Natural Resource Damage Assessment Preassessment Report

for the

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

May 10, 2019

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

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Overview

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 Report is to briefly summarize the steps 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 preassessment phase of the Natural Resource Damage Assessment and Restoration (NRDAR) process pursuant to 15 C.F.R. Part 990, Subpart D. Specifically, this Report describes the Trustees' jurisdiction to pursue restoration activities under the Oil Pollution Act (OPA), including a brief description of the incident, as well as a description of the natural resources under the trusteeship of the Trustees that may have been or may be injured as a result of the incident. This report summarizes the toxicological evaluation of No. 2 diesel fuel prepared by our contractor (US Geological Survey, Columbia Environmental Research Center) and the potential effects of the incident on aquatic organisms under our trusteeship. In the toxicological evaluation, further investigations are described which would generate toxicity threshold values based on two aquatic invertebrates and a fish. Results of this further effort would enable a more accurate estimate the adverse biological effects of the March 20, 2018 diesel spill on aquatic species found in the Big Creek watershed which would aid in the development of feasible restoration actions.

Diesel Spill Incident

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 (~4.6 miles downstream of the breach) and at Wabash Road (~7.6 miles downstream of the breach). IDEM spill response staff observed diesel arriving at the bridge at the same time the booms were deployed. This suggests the diesel fuel traveled approximately 4.6 miles from the spill site to Lower New Harmony Road in 4.5 hours. Recovery operations using skimmers to remove diesel pooled above the booms started 12 hours later at approximately 11:00 CST on March 21, 2018 (Fig. 1). By 07:00 CST on March 22, 2018 the estimated product recovery was around 80.1% of the total released. Based on this timeline there was between 36 to 48 hours for a large percentage of the spilled product to be in contact and mix with the surface water. Stream flow in the Big Creek remained relatively low at the time of the spill until March 24 2018 in response to a 1.15 inch rainfall event during the evening of March 23 and into the early morning of March 24, 2018 (Fig. 2) (NWS, 2018). At 07:00 CST on March 27, 2018 the final estimate of total diesel recovery was 84.8% of the total released.

Natural resource trustee personnel were in communication with the USEPA and IDEM spill response staff several times on March 21, 2018 being updated on the spill and providing natural resource technical assistance. Two staff from the USFWS were on-site beginning with the March

22, 2018 0800 hours morning briefing. While on-site we provided technical assistance to EPA, IDEM and Marathon Pipe Line LLC. We participated in walking the creek banks for several miles on March 22 and 23, 2018, evaluating sheen and creek bank conditions. These observations led to the retrieval of a single oiled, dead pied-billed grebe (*Podilymbus podiceps*). This grebe was placed in a plastic bag, in a cooler with ice and taken to the USFWS office where it was maintained in a locked freezer at -4ºC. After coordinating with the Marine Safety Laboratory (MSL) of the USCG, we clipped feathers from this grebe and shipped them to MSL for oil fingerprint analysis. Marathon Pipe Line LLC separately sent MSL a sample of the diesel product that was discharged due to the pipeline failure. MSL confirmed that the oil product sample provided by Marathon to MSL matched the oil from the deceased grebe's feathers (Appendix I). The analytical data from MSL provided the basic chemistry for USGS to conduct their toxicological evaluation (Appendix II; Steevens et al. 2018) for the natural resource trustees. In sum, and as explained more fully herein, the USEPA's response actions have not adequately addressed, or are not expected to address, the injuries to natural resources resulting from the incident.

Fig. 1. Diesel fuel recovery at the Lower New Harmony Road Bridge (38.001162, -87.954638) on 3-21-2018. Photo credit Blair Photo EVV.

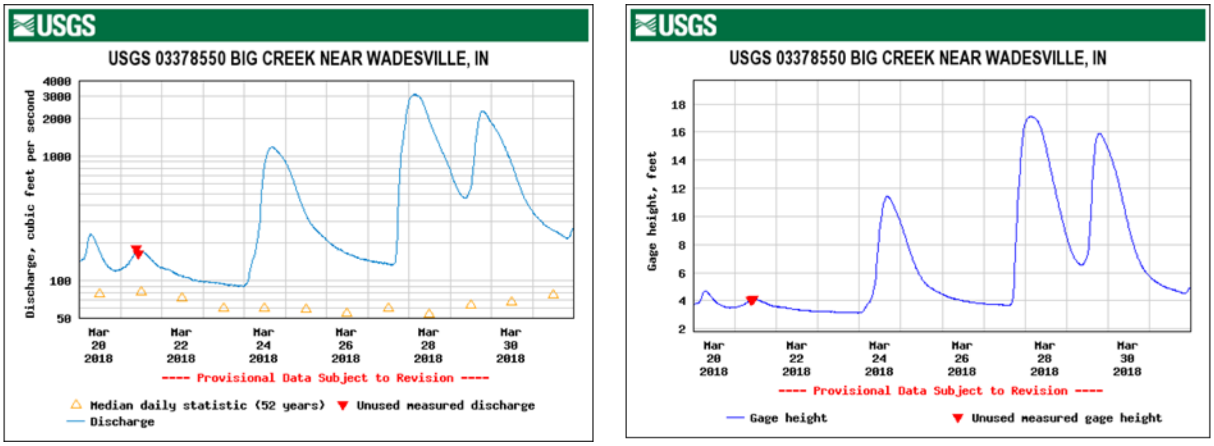

Fig. 2. USGS Stream gauge discharge and height at Big Creek Station 03378550 near Wadesville, IN.

General Site Description of Big Creek

Big Creek is in the southwestern tip of Indiana in Posey County approximately 7 miles north of Mount Vernon. The Big Creek is a tributary to the Wabash River and then the Ohio River (Fig. 3). It receives input from 198 miles of perennial streams for approximately 256 square miles (Borries, 2009). 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) located near Wadesville, IN. The mean daily discharge at that location is 110 cubic feet per second 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.

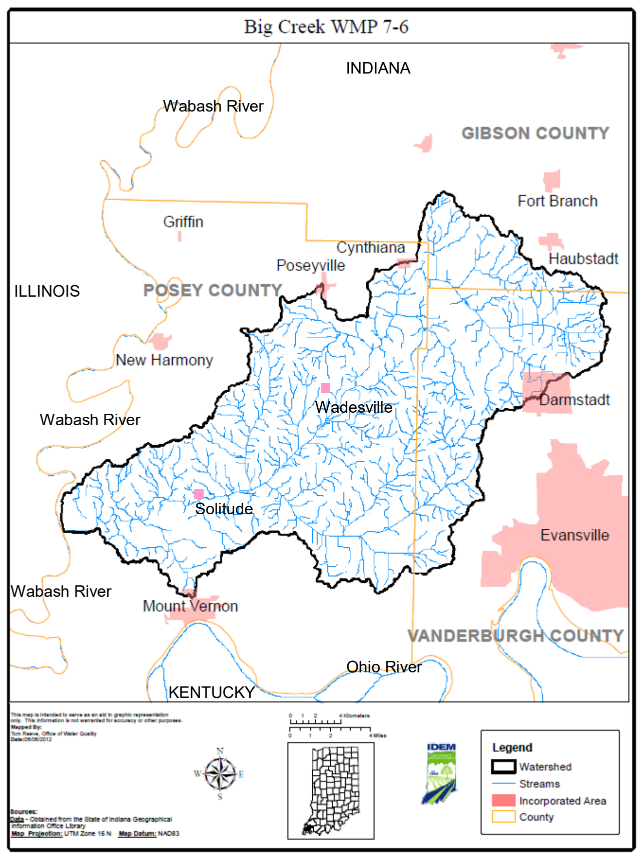

Fig. 3. Big Creek watershed. Modied from Borries 2009

The Big Creek ecosystem is home to a wide range of fish and invertebrate species. The Indiana Department of Environmental Management (IDEM) has sampled the fish community of Big Creek in 1999, 2011 and 2016 under varying hydrological conditions (Table 1). In 2009 the US EPA funded a watershed survey and management plan describing the benthic invertebrate and fish species diversity in the Big Creek and its tributaries (Borries, 2009). This survey of benthic invertebrates and fish was

Table 1. IDEM Big Creek fish community samples at varying hydrologic conditions.

	38.000232 N -87.985258 W 11-Aug-99 CR350W	38.000133 N -87.989025 W 6-Jul-11 Raben Rd	38.016621 N -87.889202 W 6-Jun-16 Johnson Rd
Bigmouth Buffalo		3	
Black Buffalo		1	
Blackspotted Topminnow	10		
Bluegill	2		2
Bullhead Minnow	1		1
Common Carp		1	1
Emerald Shiner	8		
Freckled Madtom	3		
Freshwater Drum		1	
Gizzard Shad	2	5	
Green Sunfish	4		
Highfin Carpsucker		1	
Longear Sunfish			7
Longnose Gar	1	4	1
Mississippi Silvery Minnow		10	
Orangespotted Sunfish			2
Pirate Perch			1
Quillback			1
Redear Sunfish	1		
Ribbon Shiner	1		1
River Carpsucker	3		
River Shiner	1		
Sand Shiner	1		
Shortnose Gar			5
Silver Carp		1	
Smallmouth Buffalo			1
Spotfin Shiner	28		5
Spotted Bass		1	
Spotted Gar			11
Suckermouth Minnow	1		
Western Mosquitofish	5		1
IBI Score	34	16	18

conducted at 32 sites throughout the watershed. Aquatic habitat was characterized using the Ohio Qualitative Habitat Evaluation Index (Rankin, 2006). Sites within the main channel of the Big Creek were generally characterized as poor quality due to high level of turbidity and siltation as well as channel modifications. In 2008, Bandoli et al found 40 different species of fish within Big Creek and its tributaries (Bandoli et al. 2010). Near State Highway 69 a total of 11 different species were documented, including shad, carp, shiner, silvery minnow, buffalo, catfish, and silverside. Additional information on the aquatic community of Big Creek is described more fully in Appendix III. 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.

How to Evaluate Natural Resource Injury when Simple Tools Are Not Available

It is established that diesel fuel is "toxic to aquatic life with long lasting effects" (Marathon 2016). No dead fish or mussels were collected as a result of Big Creek diesel spill response efforts. However, anecdotal information suggests workers collecting oiled debris at the Wabash River bridge "may have seen a few fish" in the oiled debris wastes they were bagging but they were not trained nor encouraged to separate dead aquatic life from debris. This is a very difficult thing to do in the best conditions even when trained. Marathon and their consultants maintained that it is their policy to separate oiled debris from oiled natural resources, and there is no reason to believe otherwise. In this response, the effort to search was limited because searching for aquatic life while removing oiled debris at booms would impede cleanup efforts and increase the risk of additional oil moving beyond containment booms. Photos were taken of what appeared to be small fish floating down the river toward the 1st recovery boom on March 21, 2019, but these are inconclusive. In fact, a second effort had to be made to return to the location where the oiled dead grebe had been observed in order to retrieve it. Where the surface of the water was disturbed, the silvery sheen (which was very difficult to see) would immediately emulsify into foam. Some sheens were observed continually escaping the last boom on the creek at Raben Rd bridge and on toward the Wabash River. Documenting adverse impacts in the Wabash River system under these conditions would pose additional serious complications.

