Natural Resource Damage Assessment Plan

for the

No. 2 Diesel Fuel Spill on Big Creek near Solitude, Posey County, Indiana, March 20, 2018

July 29, 2019

U.S. Fish and Wildlife Service, Department of the Interior

Indiana Department of Natural Resources

Indiana Department of Environmental Management

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INTRODUCTION

On March 20, 2018 a pipeline carrying No. 2 diesel fuel breached, resulting in the discharge of approximately 58,800 gallons of fuel into Big Creek, Indiana (the "incident"). The purpose of this Assessment Plan (Plan) is to identify the steps that will be taken by the natural resource Trustees; the State of Indiana Department of Environmental Management (IDEM) and Department of Natural Resources (IDNR), and the U.S. Fish and Wildlife Service; to complete the restoration planning phase of the Natural Resource Damage Assessment and Restoration (NRDAR) process pursuant to 15 C.F.R. Part 990, Subpart E. Specifically, this Plan describes the Trustees' intent to pursue injury assessment and quantification activities under the Oil Pollution Act (OPA) and its regulations, 15 C.F.R. §990.10 *et seq*.

Preassessment Summary

In May 2019, the US Fish and Wildlife Service (USFWS 2019) produced a Preassessment Report (https://www.fws.gov/midwest/es/ec/nrda/BigCreekIndiana/pdf/Preassessment_Report_Big_Creek_Diesel_Spill_4-29-2019_final_with_Appendices.pdf) describing the spill incident, chemical constituents of the discharged petroleum product, the natural resources present in the impact zone, a conceptual model for understanding injury to natural resources (including toxicity impacts), and evaluations of toxicity from the literature and simple mathematical models from what is known about specific petroleum products found in the spilled diesel. This Assessment Plan does not repeat the work of the Preassessment Report, rather it builds upon that work to address specific assessment task called for pursuant to 15 C.F.R. § 990.50 –§990.52.

The Preassessment Report identifies the injuries to aquatic resources that have or are likely to have occurred and explains the pathways linking the incident to those injuries. The conceptual site model description in the Preassessment Report illustrates the pathway between the discharge of diesel into Big Creek and its direct physical contact with the aquatic life present. The constituents of the spilled diesel were identified by the Marine Safety Laboratory (MSL 2018) (Table 1). The deceased pied-billed grebe covered with diesel was matched (fingerprinted) to the released product, which not only confirms injury to trust resources, but also confirms one of the direct exposure pathways. The aquatic life present in Big Creek at the time of the spill were directly exposed to the diesel product and its constituents, including its water accommodated fraction (WAF) as determined by the known solubility of each of these diesel constituents (Table 2). These concentrations of diesel constituents were modeled using simple mathematics and compared to known literature toxicity values (Table 3). Even using a conservative estimate of these constituents reaching 10% of their known solubility exceeded chronic and/or acute toxicity screening values for 7 of 8 diesel constituents (Table 4).

The Preassessment Report (USFWS 2019) also presents the hydrodynamics of Big Creek (flow regime), the volume of the spill, the duration which the diesel was in Big Creek before it was recovered and the residual amount of unrecovered diesel and its breakdown products. Because diesel is known to be "toxic to aquatic life with long lasting effects" (Marathon 2016), the direct exposure to diesel that happened in Big Creek directly impaired survival, and indirectly impaired growth and reproduction due to continued adverse health conditions. The diesel spilled caused direct physical and chemical habitat quality impairments in the short term and may continue to

have lingering impacts to habitat quality and structure. The proposed injury assessment work described in this Plan will fully elucidate these matters.

The primary task of the Assessment Plan will replicate the amounts (concentrations) of these released oil products and evaluate the spatial and temporal extent of the exposure. Test durations will mimic relative quantities discharged into Big Creek and the identical timeframes in which it occurred. Direct and indirect impacts will be observed. Ample chemistry samples will be taken to document exposures, pathways, and the extent of toxicity. Test organisms selected are suitable surrogates for the aquatic resources of concern, a common freshwater mussel for the endangered fat pocket book freshwater mussel (Table 5), a Cladoceran (representing a basic food source for the aquatic ecosystem) (Table 6), and a fish representing all the many species of fish present (Table 7). This assessment will directly answer the questions of quantifying injury for each of these groups by identifying the degree of toxicity relative to the oil constituents, and will be able to observe the spatial and temporal extent of injury to a natural resource. It will be the same exposure pathways, and in this setting we will be able to gather the evidence of adverse change or impairment that constitutes injury. It is likely that additional evidence of the mechanism by which toxicity occurred will be observed. The proposed longer term study will address the potential for indirect impacts and provide information useful in considering the potential for natural recovery. Because we are working with aquatic resource surrogate species, we will have good information regarding feasible restoration actions. The actual laboratory time to run these microcosm reenactments will be less than a month. There will be some lead up time in preparation for this work, and a few months to analyze and report on the data. The detailed cost breakdown for the primary Assessment Plan task is $216,440 (Table 8).

