/2019	2.28	J	2.35	J	90	Ppro
Ctox-Ppro-25-0-1	4/23/2019	1904046-04	5/7/2019	0.94	J	1.01	J	91	Ppro
Ctox-Ppro-0-0-1	4/23/2019	1904046-05	5/7/2019	0.2	J	0.45	UJ	93	Ppro
Ctox-Ppro-LabCont-0-1	4/23/2019	1904046-06	5/7/2019	0.17	UJ	0.43	UJ	93	Ppro
Ctox-Aaff-100-0-1	4/23/2019	1904046-07	5/7/2019	3.94		3.84		88	Aaff
Ctox-Aaff-100-0-2	4/23/2019	1904046-08	5/14/2019	2.84		3.67		88	Aaff
Ctox-Aaff-50-0-1	4/23/2019	1904046-09	5/14/2019	1.41		1.59		94	Aaff
Ctox-Aaff-25-0-1	4/23/2019	1904046-10	5/14/2019	0.67		0.58		92	Aaff
Ctox-Aaff-0-0-1	4/23/2019	1904046-11	5/14/2019	0.22		0.48	U	84	Aaff
Ctox-Aaff-LabCont-0-1	4/23/2019	1904046-12	5/15/2019	0.17	U	0.42	U	81	Aaff
Ctox-Aaff-100-24-1	4/24/2019	1904046-13	5/15/2019	2.74		3.51		74	Aaff
Ctox-Aaff-50-24-1	4/24/2019	1904046-14	5/15/2019	0.43		0.48	U	75	Aaff
Ctox-Aaff-25-24-1	4/24/2019	1904046-15	5/15/2019	0.62		0.57		85	Aaff
Ctox-Ppro-100-24-1*	4/24/2019	1904046-16	5/15/2019	0.22		0.48	U	74	Ppro
Ctox-Ppro-50-24-1	4/24/2019	1904046-17	5/15/2019	0.19	U	0.49	U	77	Ppro
Ctox-Ppro-25-24-1	4/24/2019	1904046-18	5/15/2019	0.81		0.85		88	Ppro
Ctox-Ppro-100-48-1	4/25/2019	1904072-01	5/15/2019	3.76		5.51		90	Ppro
Ctox-Ppro-100-48-2	4/25/2019	1904072-02	5/15/2019	3.98		5.83		88	Ppro
Ctox-Ppro-50-48-1	4/25/2019	1904072-03	5/21/2019	1.8		2.53		90	Ppro
Ctox-Ppro-25-48-1	4/25/2019	1904072-04	5/21/2019	0.93		1.29		102	Ppro
Ctox-Aaff-100-48-1	4/25/2019	1904072-05	5/21/2019	3.06		4.27		99	Aaff
Ctox-Aaff-100-48-2	4/25/2019	1904072-06	5/21/2019	3.22		4.66		95	Aaff
Ctox-Aaff-50-48-1	4/25/2019	1904072-07	5/21/2019	1.43		1.92		100	Aaff
Ctox-Aaff-25-48-1	4/25/2019	1904072-08	5/21/2019	0.65		0.68		103	Aaff
Ctox-Aaff-100-72-1	4/26/2019	1904072-09	5/21/2019	3.19		4.72		101	Aaff
Ctox-Aaff-50-72-1	4/26/2019	1904072-10	5/21/2019	1.35		1.71		105	Aaff
Ctox-Aaff-25-72-1	4/26/2019	1904072-11	5/21/2019	0.62		0.63		99	Aaff
Ctox-Ppro-100-72-1	4/26/2019	1904072-12	5/21/2019	3.93		6.52		93	Ppro
Ctox-Ppro-50-72-1	4/26/2019	1904072-13	5/21/2019	1.97		3.15		109	Ppro
Ctox-Ppro-25-72-1	4/26/2019	1904072-14	5/21/2019	0.86		1.15		110	Ppro
Ctox-Ppro-100-96-1	4/27/2019	1904072-15	5/21/2019	4		6.59		97	Ppro