Several additional factors impeded Trustees ability to observe / collect dead or impaired aquatic life in Big Creek, especially impaired freshwater mussels. In the case of mussel mortality, this often takes weeks or more to observe in streams that are easier to study (i.e. Fish Creek, Indiana; D. Sparks, personal observation). In cold water temperatures, dead fish sink for hours to days. Sampling efforts in cold water conditions increases human health safety considerations, especially the risk of hypothermia. Big Creek is known for its turbidity, especially during high water conditions and in the spring. Turbidity interferes with effective sampling. It should be pointed out however, turbidity readings for much of Big Creek are below Indiana's statewide average (see Appendix III, Fig. 4). Big Creek has a problem with streambank stability partially due to how the county drainage board manages the legal drain areas. Unstable streambanks impair sampling efforts and raise human health hazards, especially after recent rains and recently receding water levels. There were 3 high flow events in Big Creek within 9 days of the initial spill event, which made evidence of injury exceedingly more difficult to find / observe. And lastly, as is often the case, evidence of effective scavenging (raccoon and coyote tracks) were very evident throughout the spill zone.

Conceptual Model for No. 2 Diesel Fuel Spill in Big Creek

As part of the Trustees' preassessment work, and in order to evaluate the impacts of the diesel spill to Big Creek, the Trustees worked through a conceptual model of what likely happened. A simple conceptual model identifies the most likely exposure pathways from the diesel fuel into the Big Creek and natural resource receptors (Fig. 4). Using a combination of simple physical, chemical and mathematical principles, combined with relevant toxicity literature, a representation of the Big Creek aquatic system could be simulated to help the Trustees understand what happens to aquatic life when diesel enters the water. Specifically, a simulated system could address the following questions: What are the components of diesel? Which components are the most toxic? Where do these components go and at what concentrations? At what concentrations are these components considered harmful and what are the most relevant receptors that may be affected by the diesel and in what ways? Many of these questions have been evaluated in the "Toxicological Evaluation" report prepared for the USFWS by USGS (Steevens et al. 2018) utilizing standard values in guidance documents and hydrologic conditions in the Big Creek (attached as Appendix II).

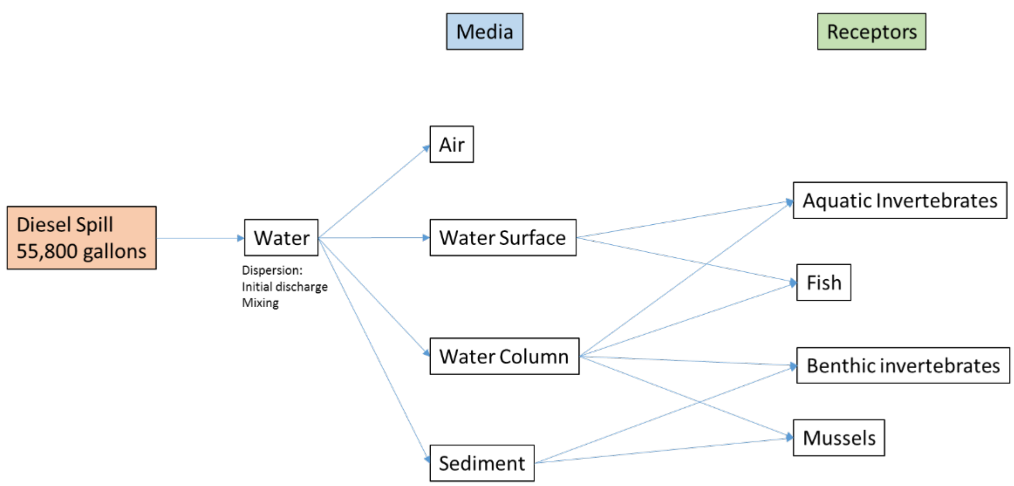

Fig. 4. Simplified conceptual model for the diesel spill in Big Creek, IN.

Concentrations of Diesel and Constituents in the Big Creek

It is estimated that approximately 58,800 gallons of No. 2 diesel fuel was released into the Big Creek. The breach in the pipeline was in the right descending bank of the creek, and due to pressure of the line, diesel released directly into the water. This dispersion presumably resulted in initial mixing of the diesel into the water column; however, the density of diesel is approximately 0.85 g/cm^3 and therefore rapidly rises to the surface of the water (Marathon, 2016). At the surface of the water, the diesel will partition into the water column, water surface, and air phases. Minor volatile components, such as benzene, toluene, ethylbenzene and xylene (BTEX), are likely to rapidly volatilize and are unlikely to contribute to the water column pathway. However, several of the constituents including polycyclic aromatic hydrocarbons (PAH) and alkanes contribute to the exposure pathway at the water surface and water column. In addition, there is a potential that some diesel can partition to sediment after weathering or through direct contact with the sediment.

The toxicological evaluation (Appendix II; Steevens et al. 2018) focused on the partitioning of the soluble fraction of diesel into the water column as the primary exposure pathway for aquatic invertebrates and fish. Secondary exposure pathways include exposure at the water surface and sediment. As discussed more fully in the report, to evaluate the potential for toxicity, the concentration of diesel constituents was estimated and compared to existing toxicity screening and literature values.

Comprehensive sampling of water or sediment was not done immediately following the spill nor during the diesel recovery process. Therefore, concentrations of diesel constituents in the water column can be estimated using physiochemical properties and stream conditions. The water accommodated fraction (WAF) is commonly used to describe the level of dissolved petroleum constituents in the water column (Anderson et al. 1974). To estimate a WAF, Steevens et al. (2018) used standard chemical concentrations of No. 2 diesel fuel (USEPA, 2003), chemical compounds identified in the US Coast Guard fingerprint analysis of No. 2 diesel fuel obtained from Marathon Oil, and solubility values frequently reported in literature for modeling the fate of petroleum compounds in the water.

A total of 8 PAHs, pristine, phytane, and alkanes were identified in the sample of oil fingerprint obtained from Marathon. To determine the maximum amount of these compounds in the WAF we assumed the total amount of diesel in the Big Creek was sufficiently large as compared to the relatively small amount of water and volatilization in the air and therefore the only limiting factor was water solubility. If solubility is the limiting factor, then the maximum theoretical concentration of diesel in the water is the sum of solubility for the major constituents which is 100 mg/L. However, it is unlikely the water reached the maximum saturation of the constituents in the diesel. Due to other factors that affect solubility (e.g., mixing, input of fresh water, removal of product from the river) Steevens et al. (2018) applied an uncertainty factor of ten and estimated the dissolved fraction was 10% of the sum solubility of major constituents or 10 mg/L. This value is also consistent with levels of petroleum hydrocarbons reported in literature where WAF are experimentally derived. Because the stream flow was sufficiently low for the first 96 hours post spill, it is assumed the concentration of diesel in the WAF of Big Creek was 10 mg/L over the 96-hour period.

Modeling the Diesel Spill

While there are several models that have been developed to assess exposures to spilled contaminants (Pistocchi, 2008; McCready and Williams, 2011; Granato, 2013), a detailed analysis was not possible in this preassessment effort. However, the exposure vulnerabilities to No. 2 diesel along the length of Big Creek was estimated by utilizing a hydraulic model based on the National Hydrography dataset (Steevens et al. 2018). The model assumed that of the total captured volume (48,000 gallons), 80% was captured at the first boom located approximately 4.7 miles downstream from the spill, and the remaining 20% was captured by the second boom located at 7.6 miles downstream from the spill (Fig. 5). The vulnerability analysis suggests that the greatest exposure risks occur between the spill origin and the first retention boom, with vulnerability decreasing with increasing distance from the spill origin. In this analysis, the unrecovered diesel (approximately 10,800 gallons) was assumed to be transported downstream to the Wabash River. However, it is possible that some of this volume remained in sediments and debris along Big Creek. One key variable that this hydraulic model did not factor in was the long retention time of the diesel plume at the first boom prior to its recovery. Additional variables that should be considered in a more comprehensive assessment include properties of No. 2 diesel fuel (e.g., density, specific gravity, weathering properties), changes in stream flow through time and space (drainage-area discharge ratios, and some estimate of in-stream or bank retention capability (e.g., roughness) (Emerson et al. 2005).

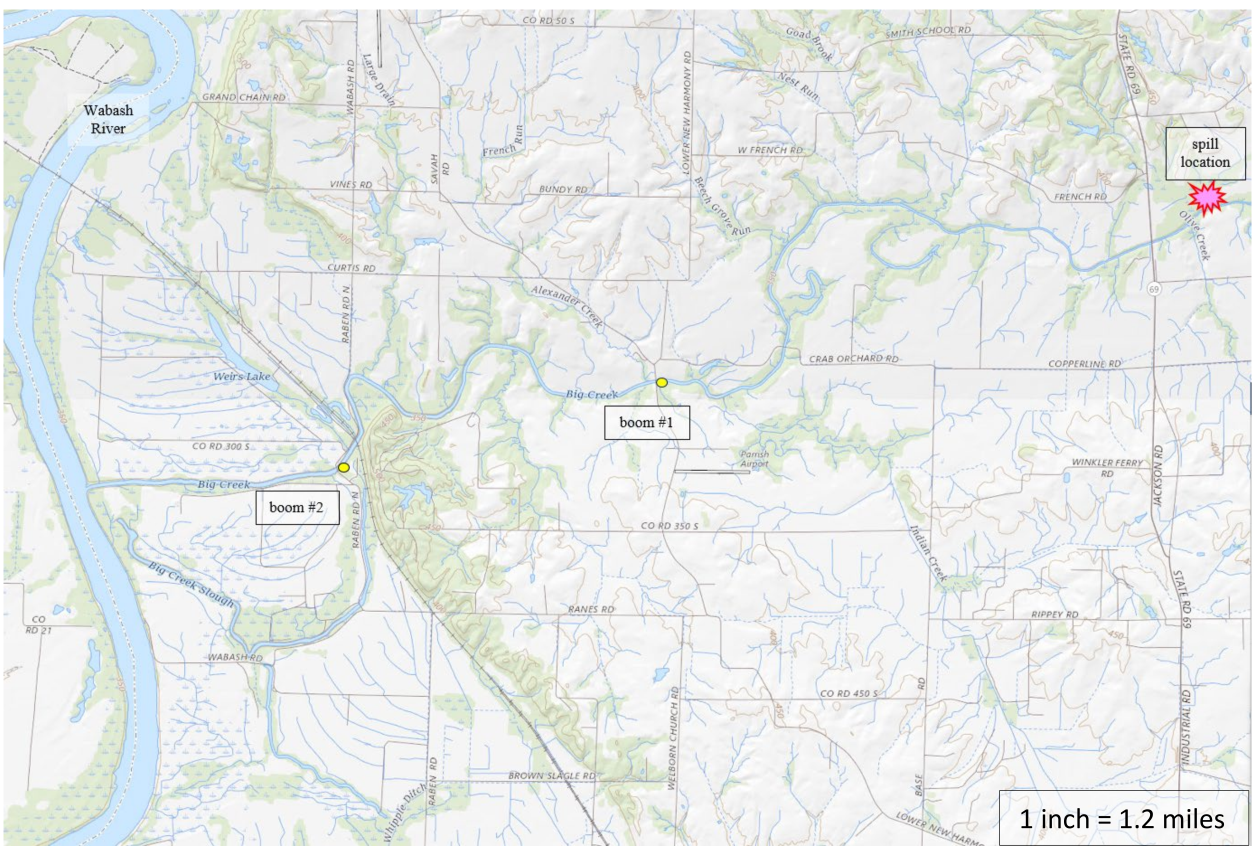

Fig. 5. Big Creek with key points of interest shown.