The assessment work described in this Plan is intended to illuminate the range of sensitivity and vulnerability that exists within the injured aquatic community. These chosen test organisms are not known to be overly sensitive species; however, there is evidence in the literature that many freshwater mussels are extremely sensitive to pollutants such as the components of diesel that we will be studying. The scope of the fat pocketbook mussel's sensitivity is not specifically known; however, its numbers and its geographic range have declined greatly over the past 30 years thereby indicating that natural recovery is not sufficient to overcome continuing oil spills. As compared to the fat pocketbook mussel, the time for natural recovery of the fish species impacted is likely rather swift (years not decades) without restoration. Big Creek and its tributaries serve as an important refugia and nursery area for the lower Wabash River aquatic ecosystem, making it important for its reproductive and recruitment potential. The presence of Big Creek contributes to the resistance and resilience (stability) of the Wabash River ecosystem in light of the many insults to its physical and chemical processes.

Big Creek and the Diesel Spill Incident

Big Creek is in the southwestern tip of Indiana in Posey County approximately 7 miles north of the city of Mount Vernon. The Big Creek is a tributary to the Wabash River and then the Ohio River (Fig. 1)(Borries 2009). It's watershed is approximately 256 square miles, and land use in this region of Indiana is predominantly agricultural (71.4%) with corn and soybeans as the two major crops (USDA 2015). There is a US Geological Survey gaging station (USGS 03378550)

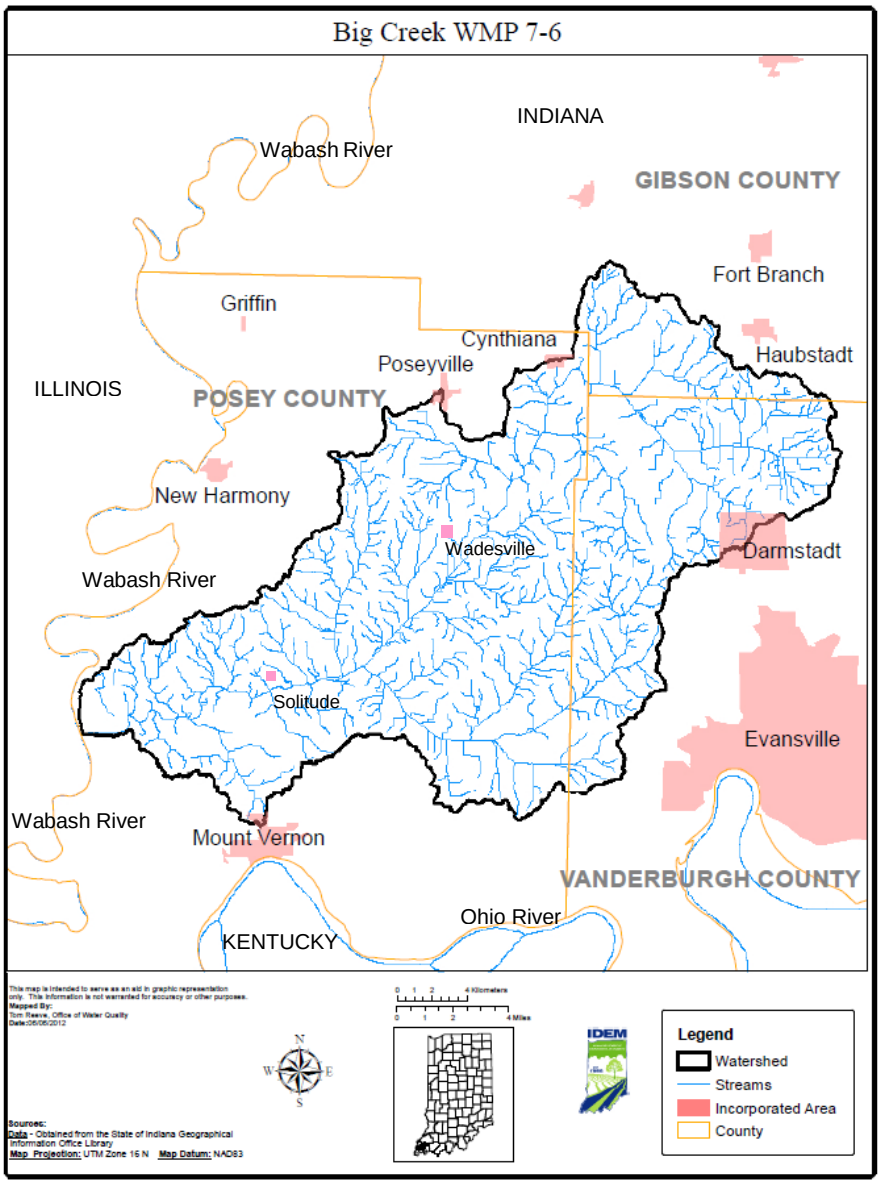

Fig. 1. Big Creek watershed. Modified from Borries (2009)

located near Wadesville, IN. The mean daily discharge at that location is 110 cubic feet per second (cfs) with highest discharge typically during the spring from March to May. Due to the high level of agriculture in this region the creek receives overland flow from fields following storm events and the levels may rise and fall rapidly.

On March 20, 2018 at 18:24 CST a 10 inch pipeline carrying No. 2 diesel fuel breached releasing 58,800 gallons into Big Creek (USCG, 2018). The location of the breach was approximately 0.4 miles upstream of the Indiana state highway 69 bridge near Solitude, IN (LAT: 38.013152N LONG: -87.899594W). In response to the incident, at approximately 23:00 CST the same day, Marathon Pipe Line LLC and its contractors installed booms at the Lower New Harmony Road (approximately 4.6 miles downstream of the breach) (Fig. 2) and later at Wabash Road

Fig. 2. Diesel fuel recovery at the Lower New Harmony Road Bridge (38.001162N, -87.954638W) on March 21, 2018 (Photo credit Blair Photo EVV).