Ctox-Ppro-100-96-2	4/27/2019	1904072-16	5/21/2019	4.12		7.07		98	Ppro
Ctox-Ppro-50-96-1	4/27/2019	1904072-17	5/21/2019	1.82		2.82		104	Ppro
Ctox-Ppro-50-96-2	4/27/2019	1904072-18	5/21/2019	1.76		2.67		97	Ppro
Ctox-Ppro-25-96-1	4/27/2019	1904072-19	5/21/2019	0.83		0.88		107	Ppro

Sample ID	sample date	MEL ID	analysis date	DRO (mg/L)		RRO (mg/L)		% spike rec.	species
Ctox-Aaff-100-96-1	4/27/2019	1904072-23	5/21/2019	4.43		7.03		104	Aaff
Ctox-Aaff-100-96-2	4/27/2019	1904072-24	5/22/2019	2.76		3.91		99	Aaff
Ctox-Aaff-50-96-1	4/27/2019	1904072-25	5/22/2019	1.23		1.41		105	Aaff
Ctox-Aaff-50-96-2*	4/27/2019	1904072-26	5/22/2019	0.27		0.44	U	77	Aaff
Ctox-Aaff-25-96-1	4/27/2019	1904072-27	5/22/2019	0.55		0.62		106	Aaff
Ctox-Ppro-100-120-1†	4/28/2019	1904072-31	5/22/2019	2.42		3.72		70	Ppro
Ctox-Ppro-50-120-1†	4/28/2019	1904072-32	5/22/2019	3.11		5.1		99	Ppro
Ctox-Ppro-25-120-1	4/28/2019	1904072-33	5/22/2019	0.95		1.16		101	Ppro
Ctox-Aaff-100-120-1	4/28/2019	1904072-34	5/22/2019	2.97		4.31		102	Aaff
Ctox-Aaff-50-120-1	4/28/2019	1904072-35	5/22/2019	1.13		1.31		103	Aaff
Ctox-Aaff-25-120-1	4/28/2019	1904072-36	5/22/2019	0.58		0.43	U	106	Aaff
Ctox-Ppro-100-144-1	4/29/2019	1905034-01	5/22/2019	3.98		6.16		107	Ppro
Ctox-Ppro-100-144-2	4/29/2019	1905034-02	5/22/2019	4.02		6.28		105	Ppro
Ctox-Ppro-50-144-1	4/29/2019	1905034-03	5/22/2019	1.89		2.62		111	Ppro
Ctox-Ppro-50-144-2	4/29/2019	1905034-04	5/22/2019	1.75		2.33		106	Ppro
Ctox-Ppro-25-144-1	4/29/2019	1905034-05	5/22/2019	0.89		0.93		106	Ppro
Ctox-Ppro-0-144-1	4/29/2019	1905034-06	5/23/2019	0.21		0.51		92	Ppro
Ctox-Ppro-LabCont-144-1	4/29/2019	1905034-07	5/23/2019	0.17	U	0.52		93	Ppro
Ctox-Aaff-100-144-1	4/29/2019	1905034-11	5/23/2019	2.66		3.67		110	Aaff
Ctox-Aaff-100-144-2	4/29/2019	1905034-12	5/23/2019	2.69		3.79		107	Aaff
Ctox-Aaff-50-144-1	4/29/2019	1905034-13	5/23/2019	0.42		0.62		88	Aaff
Ctox-Aaff-50-144-2	4/29/2019	1905034-14	5/23/2019	1.2		1.44		107	Aaff
Ctox-Aaff-25-144-1	4/29/2019	1905034-15	5/23/2019	0.64		0.61		108	Aaff
Ctox-Aaff-0-144-1	4/29/2019	1905034-16	5/23/2019	0.17	U	0.43	U	101	Aaff
Ctox-Aaff-LabCont-144-1	4/29/2019	1905034-17	5/23/2019	0.15	U	0.37	U	99	Aaff