Aquatic Toxicity of Diesel

To interpret the estimated concentration of diesel constituents in the water column, Steevens et al. (2018) identified and reviewed the relevant literature, threshold values, and physicochemical based models to evaluate the potential for toxic effects to natural resources associated with the water column pathway. The purpose of the screening level analysis is to determine if a more comprehensive analysis is warranted. A search of the literature focused on peer-reviewed, published manuscripts to understand life history, distribution, and contaminant susceptibility of the fat pocketbook mussel, and a second search was performed with key words including No. 2. diesel fuel, aquatic toxicity (or fate, or chemistry), and fish or invertebrates or mussels. It should be noted that there were issues with missing information reported in most literature. Lack of information was especially a factor for reported chemistry measurements and characterizations.

Steevens et al. (2018) found a paucity of information in the published literature defining the toxicity of diesel on freshwater organisms. Diesel is a complex mixture of aromatic and aliphatic hydrocarbons. A fraction of the diesel has the potential to volatilize quickly (potentially a terrestrial asphyxiation risk in low lying areas), but posing little danger to aquatic organisms occupying the water column. A significant portion of diesel will remain at the surface of the water and portions of this will partition into the water column. Schein et al. (2009) defined a 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). Barron et al. (2013) reported a 5th percentile hazard concentration (HC5) of 0.285 mg TPH/L for diesel. In the current assessment of the diesel spill on Big Creek, a conservative assumption is the concentration of TPH in the WAF was 1/10 of the solubility limit, 10 mg/L. This conservative estimate is approximately 25 times the upper prediction limit of the 5th percentile hazard concentration reported by Barron et al. (2013). Please see Appendix II (Steevens et al. 2018) for the full toxicological evaluation of diesel and mussels.

Additional screening tools were reviewed by Steevens et al. (2018). This included comparing threshold values with estimated WAF concentrations (assuming 1/10th solubility) and evaluating physicochemical based models (the target lipid model [TLM] and the Petrotox model™) for potential use in a more comprehensive analysis. A comprehensive analysis would include the chemical analysis of the WAF for Marathon No. 2 diesel fuel in a manner similar to studies that have estimated the toxicity of gasoline (McGrath et al. 2005) and weathered crude oil (Di Toro et al. 2007). At this preassessment stage, comparisons can be made between conservative assumptions of the PAH constituents present in WAF that developed from Marathon's No. 2 diesel fuel, that is 1/10th of their solubility limit as previously described. These estimated WAF concentrations were compared to U.S. EPA Region 4 relevant water threshold values for PAH constituents of diesel that were derived using equilibrium partitioning (USEPA, 2018). As indicated in Table 2, at least 6 PAH would exceed the EPA Region 4 chronic surface water screening values. For example, the estimated concentration of naphthalene in the WAF is 15.9 times higher than the chronic surface water screening value. Taken together, the toxicological

Table 2. 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

evaluation of the Marathon diesel spill (Steevens et al. 2018) likely caused significant injury to natural resources in Big Creek. Therefore, more comprehensive exposure and effects assessment should be undertaken to fully explore and document this toxicity, which will allow the Trustees to identify the injuries to natural resources that have or are likely to result from the incident, and the extent of these injuries. This in turn will help inform the development of feasible restoration actions to address those injuries.

References

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Appendices

I. Marine Safety Laboratory (MSL) US Coast Guard (USCG) (2018) Oil Sample Analysis Report U.S. Fish and Wildlife Service Case Number 18514 Marine Safety Laboratory Case Number 18-078.

II. Steevens, J., Farag, A., Jones, D., Ivey, C., Besser, J., Dorman, R., Sparks, D.W. (2018). Toxicological Evaluation of No. 2 Diesel Fuel Spill on the Big Creek Indiana, March 20, 2018. US Geological Survey, Columbia Environmental Research Center, Columbia, MO

III. USFWS (2019) Natural Resource Damage Assessment: Big Creek Aquatic Community Baseline Preassessment Evaluation for the March 20, 2018 Marathon Diesel Pipeline Break on Big Creek near Solitude, Posey County, Indiana.

Appendix I

Marine Safety Laboratory (MSL) US Coast Guard (USCG) (2018)

Oil Sample Analysis Report U.S. Fish and Wildlife Service Case Number 18514 Marine Safety Laboratory Case Number 18-078.

Oil Sample Analysis Report

U. S. Fish and Wildlife Service
Case Number E18514

Marine Safety Laboratory
Case Number 18-078

U.S. Department of Homeland Security

United States Coast Guard

Manager
U.S. Coast Guard
Marine Safety Laboratory

1 Chelsea Street
New London, CT 06320
Phone: (860) 271-2704
Fax: (860) 271-2641

16450
02 May 2018

U. S. Fish and Wildlife Service
Attn: Agent in Charge
Indiana Field Office
620 South Walker Street
Bloomington, IN 474032121

Dear Agent in Charge:

The laboratory analysis of this case has been completed and our report is forwarded. The technical data supporting the report (spectrograms and chromatograms) have been archived at our facility and are available upon request. We will maintain the oil samples in refrigerated storage pending final case disposition.

Questions concerning this report or the analytical methods used should be directed to the Supervisor of Analysis.

Kristy Juaire
K. JUAIRE

Encl: (1) MSL Report 18-078

United States Coast Guard
Marine Safety Laboratory

Oil Spill Identification Analysis
Cost Recovery Documentation

Laboratory Case Number:	18-078
Requestor:	U. S. Fish and Wildlife Service
Unit Case Number:	E18514
Number of Samples:	5
Cost Per Sample Prepared:	$20.00
Total Costs of Sample Preparation:	$100.00
Number of Analyses:	12
Cost Per Sample Analyzed:	$86.00
Total Costs for Analysis:	$1,032.00
TOTAL COSTS:	$1,132.00

This documentation is provided for purposes of Phase IV - Documentation and Cost Recovery under the National Oil and Hazardous Substances Pollution Contingency Plan (40 CFR Part 300)

Signature: [signature] **Date:** 02 May 2018

United States Coast Guard
Marine Safety Laboratory
Oil Sample Analysis Report
18-078

Requestor: U. S. Fish and Wildlife Service
Unit Case/Activity Number: E18514
Received: 26-Apr-18 **Via:** Federal Express 8099 2730 1509

Number Of Samples: 2
Lab ID for Spills: 1
Lab ID for Sources: 2
Lab ID for Background: n/a

Analysis Methods:

- [x] GAS CHROMATOGRAPHY (GC)
- [x] GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)
- [ ] INFRARED SPECTROSCOPY (IR)

Laboratory's Conclusion (as explained below): MATCH

RESULTS:

1. Sample 18-078-1 was specified to be representative of spilled oil. Analysis indicates this sample contains slightly weathered light fuel oil. Non-petroleum contamination is present.

2. Suspected source sample 18-078-2 contains light fuel oil with characteristics similar to those of spill sample 18-078-1. Differences are attributable to weathering and non-petroleum contamination.

CONCLUSIONS:

1. Suspected source sample 18-078-2 and spill sample 18-078-1 are derived from a common source of petroleum oil.

SUPERVISOR OF ANALYSIS K. JUAIRE (signature) **DATE** 02-May-18

United States Coast Guard
Marine Safety Laboratory Sample
Check-In Log

MSL Case/Activity Number: 18-078

Requestor: U. S. Fish and Wildlife Service **Unit Case Number:** E18514

Federal Project Number: E18514 **Delivery Method:** Federal Express

Received Date: 26 Apr 18 **Delivery Number:** 8099 2730 1509

Priority: No **Rush:** No **Comparison:** No

Lab ID 18-078	Sample Descriptions from Sample Jars	Spill	Source
1	1 FEATHERS FROM A PIED-BILLED GREBE COLLECTED MARCH 22, 2018 FEATHERS REMOVED FROM FROZEN CARCASS APRIL 24, 2018 3-22-18 13:00	☑	☐
2	2 BIG CREEK DIESEL RETAIN 4-27-2018 13:50	☐	☑
3		☐	☐
4		☐	☐
5		☐	☐
6		☐	☐
7		☐	☐
8		☐	☐
9		☐	☐
10		☐	☐

Remarks: Sample 2 received 01MAY18. FedEx tracking # 4275 9592 1622.

Samples checked in by: MSTC RICHARD FORTE [signature] **Date:** 26 Apr 18

Sample Custodian: MST1 SEAN JANNE [signature] **Date:** 02 MAY 18

Supervisor of Analysis: K. JUAIRE [signature] **Date:** 02 May 18

Appendix II

Steevens, J., Farag, A., Jones, D., Ivey, C., Besser, J., Dorman, R., Sparks, D.W. (2018).

Toxicological Evaluation of No. 2 Diesel Fuel Spill on the Big Creek Indiana, March 20, 2018. US Geological Survey, Columbia Environmental Research Center, Columbia, MO

Toxicological Evaluation of No. 2 Diesel Fuel Spill on the Big Creek Indiana, March 20, 2018

July 27, 2018

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Overview

On March 20, 2018 a pipeline carrying No. 2 diesel fuel breached and released approximately 58,800 gallons of fuel into Big Creek, Indiana. The purpose of this memorandum is to briefly summarize toxicity information about the toxicity of No. 2 diesel fuel and the potential effects of the spill on aquatic organisms. In addition, a study is proposed to generate toxicity threshold values based on two aquatic invertebrates and a fish. Results of this study will be used to more accurately estimate the adverse biological effects of the spill on aquatic species found in the Big Creek watershed.