(approximately 7.6 miles downstream of the breach).

The Big Creek ecosystem is home to a wide range of fish and invertebrate species (see the Preassessment Report for more details). The federally listed endangered species, fat pocketbook mussel (*Potamilus capax*), has been documented in the Wabash River and Big Creek watershed just downstream of the pipeline break (incident), near the State Highway 69 bridge and at the confluence of Big Creek with the Wabash River (Cummings et al. 1992; Fisher, 2006a, b). Two additional federally listed species, the endangered Indiana bat (*Myotis sodalis*), and the threatened northern long-eared bat (*Myotis septentrionalis*) are found in the Big Creek watershed (USFWS). Accordingly, natural resources under the trusteeship of the Trustees have resulted or are likely to result from the incident.

Injury Assessment Objectives

The primary task of this injury assessment will result in the development of toxicity threshold values for a freshwater mussel (*Lampsilis siliquoidea*), a cladoceran (*Ceriodaphnia dubia*), and a fish, the fathead minnow (*Pimephales promelas*). The results of Task 1 will also enable the trustees to quantify the adverse biological effects of the March 20, 2018 diesel spill on aquatic species found in the Big Creek watershed. This in turn will help inform the development of feasible restoration actions to address those injuries.

ASSESSMENT PLAN PRIMARY TASK: Aquatic Toxicity Assessment for No. 2 diesel fuel in freshwater systems

Overview

The Preassessment Report indicates a potential for the Marathon No. 2 Diesel to adversely affect aquatic biota in the Big Creek. Due to the uncertainties in the WAF that are used to estimate toxicity and the potential for diesel to affect aquatic organisms (e.g. reduced growth, reduced survival) in the Big Creek we are proposing additional study to determine the toxicity of Marathon No. 2 Diesel. The approach will include three main components: (1) chemical analysis of diesel and partitioning to the WAF, (2) toxicity testing of diesel WAF with three aquatic organisms, and (3) modeling of diesel toxicity using physicochemical based models (the target lipid model [TLM] and the Petrotox model™)(Steevens et al. 2018). The results of this study will be used in a weight-of-evidence approach outlined by the U.S. EPA (USEPA 2016) to develop toxicity thresholds and estimate the scope of the injury of diesel spilled in Big Creek, IN to aquatic biota.

A freshwater mussel (*Lampsilis siliquoidea*), a cladoceran (*Ceriodaphnia dubia*), and a fish, the fathead minnow (*Pimephales promelas*), will be exposed to No. 2 Diesel fuel (obtained from Marathon Oil) in acute and chronic (7 days) static renewal exposures following standard toxicity methods and guidelines (USEPA, 2002; ASTM-International, 2016). The acute toxicity of the WAF will be determined in a standard water-only exposure. However, long-term effects of the diesel are more likely due to the presence of the WAF constituents and the relatively insoluble fraction of the diesel that is associated with the sediments. Therefore a long-term exposure through sediment associated contaminants will also be conducted to further evaluate this scenario. These three species were chosen to cover a range of sensitivities and routes of exposure among taxa. Endpoints will include survival, growth and biomass. Behavioral effects such as narcosis in fish will also be documented. Additional replicates may also be set-up to document photo-activated toxicity for each species.

Exposure Methods Using the WAF

Test organisms will be exposed to the WAF of No. 2 diesel fuel, which consists of components of No. 2 diesel fuel dissolved in water by mechanical mixing. Each organism will be exposed to a series of six concentrations and a control water. Water will be renewed daily with freshly prepared WAF. These solutions will be prepared by a standardized method described by Ramachandran et al. (2004). The No. 2 diesel fuel will be mixed with distilled water at a ratio of 1:9 by gentle stirring for 18 h and then left to stand for 1 h. The clear bottom layer of the mixture will be removed and used for dilutions so that test organisms are only exposed to hydrocarbons dissolved in water during the mixing process (Schein et al. 2009).

Chemical Analysis

Test solutions will be analyzed by gas chromatography-mass spectrometry (GC-MS) and fluorescence spectroscopy. The GC-MS methods are required to fully characterize diesel and the constituents in the WAF. Samples of the freshly prepared WAF on day 0 and aged WAF in the

exposure at day 7 will be analyzed using GS-MS. Due to the cost of GC-MS and the number of analyses needed to characterize the WAF throughout the exposure an alternative screening method will be used during the exposure. Fluorescence spectroscopy will be used as an alternative method to provide information on the total PAH concentration rather than individual PAHs through GC-MS (Schein et al. 2009). However, a relationship between TPH and PAH content will be established through comparison of the two methods. Test solutions will be collected for fluorescence-based analysis at test initiation, prior to each water renewal (aged sample), and for each freshly prepared water test solution. The GC-MS method will be used to confirm the analysis by GC-MS and to demonstrate a constant exposure throughout the study.