Ctox-Ppro-100-168-1-S	4/30/2019	1905034-21	5/23/2019	3.56		6.07		95	Ppro
Ctox-Ppro-50-168-1-S	4/30/2019	1905034-22	5/23/2019	1.72		2.85		101	Ppro
Ctox-Ppro-25-168-1-S	4/30/2019	1905034-23	5/23/2019	0.81		1.2		101	Ppro
Ctox-Aaff-100-168-1-S	4/30/2019	1905034-26	5/23/2019	2.86		4.48		101	Aaff
Ctox-Aaff-50-168-1-S	4/30/2019	1905034-27	5/23/2019	1.14		1.59		104	Aaff
Ctox-Aaff-25-168-1-S	4/30/2019	1905034-28	5/23/2019	0.51		0.45		111	Aaff
Ctox-Spur-100-0-1	5/1/2019	1905034-31	5/23/2019	2.83		4.62		102	Spur
Ctox-Spur-100-0-2	5/1/2019	1905034-32	5/23/2019	2.59		4.16		102	Spur
Ctox-Spur-0-0-1	5/1/2019	1905034-33	5/23/2019	0.28		0.39	U	95	Spur
Ctox-Spur-0-0-2	5/1/2019	1905034-34	5/23/2019	0.16	U	0.4	U	74	Spur
Ctox-Spur-LabCont-0-1	5/1/2019	1905034-35	5/23/2019	0.16	U	0.39	U	102	Spur
Ctox-Spur-LabCont-0-2	5/1/2019	1905034-36	5/23/2019	0.15	U	0.38	U	101	Spur
Ctox-Cdub-100-24-1	5/15/2019	1905056-01	6/13/2019	4.02	J	4.48	J	102	Cdub

Sample ID	sample date	MEL ID	analysis date	DRO (mg/L)		RRO (mg/L)		% spike rec.	species
Ctox-Cdub-100-48-1	5/16/2019	1905056-02	6/13/2019	4.13	J	4.6	J	103	Cdub
Ctox-Cdub-100-48-2	5/16/2019	1905056-03	6/13/2019	4.24		4.56		106	Cdub
Ctox-Cdub-100-72-1	5/17/2019	1905056-04	6/13/2019	4.24		4.49		104	Cdub
Ctox-Cdub-100-96-1	5/18/2019	1905056-05	6/13/2019	4.02		4.29		110	Cdub
Ctox-Cdub-100-96-2	5/18/2019	1905056-06	6/13/2019	4.13		4.56		112	Cdub
Ctox-Cdub-0-120-1	5/19/2019	1905056-08	6/13/2019	0.28		0.45	U	115	Cdub
Ctox-Cdub-LabCont-120-1	5/19/2019	1905056-09	6/13/2019	0.16	J	0.39	U	119	Cdub
Ctox-Cdub-100-0-1	5/14/2019	1905056-10	6/13/2019	3.76	J	3.97	J	112	Cdub
Ctox-Cdub-100-0-2	5/14/2019	1905056-11	6/13/2019	4.38	J	4.77	J	120	Cdub
Ctox-Cdub-0-0-1	5/14/2019	1905056-12	6/13/2019	0.22	J	0.37	J	108	Cdub
Ctox-Cdub-LabCont-0-1	5/14/2019	1905056-13	6/13/2019	0.17	J	0.4	U	109	Cdub

*sample result was considered an outlier and not included in the calculation of the mean test DRO concentration.
†samples were mislabeled and analytical results should be switched for sample 1904072-31 and -32.

Table D-4: NWPTH-Dx results with no cleanup, silica gel cleanup (SGC) and SGC with sulfuric acid (H_2SO_4).