1. General Site Description of Big Creek

Big Creek is in the southwestern tip of Indiana in Posey County approximately 7 miles north of Mount Vernon (Fig.1). The Big Creek is a tributary to the Wabash River and then the Ohio River. It receives input from 198 miles of perennial drains for approximately 256 square miles (IDEM, 2009). 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) located near Wadesville, IN. The mean daily discharge at that location is 110 cubic feet per second 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.

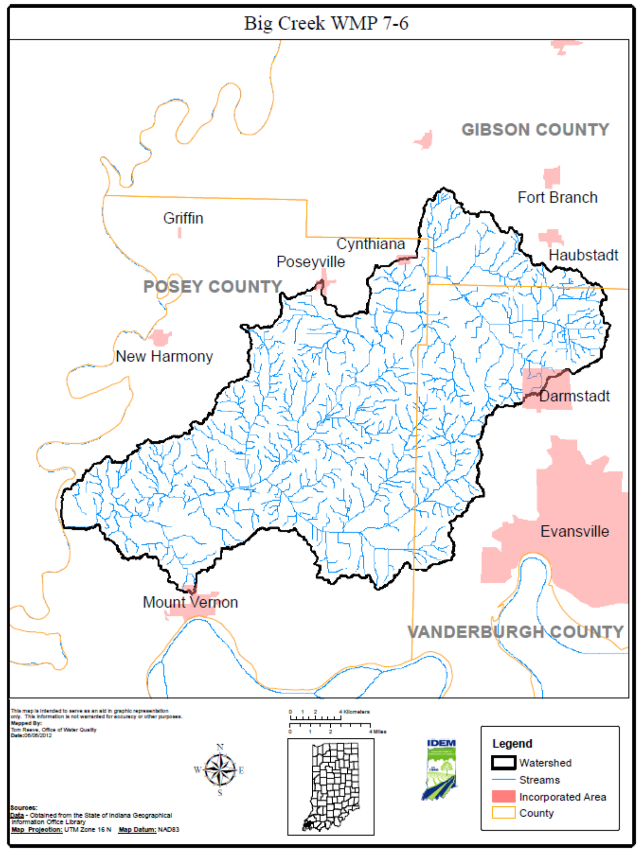


Fig. 1. Big Creek watershed. From IDEM 2009

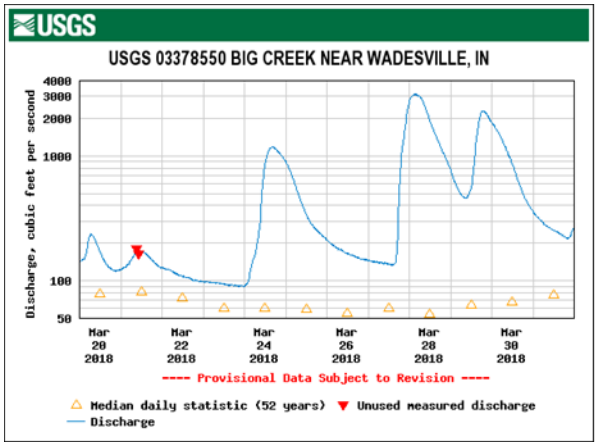
The Big Creek ecosystem is home to a wide range of fish and invertebrate species. In 2009 the Indiana Department of Environmental Management (IDEM) completed a watershed survey and management plan describing the benthic invertebrate and fish species diversity in the Big Creek and its tributaries (IDEM, 2009). A survey of benthic invertebrates and fish was conducted at 32 sites throughout the watershed. The benthic invertebrate community was characterized using the Ohio Qualitative Habitat Evaluation Index (Rankin, 2006). Sites within the main channel of the Big Creek were generally characterized as poor quality due to high level of turbidity and siltation as well as channel modifications. In 2008, Bandoli et al found 40 different species of fish within Big Creek and its tributaries (Bandoli and others, 2010). Near State Highway 69 a total of 11 different species were documented, including shad, carp, shiner, silvery minnow, buffalo, catfish, and silverside. Three federally listed endangered species, the Indiana bat (*Myotis sodalis*), northern long-eared bat (*Myotis septentrionalis*), and fat pocketbook mussel (*Potamilus capax*) are found in the Big Creek watershed (USFWS). The fat pocketbook mussel has been documented in the Wabash River and Big Creek watershed, specifically in Big Creek near the State Highway 69 bridge and at the confluence of Big Creek with the Wabash River (Cummings and others, 1992; Fisher, 2006a, b).

2. Diesel Spill Description

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). At approximately 23:00 CST the same day booms were installed at the Lower New Harmony Road (~4.6 miles downstream of the breach) and at Wabash Road (~7.6 miles downstream of the breach). The U.S.

EPA observed diesel arriving at the bridge at the same time the booms were deployed. This suggests the diesel fuel traveled approximately 4.6 miles from the spill site to Lower New Harmony Road in 4.5 hours. Recovery operations using skimmers to remove diesel pooled above the booms started 12 hours later at approximately 11:00 CST on March 21, 2018 (see Fig. 2). By 07:00 CST on March 22, 2018 the estimated product recovery was around 80.1% of the total released. Based on this timeline there was between 36 to 48 hours for a large percentage of the spilled product to be in contact and mix with the surface water. Stream flow in the Big Creek remained relatively low at the time of the spill until March 24 2018 in response to a 1.15 inch rainfall event during the evening of March 23 and into the early morning of March 24, 2018 (Fig. 3) (NWS, 2018). At 07:00 CST on March 27, 2018 the final estimate of total diesel recovery was 84.8% of the total released.

Figure 2. Diesel fuel recovery at the Lower New Harmony Road Bridge (38.001162, -87.954638) on 3-21-2018. Photo credit Blair Photo EVV.



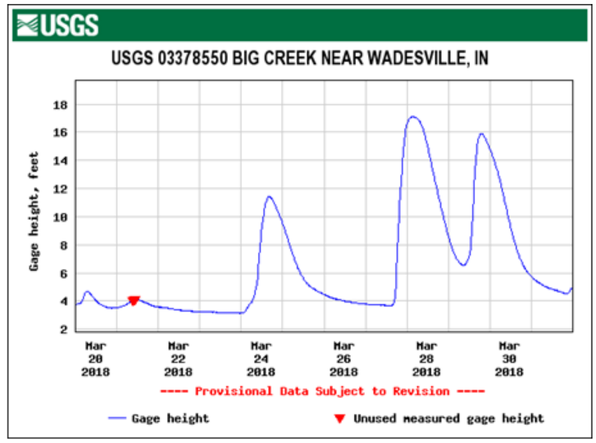


Figure 3. USGS Stream gauge discharge and height at Big Creek Station 03378550 near Wadesville, IN.

3. Conceptual Model for No. 2 Diesel Fuel Spill in Big Creek

Conceptual models are used to communicate and guide the analysis of the sources of contaminants, transport pathways, and receptors within the context of a risk-based analysis (ASTM-International, 2008). The conceptual model is initially used to structure the analysis and identify the most likely exposure pathways due to the release of the fuel into the Big Creek. The fate of diesel and its constituents is a key element of the analysis, therefore we used the conceptual model to guide decisions regarding the composition and distribution of the diesel constituents in the Big Creek system. These decisions were informed by information such as standard values in guidance documents and hydrologic conditions in the stream. Lastly, we used the conceptual model to guide the identification of the most relevant receptors that may be affected by the diesel. In the future, the detail within the conceptual model should be expanded as part of a more comprehensive analysis.

It is estimated that approximately 58,800 gallons of No. 2 diesel fuel was released into the Big Creek. The breach in the pipeline was in the right descending bank of the creek, and due to pressure of the line, diesel released directly into the water. This dispersion presumably resulted in initial mixing of the diesel into the water column; however, the density of diesel is approximately 0.85 g/cm^3 and therefore rapidly rises to the surface of the water (Marathon, 2016). At the surface of the water, the diesel will partition into the water column, water surface, and air phases. Minor volatile components, such as benzene, toluene, ethylbenzene and xylene (BTEX), are likely to rapidly volatilize and are unlikely to contribute to the water column pathway. However, several of the constituents including polycyclic aromatic hydrocarbons (PAH) and alkanes contribute to the exposure pathway at the water surface and water column. In addition, there is a potential that some diesel can partition to sediment after weathering or through direct contact with the sediment.

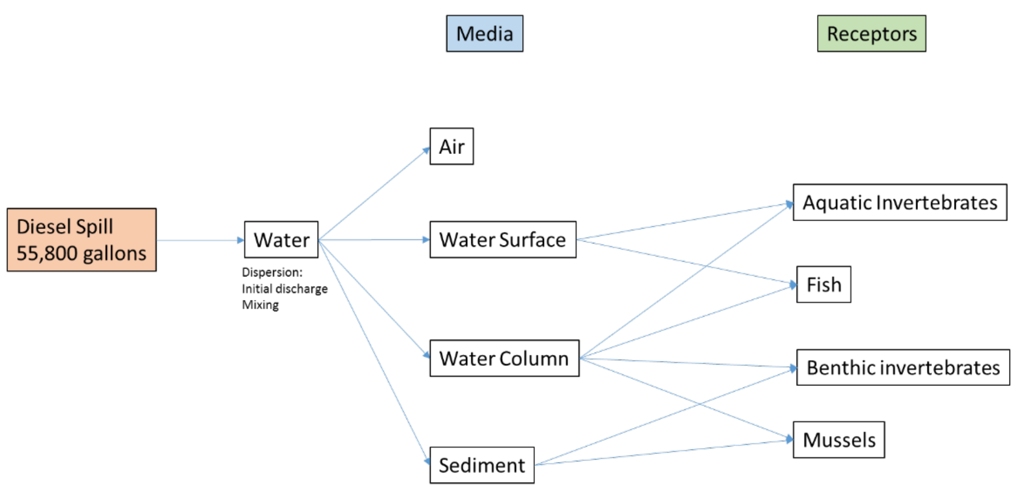


Figure 4. Simplified conceptual model for the diesel spill in Big Creek, IN.

In this analysis we focus on the partitioning of the soluble fraction of diesel into the water column as the primary exposure pathway for aquatic invertebrates and fish. Secondary exposure pathways include exposure at the water surface and sediment. To evaluate the potential for toxicity the concentration of diesel constituents will be estimated and compared to existing toxicity screening and literature values.

4. Concentrations of Diesel and Constituents in the Big Creek

No sampling of water or sediment was done immediately following the spill and during the diesel recovery process. Therefore, concentrations of diesel constituents in the water column must be estimated using physiochemical properties and stream conditions. The water accommodated fraction (WAF) is commonly used to describe the level of dissolved petroleum constituents in the water column (Anderson and others, 1974). To estimate a WAF we used standard chemical concentrations of No. 2 diesel fuel, chemical fingerprinting data from the spill, and solubility values frequently used for modeling the fate of petroleum compounds in the water.