Passive Samplers

Determination of the aqueous concentrations of PAHs in the exposure chambers will be conducted using solid-phase microextraction (SPME). The use of SPME for sampling petroleum in surface waters, pore waters, soils, and sediments is well established (Langenfeld et al. 1996, Hook et al. 2002, Hawthorne et al. 2005). In the SPME process, organic compounds are extracted from the surrounding media onto a stationary phase that is bounded to a fused silica fiber. Typically, the exposures are conducted for sufficient time to allow for equilibrium to be reached. The time for equilibrium can vary from minutes to hours depending on the fiber coating and thickness, the physicochemical properties of the chemical, and the environmental conditions of the test system. For this proposed study, SPME fibers with a stationary phase of polydimethylsiloxane with a thickness of 7 to 30 um will be equilibrated in the test waters for a pre-determined period of time. Following this equilibration period, the fibers will be extracted by immersion in an organic solvent. The extracts will then be analyzed by GC/MS.

Freshwater Mussel Bioassay

Table 5 summarizes conditions for conducting acute 7 d toxicity tests with juvenile mussels (fatmucket, *Lampsilis siliquoidea*). This toxicity test will be started with about 1-week-old fatmucket. Six test exposure concentrations will be created with a 50% dilution series plus a control. The test water used will have water quality parameters similar to Big Creek.

At the beginning of a test, ten juvenile mussels exhibiting foot movement will be impartially transferred into each of eight 300 mL glass beakers per concentration, with four replicate beakers for each of the 7 d exposures. Each beaker will contain about 200 mL of water and 10 mL of sand (<500-µm particles; Granusil #4030, Unimin Corporation, New Canaan, CT). All beakers will be held in a water bath at 25ºC. Archive samples of four replicates (10 mussels per replicate) will also be collected for measurements of initial length. The mussels will be fed 2 mL of an algae mixture twice per day. About 80% of water in each replicate beaker will be renewed daily. The pH, conductivity, hardness, alkalinity and total ammonia nitrogen will be measured on pooled replicate test solutions collected from all treatments at the beginning and end of the test.

Survival of juvenile mussels will be determined at day 4 and at the end of the 7 d exposure. Mussels with a gaped shell containing swollen or decomposed tissue and empty shells will be classified as dead. Surviving mussels will be isolated and preserved in 70% ethanol for

subsequent shell length determination. The test acceptability criterion is ≥80% control survival. Endpoints will be survival, growth, and biomass.

Ceriodaphnia Bioassay

Table 6 summarizes conditions for conducting 7 d chronic toxicity tests with *Ceriodaphnia dubia*. Toxicity tests will be conducted following standard guidance and methods (USEPA, 2002; ASTM-International, 2016). The test will consist of six test concentrations of WAF in a 50% dilution series, plus a control (7 total). The test water used will have water quality parameters similar to Big Creek. Tests will start with <24 h neonates. At the beginning of the test (day 0), neonates will be assigned impartially to test replicates by placing one organism in each of ten 30-ml plastic exposure cups containing 20 mL of equilibrated test solution. Tests will be conducted in an incubator at 25°C. *C. dubia* in each cup will be fed 0.1 ml each yeast-cerophyll-trout chow (1800 mg/L stock solution) and an algal suspension per chamber daily (*Pseudokirschneriella subcapitata*, 3.0 X 10^7 cell/mL, Aquatic Bio Systems, Fort Collins, CO).

On each day of the test, each first-generation *C. dubia* will be recorded as alive or dead. Death is considered equivalent to immobilization, which is indicated by lack of movement within 5 seconds in response to gentle prodding. Each live organism will be transferred to a new chamber containing fresh equilibrated test solution. The number of young produced over each 24 h period will also be recorded. Exposures will be conducted for 6 to 8 days, with the test ended when at least 60% of surviving first-generation *C. dubia* in the controls have produced three broods, with an average of 15 or more young per female.

Fish Bioassay

Table 7 summarizes conditions for conducting testing acute 7d toxicity tests with fathead minnow or similar fish species. A similar fish may be substituted based on species present in Big Creek. Less than 48 h fathead minnows will be acclimated to test water and test temperature (25°C) for 24 h before testing. During the acclimation period, the fish will be fed newly hatched (less than 24 h old) brine shrimp nauplii twice daily at a rate of 1 mL of a concentrated suspension of the nauplii to 2 L of water. At the beginning of a test, ten fish (<48 h old) will be impartially transferred into each replicate 1-L glass beaker containing about 250 ml of test solution. Six concentrations of the chemical will be created with a 50% dilution series plus a control (7 total). The test water will have similar water quality parameters as Big Creek. About 80% of the water will be renewed daily. The fish will be fed 0.15 mL of a concentrated suspension of less than 24 h old brine shrimp nauplii twice daily on test day 0 to 6. Fish survival will be determined daily and at the end of the test. Behavioral effects such as narcosis will also be recorded. The acceptability criterion for a toxicity test is ≥80% 7 d control survival.

Chronic exposure to sediment associated fraction of No. 2 diesel. In addition to the acute mussel and fish toxicity bioassays a parallel 28-day exposure will be conducted to assess the chronic effects of sediment associated diesel constituents. This study will be conducted to reflect the potential residual exposure that occurred in Big Creek following the partial recovery of diesel by Marathon and the rainfall event.