				no cleanup	SGC	SGC + H2SO4		no cleanup		SGC		SGC + H2SO4	
Sample ID	sample date	MEL ID	analysis date	DRO (mg/L)	DRO (mg/L)	DRO (mg/L)		RRO (mg/L)		RRO (mg/L)		RRO (mg/L)	
Ctox-Ppro-100-48-1	4/25/2019	1904072-01	5/15/2019	3.76	1.31	0.22		5.51		1.02		0.47	U
Ctox-Ppro-100-48-2	4/25/2019	1904072-02	5/15/2019	3.98	1.24	0.21		5.83		0.85		0.47	U
Ctox-Aaff-100-48-1	4/25/2019	1904072-05	5/21/2019	3.06	0.94	0.36		4.27		0.59		0.47	U
Ctox-Aaff-100-48-2	4/25/2019	1904072-06	5/21/2019	3.22	2.04	0.32		4.66		1.94		0.47	U
Ctox-Aaff-50-48-1	4/25/2019	1904072-07	5/21/2019	1.43	0.91	0.22		1.92		0.64		0.48	U
Ctox-Ppro-100-96-1	4/27/2019	1904072-15	5/21/2019	4.00	2.21	0.24		6.59		2.29		0.45	U
Ctox-Ppro-100-96-2	4/27/2019	1904072-16	5/21/2019	4.12	1.78	0.19		7.07		1.67		0.44	U
Ctox-Ppro-100-144-1	4/29/2019	1905034-01	5/22/2019	3.98	3.19	0.23		6.16		3.15		0.48	U
Ctox-Ppro-100-144-2	4/29/2019	1905034-02	5/22/2019	4.02	3.43	0.26		6.28		3.28		0.50	U
Ctox-Ppro-50-144-1	4/29/2019	1905034-03	5/22/2019	1.89	1.26	0.19		2.62		0.88		0.49	U
Ctox-Ppro-50-144-2	4/29/2019	1905034-04	5/22/2019	1.75	0.85	0.19	U	2.33		0.49	U	0.19	U
Ctox-Aaff-100-144-1	4/29/2019	1905034-11	5/23/2019	2.66	2.03	0.25		3.67		1.58		0.47	U
Ctox-Aaff-100-144-2	4/29/2019	1905034-12	5/23/2019	2.69	1.78	0.36		3.79		1.47		0.48	U
Ctox-Aaff-50-144-1	4/29/2019	1905034-13	5/23/2019	0.42	0.38	0.19	U	0.62		0.49	U	0.49	U
Ctox-Aaff-50-144-2	4/29/2019	1905034-14	5/23/2019	1.20	0.79	0.19	U	1.44		0.47	U	0.47	U
Ctox-Spur-100-0-1	5/1/2019	1905034-31	5/23/2019	2.83	2.20	0.29		4.62		2.27		0.39	U
Ctox-Spur-100-0-2	5/1/2019	1905034-32	5/23/2019	2.59	1.95	0.24		4.16		1.88		0.39	U

Table D-5: Supplemental hydrocarbon analysis of key samples from the final toxicity tests. Parameters include: volatile petroleum hydrocarbons (VPH), extractable petroleum hydrocarbons (EPH), NWTPH-gasoline fraction (Gx) with BETX, and polycyclic aromatic hydrocarbons (PAHs).

Sample ID	Ctox-Ppro-100-144		Ctox-Ppro-50-144		Ctox-Aaff-100-144		Ctox-Aaff-50-144		Ctox-Ppro-100-168		Ctox-Ppro-50-168		Ctox-Aaff-100-168		Ctox-Aaff-50-168		Ctox-Cdub-100-1	
Collection date	**4/29/2019**		**4/29/2019**		**4/29/2019**		**4/29/2019**		**4/30/2019**		**4/30/2019**		**4/30/2019**		**4/30/2019**		**5/27/2019**	
Analysis date	**5/8/2019**		**5/8/2019**		**5/8/2019**		**5/8/2019**		**5/15/2019**		**5/15/2019**		**5/15/2019**		**5/15/2019**		**5/31/2019**	
MEL ID	**1905034-08**		**1905034-09**		**1905034-18**		**1905034-19**		**1905034-24**		**1905034-25**		**1905034-29**		**1905034-30**		**1905056-14**	
NWTPH-Gx	0.07	U	0.07	U	0.07	U	0.07	U	-		-		-		-			
Benzene	1	U	1	U	1	U	1	U	-		-		-		-			
Ethylbenzene	1	U	1	U	1	U	1	U	-		-		-		-			
m,p-Xylene	2	U	2	U	2	U	2	U	-		-		-		-			