To estimate the concentration of chemicals in the WAF we first identified the constituents that are often present in No. 2 diesel fuel (USEPA, 2003). This list was compared to the compounds identified in the US Coast Guard fingerprint analysis of No. 2 diesel fuel obtained from Marathon Oil (Table 1). A total of 8 PAH, pristine, phytane, and alkanes were identified. To determine the maximum amount of these compounds in the WAF we assumed the total amount of diesel in the Big Creek was sufficiently large as compared to the relatively small amount of water and volatilization in the air and therefore the only limiting factor was water solubility. If solubility is the limiting factor the maximum theoretical concentration of diesel in the water is the sum of solubility for the major constituents, 100 mg/L. However, it is unlikely the water reached the maximum saturation of the constituents in the diesel. Due to other factors that affect solubility (e.g., mixing, input of fresh water, removal of product from the river) we applied an uncertainty factor of ten and estimated the dissolved fraction was 10% of the sum solubility of major constituents or 10 mg/L. This value is also consistent with levels of petroleum hydrocarbons reported in literature where WAF are experimentally derived. Because the stream flow was sufficiently low for the first 96 hours it is assumed the concentration of diesel in the WAF of Big Creek was 10 mg/L over the 96-hour period. However, additional spill modeling can provide a more comprehensive analysis to more accurately refine the estimated concentration of diesel based on the dilution, dispersion, and contact time with the water.

5. Modeling the Diesel Spill

To estimate potential exposure vulnerabilities to No. 2 diesel along the length of Big Creek, a flow path analysis was conducted in ArcMap (ESRI., 2018a). NHDPlus Version 2 datafiles including a 30 m digital elevation model (DEM), flow direction grid, and flow lines were downloaded for the Big Creek region (USEPA 2016b) and assembled in a Geographic Information System (GIS). A point dataset was generated denoting the spill origin, the first and second oil collection booms, and the confluence of Big Creek and Wabash River (Fig. 5). Flow accumulation and flow length (up and downstream) grids were generated for Big Creek from the NHDPlus flow direction surface using the Hydrology toolset in ArcMap (ESRI., 2018b). Values were extracted for each 30 m pixel along the length of Big Creek from the spill origin to the confluence of the Wabash and exported for analysis in R (R-Core-Team, 2018) .

Fig. 5. Big Creek with key points of interest shown.

An exponential decay function was used to estimate volumes of diesel fuel at 30 m increments along Big Creek. The function assumes that of the total captured volume (48,000 gallons), 80% was captured at the first boom located approximately 4.6 miles downstream from the spill, and the remaining 20% was captured by the second boom located at 7.6 miles downstream from the spill. The function is of the form

$$V = 58{,}471e^{-0.159d}$$

where *V* is the estimated volume of No. 2 diesel (in gallons) present at distance *d* downstream (in miles). As a coarse estimate of exposure vulnerability, the estimated incremental volumes were interpreted as a direct measure of vulnerability, where higher volumes indicate increased vulnerability (see Fig. 6). This assumption does not account for transformations of the diesel plume due to weathering or dispersion, changes in streamflow over time or moving downstream, and any additional retention of diesel by in-stream obstructions (e.g., debris, gravel bars) or other retarding mechanisms.

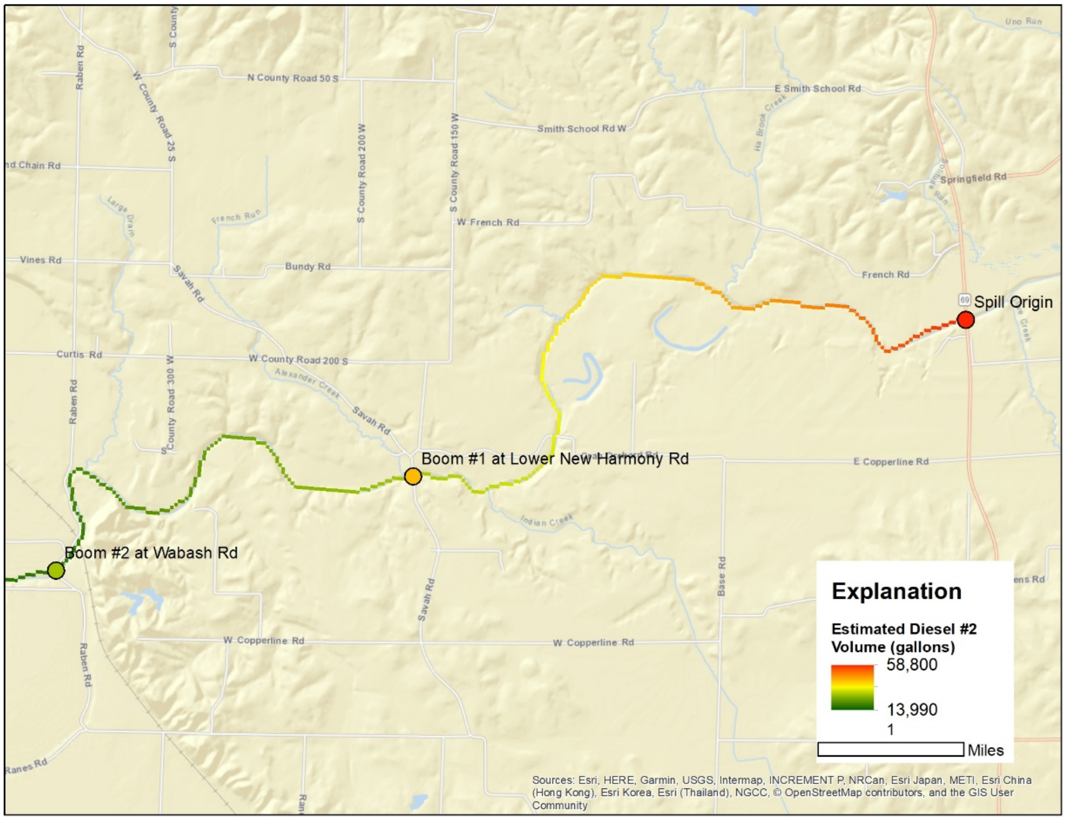


Fig. 6. Estimated volume of No. 2 diesel along Big Creek. Red sections of the stream reach are assumed to be at increased exposure risk.

The vulnerability assessment suggests that the greatest exposure risks occur between the spill origin and the first retention boom, with vulnerability decreasing with increasing distance from the spill origin. In this assessment, the unrecovered diesel (approximately 10,800 gallons) was assumed to be transported downstream to the Wabash River. However, it is possible that some of this volume remained in sediments and debris along Big Creek. This additional reservoir of diesel components would augment the volumetric estimates and exposure vulnerabilities shown here. While there are several models that have been developed to assess exposures to spilled contaminants (Pistocchi, 2008; McCready and Williams, 2011; Granato, 2013), a more detailed assessment was not possible in this assessment. Some key variables that should be considered in a more comprehensive assessment include properties of No. 2 diesel fuel (e.g., density, specific gravity, weathering properties), changes in stream flow through time and space (drainage-area discharge ratios, and some estimate of in-stream or bank retention capability (e.g., roughness) (Emerson and others, 2005).

6. Aquatic Toxicity of Diesel

To interpret the estimated concentration of diesel constituents in the water column we identified and reviewed the relevant literature, threshold values, and physicochemical based models. This information can be used as a conservative screening level assessment for the potential effects associated with the water column pathway. The purpose of the screening level assessment is to determine the need for a more refined and comprehensive analysis.

The literature review focused on peer-reviewed, published manuscripts obtained through Web of Science, Google Scholar, Defense Technical Information Center, and the Online Computer Library Center WorldCat. In addition, the Columbia Environmental Research Center Library, a specialized collection focusing on aquatic environments and restoration ecology, was used to identify any other relevant resources. An initial search of literature on the fat pocketbook mussel was performed and yielded 26 references. These manuscripts were used to understand life history, distribution, and contaminant susceptibility of the fat pocketbook mussel. A second search was performed with key words including No. 2. diesel fuel, aquatic toxicity (or fate, or chemistry), and fish or invertebrates or mussels. The second search yielded 21 references. Five of the 21 references were deemed unrelated to the current inquiry and were removed from the pool.

The remaining toxicity literature was evaluated for relevancy to the aquatic toxicity of diesel. Each study was reviewed to identify the species studied, exposure conditions, endpoints measured, and whether adequate analytical chemistry was conducted on exposure media. Each study was summarized, reviewed by three scientists, and ranked with a score of 1 – 5 with 5 being the most relevant and useful. During the search, additional manuscripts cited in relevant literature were also located and reviewed. Eight manuscripts ranked as 3 - 5 are presented in Table 3. It should be noted that there were issues with missing information reported in most literature. Lack of information was especially a factor for reported chemistry measurements and characterizations.

The literature review found there is a paucity of information defining the toxicity of diesel on freshwater organisms. Diesel is a complex mixture of aromatic and aliphatic hydrocarbons. A fraction of the diesel has the potential to volatilize quickly and as such pose little danger to organisms occupying the water column. A significant portion of diesel will remain at the surface of the water or partition into the water column. Schein (Schein and others, 2009) defined an increased toxicity of diesel for rainbow trout after mixing (also defined as the WAF). Adding dispersant initially seemed to increase toxicity, but the differences between the two became nonexistent when the toxicity was based on total hydrocarbons. This recalculation was necessary because of a disparity between nominal and measured concentrations of PAHs, especially for the WAF. The authors defined a 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). Work by Schein et al. (Schein and others, 2009) highlights the importance of adequately defined chemistry during toxicity experiments with diesel. The following information illustrates the types of effects observed, but varying units of measurement among studies makes comparisons of effect and no effect concentrations more difficult. Some field data provide evidence that benthic invertebrate community structure is affected post spill. Lytle and Peckarsky defined reduced invertebrate density and taxonomic richness 5 km downstream of a spill on the Cayuga River, NY for 3 months following a spill (Lytle and Peckarsky, 2001). While significant differences at the site 5 km downstream of the spill had improved within 12 months, the site immediately below the spill was still dominated by one taxon at 15 months, at the study’s end. Unfortunately, this study did not provide quantified PAH concentrations. Carman et al. defined that > 300 mg PAH/Kg caused excessive invertebrate mortality in an experimental mesocosm and caused one species of nematode to dominate the system (Carman and others, 1998). Keller et al. exposed freshwater mussels, *Villosa villosa* (juvenile), *Lampsilis siliquoidea* (glochidia and juvenile), *Lasmigona costata* (glochidia) to sediments collected from Fish Creek, Indiana, 2 years post-spill (Keller and others, 1998). No effects on survival were observed and there were no quantifiable concentrations of organics in the sediments at the time of testing. In addition, this study was prior to the development of methods for culturing freshwater mussels in the laboratory and therefore poor control survival may have masked any potential diesel toxicity. Martinović et al. did not observe effects on survival but documented increased heart rates of *Mytilus galloprovincialis*, a marine mussel exposed to 0.1 and 1.0 ml diesel/L (Martinović and others, 2015). Hamoutene et al. (Hamoutene and others, 2004) documented significant increases in immune functions in a marine adult mussel, *Mytilus edulis* exposed for 4 days to a WAF of diesel fuel. The authors attribute this toxicity to low molecular weight PAHs present in the diesel used in the experiments. The concentrations of LMW PAHs were greatest 7 days after an experimental spill. Marques et al. (Marques and others, 2014) dosed a mangrove to 0.17 mg diesel/g and appeared to overwhelm the GSH response and cause a decrease in GSH levels on Day 7 for *Mytella guyanensis*, a mangrove mussel. Table 3 lists additional experimental evidence of effects on survival, growth, and DNA adducts, and elevated BPMO activity for marine and freshwater fish. Organic chemistry was expressed in variable units (e.g. diesel loading rates, mg diesel/L).