A chronic study will be conducted in parallel to the acute mussel and fish bioassays. The 28 d chronic study will be composed of a 7 d sediment conditioning without organisms followed by a 21 d exposure for mussels and fish surviving the acute study. Briefly, 1 L of sediment collected from above the spill site at Big Creek will be pre-conditioned with an aqueous mixture (containing colloidal diesel) for 7 d. The conditioning will allow the WAF and less soluble fraction of diesel to become associated with the sediment. At the end of the 7 d conditioning the overlying water will be replaced with flow through water renewal for 12 hours and then mussels and fish from the acute study will be added. Chemical concentrations of diesel constituents will be monitored in the overlying water during the study and sediment at the beginning and end of the 21 d exposure. Endpoints from the chronic bioassay will include survival, growth, and biomass.

Statistical Analysis

Measured exposure concentrations will be used to estimate effect concentrations at 20% and 50% (EC20 and EC50s) for survival, dry weight, or biomass (total dry weight of surviving organisms per replicate). Toxicity Relationship Analysis Program (TRAP) software (Ver. 1.31a) will be used to fit Gaussian (normal) distribution to log-transformed concentrations to calculate EC50s for survival and the nonlinear regression analysis with a logistic equation model will be used for dry weight and biomass EC20s. If a TRAP model cannot be produced (because of an insufficient number of treatments with partial effects), for the chronic test, no-observed-effect concentration (NOEC) and lowest–observed-effect concentration (LOEC) will also be determined by analysis of variance, with mean comparison made by one-tailed Dunnett's test, using TOXSTAT® software (version 3.5, Western EcoSystem). The level of statistical significance will be set at α=0.05.

CONCLUSION

As described herein, the purpose of this Assessment Plan is to identify the steps that will be taken by the Trustees to complete the injury assessment and restoration planning phase of the NRDAR process pursuant to OPA and its implementing regulations, 15 C.F.R. Part 990, Subpart E. This Plan describes the Trustees' intent to pursue injury assessment and quantification activities. The assessment work described in this Plan is intended to illuminate the range of sensitivity and vulnerability that exists within the aquatic community that was injured as a result of the March 20, 2019, diesel fuel spill in Big Creek, Indiana. The assessment work is intended to help the Trustees identify a reasonable range of restoration alternatives to address the natural resource injuries associated with the incident.

Table 1. The constituents of the spilled diesel identified by the Marine Safety Laboratory (MSL 2018).

Quantitation Report (QT Reviewed)

Data Path : D:\18-078\
Data File : 1807801.D
Acq On : 1 May 2018 6:19 pm
Operator : SDJ
Sample : 18-078-1, SP
Misc :
ALS Vial : 4 Sample Multiplier: 1

Quant Time: Jun 27 11:23:04 2018
Quant Method : C:\MSDCHEM\1\METHODS\BIOMARK3.M
Quant Title :
QLast Update : Mon Mar 28 07:37:49 2011
Response via : Initial Calibration

Internal Standards	R.T.	QIon	Response	Conc Units	Dev(Min)
Target Compounds					Qvalue
1) 85-SATURATED HYDROCARBONS	15.512	85	173838927	No Calib	
2) n-C17	19.250	85	4808679	No Calib	
3) PRISTANE	19.341	85	1910153	No Calib	
4) n-C18	20.973	85	4012779	No Calib	
5) PHYTANE	21.109	85	1613271	No Calib	
6) 113-SATURATED HYDROCAR...	19.341	113	34551057	No Calib	
7) ACYCLIC ISOPRENOIDS/AL...	19.341	183	12783397	No Calib	
8) C2-NAPHTHALENES	13.577	156	8750735	No Calib	
9) C3-NAPHTHALENES	15.810	170	9079246	No Calib	
10) C4-NAPHTHALENES	18.425	184	4890097	No Calib	
11) PHENANTHRENE/ANTHRACENE	20.186	178	938660	No Calib	
12) BENZONAPHTHIOPHENE	28.205	234	649844	No Calib	
13) DIBENZOTHIOPHENE	19.607	184	22424	No Calib	
14) C1-DIBENZOTHIOPHENE	19.113	198	1574352	No Calib	
15) C2-DIBENZOTHIOPHENE	23.675	212	20749	No Calib	
16) C3-DIBENZOTHIOPHENE	25.327	226	13061	No Calib	
17) C1-PHENANTHRENES	22.201	192	2368079	No Calib	
18) C2-PHENANTHRENES	24.308	206	2674905	No Calib	
19) C3-PHENANTHRENES	26.092	220	1450781	No Calib	
20) TRITERPANES/HOPANES	0.000	191	0	N.D.	
21) HOPANE A	0.000	191	0	N.D.	
22) HOPANE B	0.000	191	0	N.D.	
23) 14 a(H) STERANES	0.000	217	0	N.D.	
24) 14 b(H) STERANES	34.830	218	42	No Calib	
25) TRI-AROMATIC STERANES	0.000	231	0	N.D.	
26) METHYLHOPANES	0.000	205	0	N.D.	
27) NORHOPANES	0.000	177	0	N.D.	
28) PYRENE/FLUORANTHENE	25.348	202	755647	No Calib	
29) METHYL PYRENE	27.440	216	657197	No Calib	
30) FLUORENE	16.712	166	269010	No Calib	
31) BICYCLONAPHTHALENES	21.083	208	1760066	No Calib	
32) CHRYSENE	30.085	228	99881	No Calib	
33) C1-CHRYSENE	31.604	242	65664	No Calib	
34) C2-CHRYSENE	33.059	256	45491	No Calib	
35) C3-CHRYSENE	34.401	270	18534	No Calib	
36) C4-CHRYSENE	0.000	284	0	N.D.	
37) SESQUITERPANES	0.000	123	0	N.D.	