o-Xylene	1	U	1	U	1	U	1	U	-		-		-		-			
Toluene	1	U	1	U	1	U	1	U	-		-		-		-			
VPH, C5-C6 Aliphatics	50	U	50	U	50	U	50	U	-		-		-		-			
VPH, >C6-C8 Aliphatics	50	U	50	U	50	U	50	U	-		-		-		-			
VPH, >C8-C10 Aliphatics	50	U	50	U	50	U	50	U	-		-		-		-			
VPH, >C10-C12 Aliphatics	50	U	50	U	50	U	50	U	-		-		-		-			
VPH, >C8-C10 Aromatics	50	U	50	U	50	U	50	U	-		-		-		-			
VPH, >C10-C12 Aromatics	50	U	50	U	50	U	50	U	-		-		-		-			
VPH, >C12-C13 Aromatics	50	U	50	U	50	U	50	U	-		-		-		-			
EPH, C8-C10 Aliphatics	-		-		-		-		40	U	40	U	40	U	40	U		
EPH, >C10-C12 Aliphatics	-		-		-		-		40	U	40	U	40	U	40	U		
EPH, >C12-C16 Aliphatics	-		-		-		-		40	U	40	U	40	U	40	U		
EPH, >C16-C21 Aliphatics	-		-		-		-		40	U	40	U	40	U	40	U		
EPH, >C21-C34 Aliphatics	-		-		-		-		40	U	40	U	40	U	40	U		
EPH, C8-C10 Aromatics	-		-		-		-		40	U	40	U	40	U	40	U		
EPH, >C10-C12 Aromatics	-		-		-		-		40	U	40	U	40	U	40	U		
EPH, >C12-C16 Aromatics	-		-		-		-		40	U	40	U	40	U	40	U		
EPH, >C16-C21 Aromatics	-		-		-		-		40	U	40	U	40	U	40	U		
EPH, >C21-C34 Aromatics	-		-		-		-		40	U	40	U	40	U	40	U		
1-Methylnaphthalene	-		-		-		-										0.0498	UJ

Sample ID	Ctox-Ppro-100-144		Ctox-Ppro-50-144		Ctox-Aaff-100-144		Ctox-Aaff-50-144		Ctox-Ppro-100-168		Ctox-Ppro-50-168		Ctox-Aaff-100-168		Ctox-Aaff-50-168		Ctox-Cdub-100-1	
Collection date	**4/29/2019**		**4/29/2019**		**4/29/2019**		**4/29/2019**		**4/30/2019**		**4/30/2019**		**4/30/2019**		**4/30/2019**		**5/27/2019**	
Analysis date	**5/8/2019**		**5/8/2019**		**5/8/2019**		**5/8/2019**		**5/15/2019**		**5/15/2019**		**5/15/2019**		**5/15/2019**		**5/31/2019**	
MEL ID	**1905034-08**		**1905034-09**		**1905034-18**		**1905034-19**		**1905034-24**		**1905034-25**		**1905034-29**		**1905034-30**		**1905056-14**	
2-Chloronaphthalene	-		-		-		-										0.0498	UJ
2-Methylnaphthalene	-		-		-		-										0.0498	UJ
Acenaphthene	-		-		-		-										0.0498	UJ
Acenaphthylene	-		-		-		-										0.0498	UJ
Anthracene	-		-		-		-										0.0498	UJ
Benz[a]anthracene	-		-		-		-										0.0498	UJ
Benzo(a)pyrene	-		-		-		-										0.0498	UJ
Benzo(b)fluoranthene	-		-		-		-										0.0498	UJ
Benzo(ghi)perylene	-		-		-		-										0.0498	UJ
Benzo(k)fluoranthene	-		-		-		-										0.0498	UJ
Carbazole	-		-		-		-										0.0498	UJ
Chrysene	-		-		-		-										0.0498	UJ
Dibenzo(a,h)anthracene	-		-		-		-										0.0498	UJ
Dibenzofuran	-		-		-		-										0.0498	UJ
Fluoranthene	-		-		-		-										0.0498	UJ
Fluorene	-		-		-		-										0.0498	UJ
Indeno(1,2,3-cd)pyrene	-		-		-		-										0.0498	UJ
Naphthalene	-		-		-		-										0.0498	UJ
Phenanthrene	-		-		-		-										0.0498	UJ
Pyrene	-		-		-		-										0.0498	UJ
Retene	-		-		-		-										0.0498	UJ