A species sensitivity distribution (SSD) is used to evaluate the reported toxicity of multiple species to a specific chemical. Barron et al reported the development of SSD for several oil products including No. 2 diesel fuel (Barron and others, 2013). To assemble data for the SSD, a literature review was conducted to identify acute effect values (48-96-hr) from exposures to WAF. The SSD generated for diesel included studies reporting the toxicity of unweathered WAF on marine invertebrate and fish species. All studies used in the SSD required chemical analysis or confirmation of the exposures as total petroleum hydrocarbons (TPH). A total of 4 different marine mollusks, 6 fish, and 11 other invertebrates were included in the modeled distribution. The resulting screening value developed from the SSD was a 5^{th} percentile hazard concentration (HC5) of 0.285 mg TPH/L (0.202- 0.403 lower and upper prediction limits). In the current assessment of the diesel spill on Big Creek, a conservative assumption is the concentration of TPH in the WAF was 1/10 of the solubility limit, 10 mg/L. This conservative estimate is approximately 25 times the upper prediction limit of the 5^{th} percentile hazard concentration reported by Barron et al.

Another model used to evaluate the effects of non-polar organic chemicals, such as diesel constituents, is the target lipid model (TLM). This approach utilizes a relationship between chemical (K_{ow}) and biological (LC_{50}) data to develop critical body burdens (CBBs) (Di Toro and others, 2000; McGrath and others, 2005). For non-polar organic chemicals with a similar mode of action, relationships have been derived to predict toxicity for a mixture of compounds based on water concentration (Deneer and others, 1988; Nirmalakhandan and others, 1994; Broderius and others, 1995). In a similar way, the TLM approach includes extensive chemical and biological data in an approach to determine the concentration of a chemical in an organism's tissue which results in an adverse effect.

For application of the TLM to estimate effects on aquatic organisms, it is useful to derive a range of values that offers a level of protection from effects. Data used in the derivation of the TLM is primarily from studies which reported acute toxic effects. Application factors were derived from different classes of chemicals based on a compilation of CBBs (Di Toro and others, 2000). A final acute value (FAV) is the concentration of chemical, based on experimental data, that will not (based on probability) have an acute narcotic effect on 95% of the organisms. In other words, the value is protective of 95% of all species. These values are calculated following guidance for the derivation of water quality criteria (Stephan and others, 1985). The FAV reported in DiToro et al. (2000) and McGrath et al. (2004) were 35.3 and 36.2, respectively. Because of the need to protect species from longer-term exposures a range of values protective of chronic effects is of interest. Approaches used for deriving water quality criteria use the acute to chronic ratio (ACR) which is a single value (slope) derived from regression of acute and chronic studies.

The Petrotox model™ is a simplified spreadsheet that relies on the TLM to estimate the toxicity of non-polar hydrophobic compounds to aquatic organisms based on the TLM. It requires chemical analysis of the water as input; therefore it will be considered in a comprehensive analysis that will include chemical analysis of the WAF for Marathon No. 2 diesel fuel similar to the approach used to estimate the toxicity of gasoline (McGrath and others, 2005) and weathered crude oil (Di Toro and others, 2007).

Regional threshold values were sought to interpret the estimated exposure concentrations in the Big Creek. U.S. EPA Region 5 does not have applicable screening values. U.S. EPA Region 4 has developed relevant water and sediment screening values for the use in ecological risk assessment (USEPA, 2018). Surface water screening values for PAH constituents of diesel were derived using equilibrium partitioning. These values are shown in the table below and can be used to compare modeled or measured concentrations in the water column.

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

A conservative assumption is that PAH constituents in the No. 2 diesel were 1/10 of their solubility limit in the WAF. Based on this conservative assumption at least 6 PAH would exceed the EPA Region 4 chronic surface water screening values. For example, the solubility limit of naphthalene is 31,000 ug/L. Assuming the concentration of naphthalene in the WAF was 1/10 of the solubility limit we estimate the concentration in the Big Creek to be 3100 μg/L or 15.9 times the chronic surface water screening value. However, it is recognized the purpose of these values is to support a screening level assessment and only used to identify where potential adverse effects may occur. Therefore, additional more comprehensive exposure and effects analysis is required.

7. Summary of Findings.

- The Marathon Oil No. 2 diesel spill released approximately 58,800 gallons of fuel to the Big Creek. Approximately 48,000 gallons was recovered leaving approximately 10,800 gallons in the Big Creek.
- Samples collected from an oiled bird were matched to Marathon No. 2 diesel fuel by the US Coast Guard. The natural resource trustees are not aware of other analytical samples being collected in Big Creek in response to this spill.
- Due to the lack of analytical data from the spill, conservative screening assumptions and models were used to determine the greatest exposures to diesel occurred between New Harmony road and the spill location (4.3 miles of Big Creek). The remaining 10,800 gallons of diesel was distributed from the spill location to the confluence of the Wabash River.
- Multiple fish species and one endangered mussel species are found in the area contaminated by the diesel spill. Based on a screening level assessment of the spill distribution organisms in this region are likely to be exposed to diesel on the surface, the water accommodated fraction, and sediment.
- Using a screening level approach, the concentration of diesel constituents (as total petroleum hydrocarbons) is estimated to be 10 mg/L in the water column of the Big Creek.
- The estimated concentration of diesel constituents in the water column exceeds the level where effects on aquatic organisms are reported in the literature and relevant SSD developed by U.S. EPA.
- The estimated concentration of 6 different PAH in the water column exceed U.S. EPA risk-based screening thresholds.
- There are several limitations of this screening level assessment. Firstly, the lack of chemical data and reliance on conservative assumptions to estimate a water concentration introduces significant uncertainty in the exposure assessment. The effects assessment does not address the potential effects on endangered species that are found in the area affected by the spill. The assessment does not address any chronic effects of PAH such as blue sac disease or developmental effects on fish species. Phototoxicity of the PAH constituents was not evaluated in the screening level assessment.
- A more comprehensive analysis of diesel constituents in the water column, sediment, water surface and associated effects to aquatic species is recommended.

8. Proposed study to develop aquatic toxicity threshold values for No. 2 diesel fuel in freshwater systems

8.1. Overview. The screening assessment on the effects of diesel 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 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 TLM or another appropriate model. 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 potential 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 (Ramachandran and others, 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 and others, 2009).

8.2. 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 and others, 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.

8.3. 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 and others, 1996; Hook and others, 2002; Hawthorne and others, 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.

8.4. Mussel bioassay. Table 4 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.

8.5. *Ceriodaphnia* bioassay. Table 5 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.

8.6. Fish bioassay. Table 6 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.

8.7. 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.

8.8. 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.

8.9. Final Product. The final product will be a peer-reviewed publication that summarizes the toxicity thresholds developed based on the toxicity bioassays for these three organisms. In addition, a literature review of other existing freshwater toxicity data will be used to generate a species sensitivity distribution for the WAF of diesel. This product will be valuable for estimating the acute effects of diesel on freshwater organisms based on measured and estimated concentrations of diesel fuel.

Table 1. 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	2
	Phenanthrene	2293	1.34%	X	1.2	2
	Dibenzothiophene	312	0.18%	X	3.07	3
	Fluorene	2481	1.45%	X	2	2
	Chrysene	0.09	0.00%	X	0.0016	2
	Biphenyl	839.73	0.49%			
	Acenaphthalene	34.87	0.02%			
	Acenaphthene	153.55	0.09%			
	Anthracene	13.08	0.01%	X	0.043	2
	fluoranthene	6.6	0.00%	X	0.21	2
	Pyrene	30.88	0.02%	X	0.14	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] Indiana Department of Environmental Management, 2009

[3] Petrotox model default parameters

[4] Massachusetts Department of Environmental Protection, 2002

Table 2. Fingerprint analysis of Marathon No. 2 diesel fuel oil. Sample was obtained from Marathon and analyzed by Kristy Juaire, Supervisory Chemist, US Coast Guard Marine Safety Laboratory, New London, CT.

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 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. 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 5. Summary of test conditions for conducting chronic water-only toxicity tests with the cladoceran, *Ceriodaphnia dubia*, following standard methods recommended by ASTM (2015) 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 6. 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

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Cost estimate for 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 analyiss 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	Overhead	Rate:	7%	$14,160	
E. Total funding				**$216,440**	

Appendix III

USFWS (2019)

Natural Resource Damage Assessment: Big Creek Aquatic Community Baseline Preassessment Evaluation for the March 20, 2018 Marathon Diesel Pipeline Break on Big Creek near Solitude, Posey County, Indiana

Appendix III

Natural Resource Damage Assessment:

Big Creek Aquatic Community Baseline Preassessment Evaluation

for the

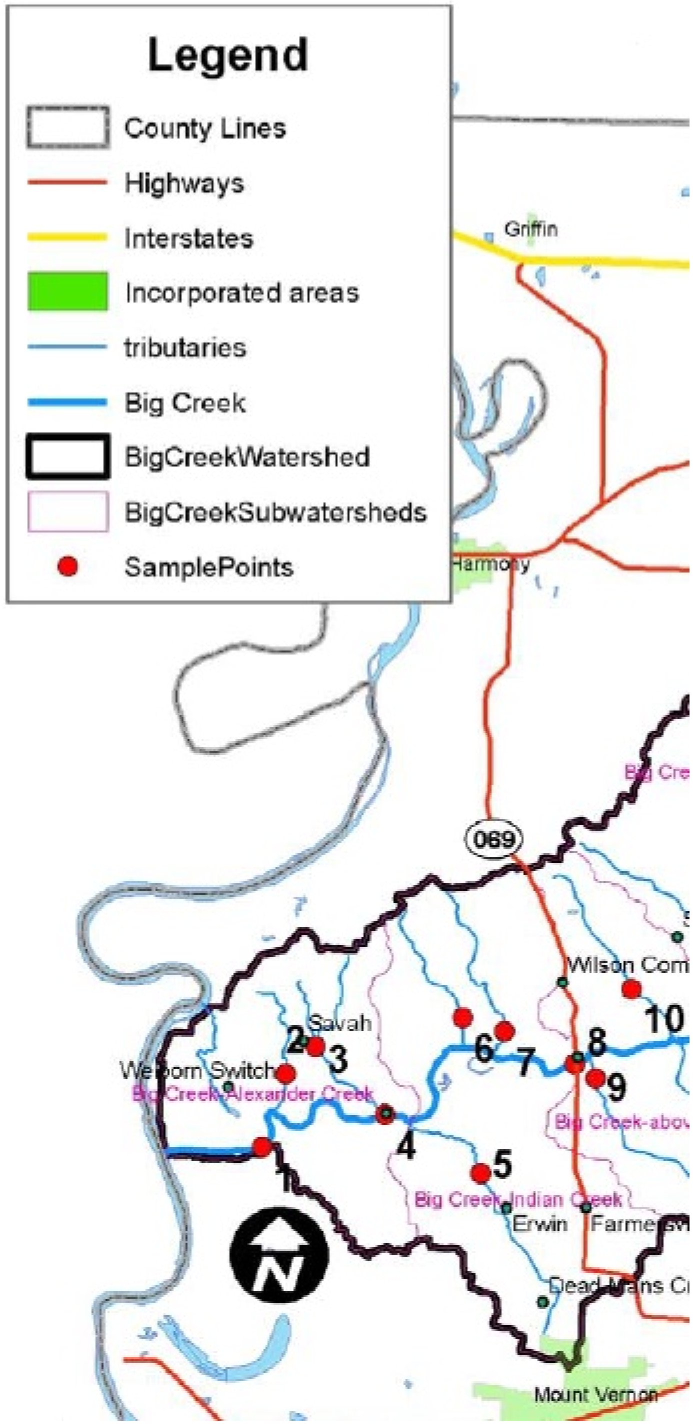
March 20, 2018

Marathon Diesel Pipeline Break

on

Big Creek near Solitude, Posey County, Indiana

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

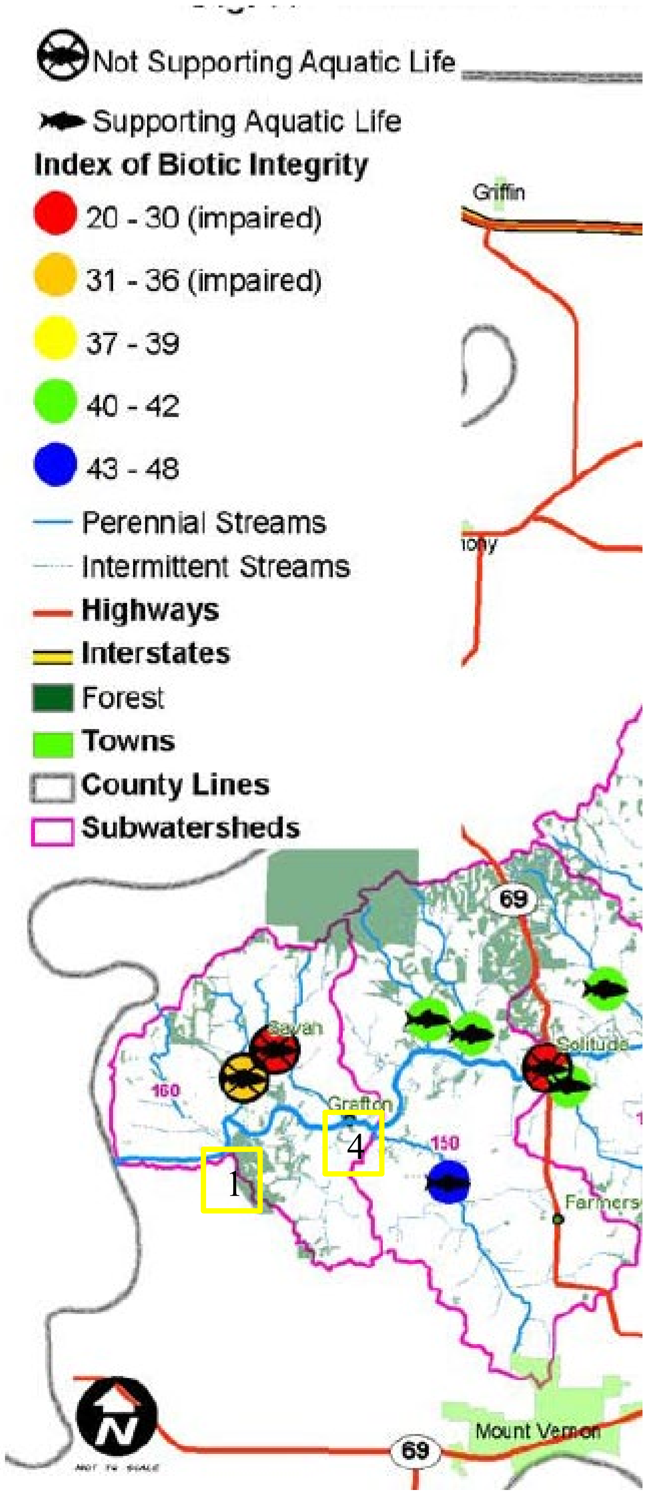


In 2009 a watershed management plan was finalized by the Posey County Soil and Water Conservation District after a couple of years of investigation, public outreach and analysis. It summarizes a great deal of data on Big Creek, and we have pulled aspects of this information out and present here in order to present a better understanding of background ecological conditions of this area relative to where the Marathon Pipeline diesel spill occurred in March 2018. This watershed management plan should be cited as: Borries B (2/21/2009) Big Creek Watershed Management Plan, Final Draft. Posey County Soil & Water Conservation District: funding from the EPA 205(j) Grant. It can be found at:

https://www.in.gov/idem/nps/3264.htm

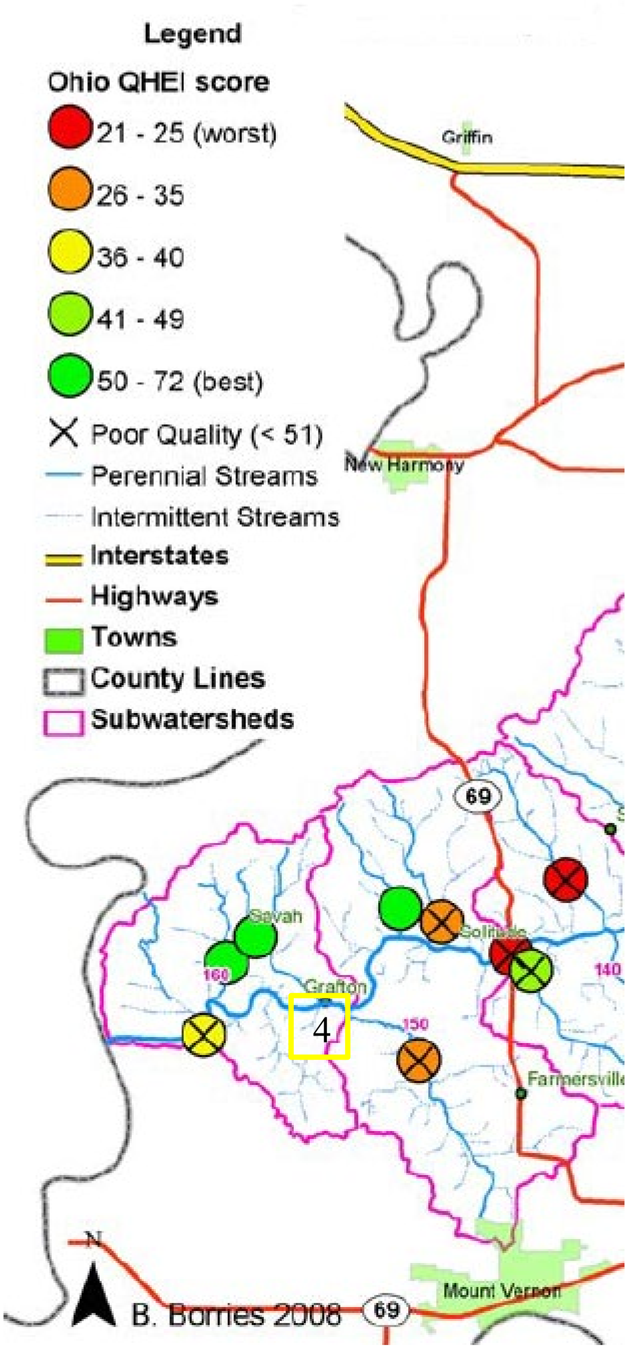
These are the sample locations in the Watershed Study that are in the Big Creek spill zone. The Highway 69 bridge (sample site #8) is just down-stream of the pipeline break.

Fig. 1. From: Borries B (2/21/2009) Big Creek Watershed Management Plan, Final Draft. Posey County Soil & Water Conservation District: funding from the EPA 205(j) Grant



University of Southern Indiana professor Dr. Jim Bandoli and his students, data was made available on the fish communities at each sample point (sites 1 and 4 were not completed because they were thought to more accurately represent fish communities of the Wabash River and not Big Creek). **This is a really important concept to grasp. Big Creek is an important nursery area for the Wabash River.** During the summer of 2008, Dr. Bandoli and his students collected fish using a multi-habitat approach. Block nets were utilized to collect organisms after they were ushered out of refuge by student assistants. Organisms were identified to the species level in the field and in a laboratory by Dr. Bandoli. Dr. Bandoli provided the data to the watershed coordinator and it was analyzed using Indiana's Index of Biotic Integrity (Simon and Dufour 1998). The results of the analysis are shown in Figure 3.26-A: Fish Index of Biotic Integrity in the Big Creek Watershed. Sites are color-coded based on their IBI score. Blue sites have the highest (best quality) score followed by green, yellow, orange, and red. Sites that are at or below the standard for the IBI are considered to have Impaired Biotic Communities (IBC). Sites 2, 3, 8, 14, 15, 22, 24, 26, 27, 28, 29, and 32 were found to be not supporting aquatic life due to IBC. There is no single thing that seems to be common among these sites; however, they all either have low QHEI scores or were found to exceed the criteria for nutrients and sediment.