(#) = qualifier out of range (m) = manual integration (+) = signals summed

Table 2. Composition of No. 2 diesel fuel and comparison to fingerprinting from Marathon spill.

Class	Constituent	Concentration (ug/g) [1]	% Composition (by weight)	Confirmed by Fingerprinting	Solubility (mg/L)	
Volatiles	Benzene	136	0.08%			
	Toluene	1024	0.60%			
	Ethylbenzene	619	0.36%			
	Xylenes	3774	2.21%			
	C_3-Benzenes	13780	8.06%			
PAH	Naphthalene	20852	12.20%	X	31.7	[2]
	Phenanthrene	2293	1.34%	X	1.29	[2]
	Dibenzothiophene	312	0.18%	X	3.07	[3]
	Fluorene	2481	1.45%	X	1.98	[2]
	Chrysene	0.09	0.00%	X	0.0020	[2]
	Biphenyl	839.73	0.49%			
	Acenaphthalene	34.87	0.02%			
	Acenaphthene	153.55	0.09%			
	Anthracene	13.08	0.01%	X	0.073	[2]
	fluoranthene	6.6	0.00%	X	0.26	[2]
	Pyrene	30.88	0.02%	X	0.135	[2]
	Benz(a)anthracene	0.25	0.00%			
Alkanes	Pristane	3810	2.23%	X	3.02E-07	[3]
	Phytane	2520	1.47%	X	8.02E-08	[3]
	C5-C8	1150	0.67%	X	11	[4]
	C9-C10	15170	8.87%	X	51	[4]
	C11-C36	101970	59.64%	X	3.00E-02	[4]

[1] Composition of No.2 diesel fuel from EPA (2003)
[2] Gui-Ning Lu et al. (2008)
[3] Petrotox model default parameters
[4] Massachusetts Department of Environmental Protection (2002)

Table 3. Description of most relevant literature for comparisons to potential diesel fuel toxicity, resilience, or recovery.

Species	Freshwater /Marine	Chemical	Water /sediment	Field/lab	Exposure Levels	Age/Size of Organism	Duration	Endpoints	Description/Effects	Reference
Blennius pavo and *Microcosmos sulcatus*	Marine	Diesel No 2	Water	Laboratory	170 ppb diesel per ml seawater	Fish size 1-6 grams	30 days	BPMO activity (benzo pyrene mono oxygenase)	Microcosmos sulcatus liver had no measurable enzyme activity change, but the Blennius pavo were first elevated at day 3, peaked at day 14 and elevation continued to 30 d. Additoinal fish were monitored for an additional 30 d and still had elebated MBPO.	Kurelec et al. 1977
Cyprinus carpio	Freshwater	Diesel No 2	Water	Laboratory	50 ug Kuwait oil equivalents/liter. Analyzed by 1982 IOC method with Picer modification (1985).	1 yr old (20-30 g)	28 days	BPMO activity and DNA adducts in liver	Laboratory prepared oil slicks caused DNA damage in carp and the damage accumulated proportionately over time. The measured concentration of diesel was as hydrocarbons in both Kuwait and chrysene equivalents. Flourescence was measured in a Zeiss PMG-3 spectroflourometer but no data given.	Kurelecet al. 1992
Villosa villosa , *Lampsilis siliqoidea* , *Lasmigona costata*	Freshwater	Diesel from spill	Sediment	Laboratory	Poorly quantified. No PAHs detected, 4 diesel constituents detected but below RL	Glochidia and juvenile	24 and 48 hr glochidia exposures; 9 day juvenile exposures	Survival	2 years post diesel spill, no effect on mussels that were exposed to field (Fish Creek, IN) collected sediments in the laboratory.	Keller et al. 1998
Various: food web: namely copepods (e.g. *Cletocamptus deitersi*) and nematode	Marine (coastal saltmarshes) sediments 0 - 80 mg/g	PAHs from diesel	Sediments	Field (microcosm)	>300 ppm PAH final concentration; 0 625 mg/g for gobi exposures	Adults	14 days	Mortality and grazing rates	High mortality to all copepods expcet one species and nematode abundance increased. grazing increased due to less competition. At >78 mg/g PAH feeding behavior of gobi reduced 60% and at >300 mg/g all feeding inhibited. Nitrogen increased	Carman et al. 1999
Benthic invertebrate survey	Freshwater	Diesel No 2	Water, Sediment	Field	Not quantified, 26,500L spill in field	Multiple	3 weeks, 3-4, 12, 15 months	Benthic index	A train accident in Nov 1997 released 26,500 L of diesel into the Cayuga River. The study evaluates the invertebrate index above (ref) and below the spill (.7, 5, 11.8 miles) over a a period of time up to 15 months. Effects on invertebrates were observed 5Km downstream for as long as 3 months. However, the entrie reach was dominated by a single species through the 15 month period.	Lytle and Peckarsky 2001.
Mytilus edulis	Marine	Diesel (water soluble fraction) or corexit 9527	in vitro and in vivo water	Laboratory	0.5 to 11 ppm WSF measured by fluorescence	hemocytes obtained from adults ranging 5-10 cm in length	in vitro and 4 days in vivo	Non significant downward trend of phagocytosis invitro at 2.2, 8.22 and 11 mg/L; significant increase of immune respose at 8,22 and 11 mg/L WAF	Non significant downward trend of phagocytosis invitro at 2.2, 8.22 and 11 mg/L; significant increase of immune respose at 8,22 and 11 mg/L WAF	Hamoutene et al. 2004
Oncohynchus mykiss (Rainbow trout) and *Daphnia magna*	Freshwater	Biodiesel and Diesel	Water	Laboratory	D. magna = 1.57, 3.13, 6.25, 12.5, 25, 50 ppm O. mykiss= 100,300,600,900,120	Trout = fry; D. magna = juveniles	D. magna = 24hr O. mykiss= 96hr	LC50s	Diesel was more toxic than Biodiesel/biodiesel blends. Good LC50 are provided but there is no description of chemistry sampling and concentrations were determined.	Khan, N. et al. 2007
Oncorhynchus mykiss (rainbow trout)	Freshwater	Ultra low sulfur (ULS) Diesel No. 2 (CAS 68476-34-6); Low molecular weight (2–3 rings) PAHs (naphtalene and phenanthrene) more abundant	Water	Laboratory	Test concentrations expressed as loading rates, i.e. the ratio of diesel to dilution water Six loading rates we 0.3, 1.5, 8, 40, 200, and 1000 mg/L tested	13 days post swim-up	14 day static; daily renewal of oiled water	survival, growth (7 and 14 day) and gene expression; gene expression considered affected if significantly alter p 0.05 in either direction.	Survival (EC 20 26.7) and gene expression (EC 20 2.1) were significantly altered at the 40 mg/L diesel exposure dose and above; growth was not altered likely due to short exposure time. Also effecrs on swimming equilibrium and gill operculatio. Observed downregulation of the Hemoglobin gene which supports this observed behavior. Downregulation of genes related to immunity function were also noted.	Mos et al. 2008
Oncorhynchus mykiss (rainbow trout)	Freshwater	Ultralow sulfur Diesel No. 2 Prepared WAF and CEWAF	Water	Laboratory	rainbow trout Exposures of WAF (0.01–1.0% v/v) or CEWAF (0.001–0.1% v/v)	Early life stage	24 hr for EROD; hatch to swim-up 24 days	EROD, blue sac disease, Growth, Survival	Median lethal concentration of 8 mg total hydrocarbons/L. A sublethal median effective concentration ranged from 1.3 to 6.1 mg total hydrocarbons/L as defined by the presence of blue sac disease and effects on growth (growth effects resulted from delayed yolk absorption).	Schein et al. 2009
Mytella guyanensis (mangrove mussel)	Marine	Diesel fuel (2L/m2) measured as PAHs	Sediments	Field	2L of marine diesel fuel per meter squared measured as sum PAHs ~170,000 ng.g (high); 0.17 mg/g	Adult	7 days	Biomarker-GSH (glutathione activity)	No effect at 2 d post spill, significant decrease in GSH 7 d post spill	Marques et al. 2015
Mytilus galloprovincialis	Marine	Diesel and dispersant	Water	Laboratory	0.1 and 1 ml/L diesel 2.	Field collected adult mussels. Age and size not indicated.	72 hours	Survival and heart rate	No effect on suvival. Increase in heart rate at 0.1 and 1.0 ml diesel/L.	Martinović et al. 2015

Table 4. Surface water screening values for PAH constituents of diesel derived using equilibrium partitioning.

Chemical	Surface Water Screening Value (ug/L)		1/10 Solubility Limit (ug/L)	Exceed Screening Value?
	Chronic	Acute		
Naphthalene	194	402	3100	Yes
Fluorene	39	82	200	Yes
Anthracene	21	43	4.3	No
Phenanthrene	19	40	120	Yes
Dibenzothiophene	48	100	307	Yes
Fluoranthene	7.1	15	21	Yes
Pyrene	10	21	14	Yes
Chrysene	2	4.2	0.16	No

Table 5. Summary of test conditions for conducting 7- and 10-day toxicity tests with juvenile mussel (fatmucket, Lampsilis siliquoidea) in basic accordance with ASTM International (2016) and USEPA (2002)

Parameter	**Conditions**
Test species	Fatmucket (*Lampsilis siliquoidea)*
Test chemicals	No. 2 Diesel fuel (Marathon Oil)
Test type	Static renewal
Test Duration	7 days
Temperature	25°C
Light quality	Ambient laboratory light
Light intensity	500 lux (16 h light/8 h dark)
Test chamber size	300 ml (10 ml of fine silica sand)
Test solution volume	200 ml
Renewal of solution	Daily (about 80% replacement of water)
Age of test organism	About 1 week after transformation
Organism/replicate	10
Replicate #	4
Feeding	2ml algal mixture 2X daily
Aeration	None
Dilution factor	0.5
Test concentrations	WAF + 50% serial dilution (5 concentrations + a control)
Chemical analyses	Water samples for chemical analysis will be collected from each exposure concentration at the beginning and the end of test and daily before renewals.
Water quality	Dissolved oxygen (daily); pH, conductivity, hardness, alkalinity, and ammonia at beginning and end of tests.
Endpoints	Survival (4 and 7 d), growth (shell length), biomass and narcosis
Test acceptability criterion	≥ 80% control survival

Table 6. Summary of test conditions for conducting chronic water-only toxicity tests with the cladoceran, *Ceriodaphnia dubia*, following standard methods recommended by ASTM (2016) and USEPA (2002).