Fig. 2. From: Borries B (2/21/2009) Big Creek Watershed Management Plan, Final Draft. Posey County Soil & Water Conservation District: funding from the EPA 205(j) Grant



Biological monitoring was included in the watershed assessment as a response to the 303(d) listing of two sub-watersheds on the basis of impaired biological communities. Habitat assessments provide a way to analyze the non-chemical stressors that lead to poor aquatic communities. The Ohio Qualitative Habitat Evaluation Index (QHEI) was used to evaluate habitat at each of the sample points where chemical monitoring occurred (assessment at site 4 was not completed due to a lack of resources). The Ohio QHEI assigns a numeric score to a stream reach based on 7 metrics: substrate, in-stream cover, channel morphology, riparian zone, pool quality, riffle quality, and map gradient (Rankin 2006). Sites may receive a maximum score of 100. IDEM's Office of Water Quality Assessment Branch has set a standard for a site to be impaired due to habitat. IDEM has determined that a score of less than 51 indicates poor habitat. However, a site will not be listed on the 303(d) if it is only impaired based on habitat; rather the QHEI criteria allows for the determination of the stressor as a non-chemical habitat related stressor instead of a chemical one.

Figure 3.2.4: Qualitative Habitat Evaluation Index Results in the Big Creek Watershed shows the results of the Ohio QHEI. Overall, most sites exhibited poor quality according to IDEM's criteria. Sites exhibiting poor habitat include sites 1, 5, 7, 8, and 9. The most common metrics resulting in low scores were those related to morphology and the riparian zone.

Fig. 3. From: Borries B (2/21/2009) Big Creek Watershed Management Plan, Final Draft. Posey County Soil & Water Conservation District: funding from the EPA 205(j) Grant

No state standard exists for turbidity or for the similar measurement of total suspended solids.

Turbidity is a measure of the clarity of water. The turbidity (y-axis) in NTUs is graphed against the sample point where it was measured (x-axis). Samples taken from Big Creek are shown as black diamonds, Little Creek is shown as purple squares, and the remaining tributaries are shown as green triangles.

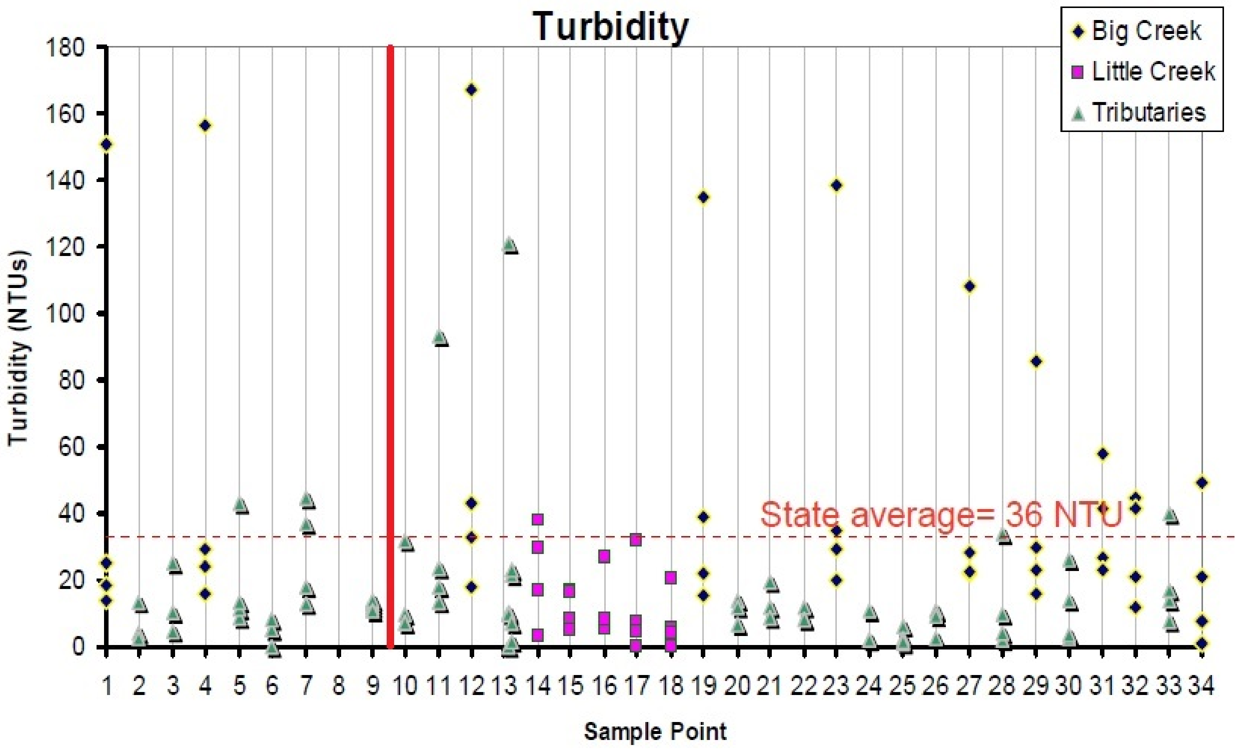


Fig. 4. From: Borries B (2/21/2009) Big Creek Watershed Management Plan, Final Draft. Posey County Soil & Water Conservation District: funding from the EPA 205(j) Grant

The spill zone portion of Big Creek is to the left of the vertical **red** line. Nearly 80% of the turbidity samples in the spill zone are below the average Indiana turbidity score.

The fish community in the lower reaches of Big Creek is strongly influenced by the lower Wabash River. It is not easy to sample at high water, but as you can see from Table 1, when conditions are wadeable in times of low water, sampling efficiency improves greatly and significantly higher fish community diversity scores can be recorded (as with the 1999 sampling effort). The lower Wabash River has significant ecological values to migratory birds, bats, and all manner of aquatic life.

Freshwater mussel diversity historically has been very high in the lower Wabash River. Water quality and habitat loss have caused significant declines in mussels in past decades. Despite this, IDEM Big Creek fish community samples at varying hydrologic conditions.

	38.000232 N -87.985258 W 11-Aug-99 CR350W	38.000133 N -87.989025 W 6-Jul-11 Raben Rd	38.016621 N -87.889202 W 6-Jun-16 Johnson Rd
Bigmouth Buffalo		3	
Black Buffalo		1	
Blackspotted Topminnow	10		
Bluegill	2		2
Bullhead Minnow	1		1
Common Carp		1	1
Emerald Shiner	8		
Freckled Madtom	3		
Freshwater Drum		1	
Gizzard Shad	2	5	
Green Sunfish	4		
Highfin Carpsucker		1	
Longear Sunfish			7
Longnose Gar	1	4	1
Mississippi Silvery Minnow		10	
Orangespotted Sunfish			2
Pirate Perch			1
Quillback			1
Redear Sunfish	1		
Ribbon Shiner	1		1
River Carpsucker	3		
River Shiner	1		
Sand Shiner	1		
Shortnose Gar			5
Silver Carp		1	
Smallmouth Buffalo			1
Spotfin Shiner	28		5
Spotted Bass		1	
Spotted Gar			11
Suckermouth Minnow	1		
Western Mosquitofish	5		1
IBI Score	34	16	18

the federally endangered fat pocketbook pearly mussel (*Potamilus capax*) has been found in Big Creek near the pipeline break and in the Wabash River at its confluence with Big Creek. The fat pocketbook has been frequently found in the Wabash River many miles both upstream and downstream of Big Creek.

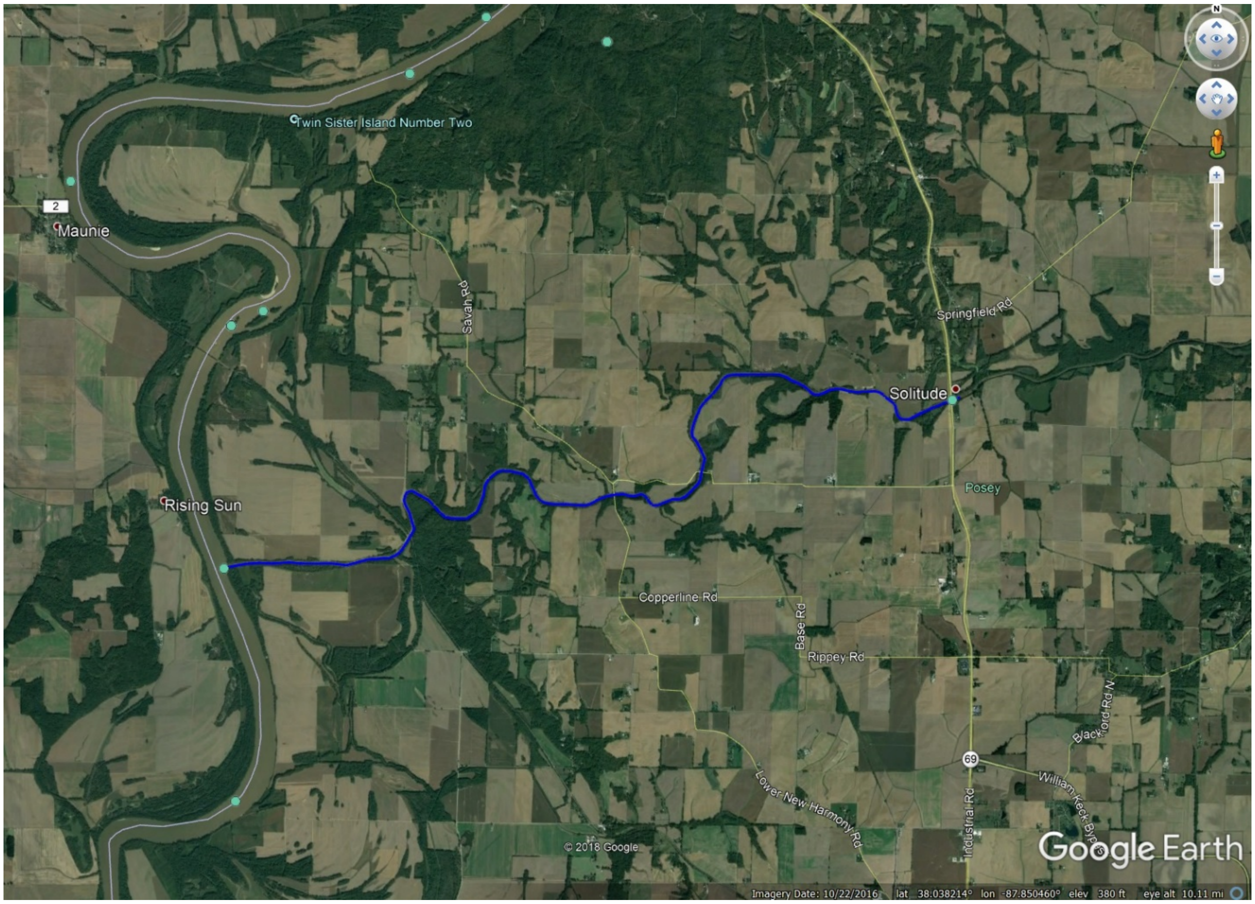


Fig. 5. Records of Fat Pocketbook pearly mussel (light green circles) in the Wabash River and Big Creek (outlined in blue).

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