Parameter	**Conditions**
Test species	Cladoceran (*Ceriodaphnia dubia*)
Test chemical	No. 2 Diesel fuel (Marathon Oil)
Test duration	7-8 days
Temperature	25°C
Light quality	Algal growth incubator (about 700 lux); 16 h light/8 h dark
Test chamber size	30 ml
Test solution volume	20 ml
Renewal of solution	Transfer to fresh test solution (after equilibration for 24 hr) daily
Age of test organism	<24 hr old
Organisms/replicate	1
Replicate #	10
Feeding	0.1 ml YCT (1800 mg/L stock) and 0.1 ml algal (*P. subcapitata*) suspension (3.0 - 3.5 X 107 cell/mL) daily
Aeration	None
Dilution factor	0.5
Test concentrations	WAF + 50% serial dilution (5 concentrations + a control)
Chemical analyses	Water samples for chemical analysis will be collected from each exposure concentration at the beginning and the end of test and daily before renewals.
Water quality	Dissolved oxygen, pH, conductivity, hardness, alkalinity, and ammonia measured in selected treatments at the beginning and end of test.
Endpoints	Survival and reproduction (both recorded daily)
Test acceptability criterion	≥ 80% control survival, ≥15 young/female in controls, and ≥60 of surviving control females have three broods

Table 7. Summary of test conditions for conducting static-renewal toxicant tests with fathead minnow in basic accordance with ASTM (2013) E729.

Parameter	**Conditions**
Test species	Fathead minnow (*Pimephales promelas*)
Test chemicals	No. 2 Diesel fuel (Marathon Oil)
Test type	Static-renewal
Test Duration:	7 d
Temperature	25°C
Lighting quality	Ambient laboratory light, about 500 lux; 16 hour light/8 hour dark
Test chamber size	1 L
Test solution volume	250 ml
Renewal of solution	Replace about 80% of volume daily
Age of test organism:	<48 h
Organisms/replicate	10
Replicate #	Minimum 2
Feeding	None
Aeration	None
Dilution factor	0.5
Test concentrations	WAF + 50% serial dilution (5 concentrations + a control)
Chemical analyses:	Water samples for chemical analysis will be collected from each exposure concentrations at the beginning and the end of test and daily before renewals.
Water quality:	Dissolved oxygen (daily); pH, conductivity, hardness, alkalinity, and ammonia at beginning and end of test.
Endpoint:	Lethality (or immobilization; recorded daily)
Test acceptability criterion	≥90% control survival

Table 8. Cost estimate for the Assessment Plan Primary Task: Diesel Toxicity Study.

Components		Units	Unit cost	Subtotal	Notes
A. Toxicity Bioassays					
1	7-day mussel bioassay	1	$12,000	$12,000	6 concentrations + control; Assumes data for 24, 48, 72, 96 and 7 day time points; GC on initial and final treatments; Fluorescence on fresh/aged daily for low, med, high
2	7-day invertebrate bioassay	1	$12,000	$12,000	6 concentrations + control; Assumes data for 24, 48, 72, 96 and 7 day time points; GC on initial and final treatments; Fluorescence on fresh/aged daily for low, med, high
3	7-day fish bioassay	1	$10,000	$10,000	6 concentrations + control; Assumes data for 24, 48, 72, 96 and 7 day time points; GC on initial and final treatments (14); Fluorescence on all treatments on initial and final, other days fresh/aged daily for low, med, high (50)
4	21-day chronic sediment exposure	2	$12,000	$24,000	Fish and mussel exposures; 3 concentrations + control; GC on initial and final treatments (24); Fluorescene analysis of overlying water (12)
	Toxicity testing subtotal			**$58,000**	
B. Chemical Analysis					
1	WAF preparation study	1	$16,500	$16,500	Includes calibration of WAF production prior to start of experiment
2	TPH by GCMS	74	$540	$39,960	50 samples from water only exposure and 24 from chronic exposure
3	TPH by fluorescence	162	$110	$17,820	
4	Passive sampler	10	$500	$5,000	
	Chemistry subtotal			**$79,280**	

C. Miscellaneous					
1	Culturing and IACUC	1	$7,000	$7,000	assumes fish is fathead minnow, invertebrate is ceriodaphnia or hyalella, and mussel is fatmucket
2	Field collected sediment, chemicals, and consumables	1	$3,000	$3,000	$2,000 for field collection and $1,000 for consumables
3	Waste disposal	1	$2,500	$2,500	diesel disposal costs and laboratory waste
4	Data compilation and analysis	1	$17,500	$17,500	
5	Reporting	1	$35,000	$35,000	Includes development of toxicity threshold values and comparison to other reported toxicity values.
	Data analysis subtotal			**$65,000**	
D. Subtotal					
1	Subtotal			$202,280	
2	USGS Overhead	Rate:	7%	$14,160	
E. Total Toxicity Assessment funding				**$216,440**	

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