Characterization of Oil and Water Accommodated Fractions Used to Conduct Aquatic Toxicity Testing in Support of the *Deepwater Horizon* Natural Resource Damage Assessment

Technical Report

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Abstract

In April 2010, the *Deepwater Horizon* (DWH) blowout resulted in the release of millions of barrels of crude oil 80 km off the coast of Louisiana. As part of the Trustees' Natural Resource Damage Assessment, a laboratory testing program was implemented to evaluate the toxicity of DWH oil and oil/dispersant mixtures to aquatic organisms of the Gulf of Mexico. Because of the variety of exposures that likely occurred during and after the spill, the testing program included four DWH oils, which encompassed a range of oil weathering states, and three different oil-in-water mixing methods, for a total of 12 chemically unique water accommodated fractions (WAFs). To better understand exposure chemistry across the toxicity testing program, we examined the effect of various WAF preparation parameters – including mixing energy, starting oil composition, and oil-to-water mixing ratios – on the chemical profiles and the final concentrations of these 12 WAFs.

In this report, we present the chemistry of the four DWH oils and 12 WAFs prepared with these oils. Our results show that both the degree of oil weathering and the mixing energy play important roles in determining polycyclic aromatic hydrocarbon (PAH) composition and concentration in each WAF. We found that the use of different oil-to-water mixing ratios to prepare WAFs can also affect composition. For the WAFs containing oil droplets, we found that the dissolved PAH concentrations were a small fraction of the total PAH concentration for the high-concentration stock WAFs, but became the dominant fraction as the concentration of the WAF decreased. Finally, we found that the mean diameters of the droplets in these WAFs were about 5–10 μm, with few droplets over 30 μm.

1. Introduction

As the largest maritime oil spill in U.S. history, the *Deepwater Horizon* (DWH) incident released oil that infiltrated and affected many different habitats over a long period of time. Because of the extended and extensive nature of the DWH spill, Gulf of Mexico (GoM) species likely were (and possibly continue to be) exposed to oil in a wide range of weathering states and physical conditions. To account for these various exposures, the Trustees developed a comprehensive toxicity testing program that included four different DWH oil samples, ranging from fresh to weathered, and three methods for creating oil/water mixtures, called water accommodated fractions (WAFs; Morris et al., 2015).

As oil moves through the marine environment, a variety of physical and chemical processes can occur that weather the oil, including biodegradation, evaporation, dissolution, emulsification/dispersion, and photo-oxidation (Jordan and Payne, 1980). Oil weathering affects both the chemical composition, as well as the physical condition of the oil (Jordan and Payne, 1980; Neff and Anderson, 1981; NRC, 2003, 2005); however, the extent that these processes alter the oil depend not only on the oil type and characteristics, but also on the different environmental locations and conditions that exist when and where the oil was released. Ultimately, the weathering state of oil in the environment can influence the manner and extent to which organisms are exposed to the oil, as well as the toxicity of oil/water mixtures (NRC, 2003).

In this report, we present a detailed description of the oils and aqueous test media used in the natural resource damage assessment (NRDA) toxicity testing program. In addition, we synthesize the results of numerous chemical analyses that we performed to characterize the 12 combinations of oil type/WAF preparation methods. We also examine various WAF preparation parameters to better understand their influence on resulting chemical composition and concentration. We distinguish the particulate and dissolved fractions in each WAF, examining how these two fractions change over time, as well as how they are affected by dilution. Finally, we present droplet size distributions for each WAF.

2. Experimental Section

2.1 DWH Oil Types

The Trustees' DWH NRDA toxicity testing program used four types of DWH oil, described below. The first three of these oils were collected from the field.

1. Mississippi Canyon Block 252 (MC252) crude oil, collected on July 26, 2010, from the hold of the barge *Massachusetts*, which received oil from the hold of the *Discoverer Enterprise*, which was receiving oil directly from the Macondo well riser. Hereafter, we refer to this oil as Source oil.

2. Surface slick oil, collected on July 29, 2010, from the hold of barge number CTC02404, which was receiving surface slick oil from various skimmer vessels near the Macondo well. Hereafter, we refer to this oil as Slick A.

3. A more weathered surface slick oil, collected on July 19, 2010, by the skimmer vessel *USCGC Juniper*. Hereafter, we refer to this oil as Slick B.

4. In addition, a fourth oil was produced by artificially weathering the Source oil. This oil type provided an intermediate weathering state between the Source oil and the field-collected slick oils. The artificial weathering procedure is described in the next section. Hereafter, we refer to this oil as Artificially Weathered Source oil.

For the three field-collected oils (Source, Slick A, and Slick B), TDI-Brooks International (TDI; College Station, TX) prepared smaller aliquots from the larger containers in which the oils were originally stored. Before preparing these aliquots, the technicians removed any excess water that had separated from the oil within the first few months of storage, and thus was not part of a stable water-in-oil emulsion. They then mixed the oil in the original collection containers (i.e., 55-gallon drums or 126-gallon square totes) using large paddle mixers. They continued mixing the oil until three samples pulled from different depths of the container showed similar hydrocarbon concentrations. TDI stored the aliquots at 4°C until they were distributed to various laboratories for toxicity testing. The toxicity testing laboratories continued to store the oil at 4°C until use. During the mixing procedure, TDI took specific precautions to ensure homogeneity and to minimize loss or change in the chemical characteristics of the oil aliquots. However, the chemical and physical properties we report for the field-collected oils reflect the characteristics of the oils used in the bioassays, which might not exactly match the characteristics of those oils when they were collected in the field.

2.2 Artificial Weathering of MC252 Source Oil

TDI conducted the artificial weathering of the Source oil based on a method modified from Carls et al. (1999). The Trustees artificially weathered the Source oil several times throughout the toxicity testing program, and identified each batch by date of weathering procedure. Approximately 3.5 L of Source oil were heated in a 4-L beaker to 90–105°C using a digital hot plate (model# C-MAGHP751, IKA, Wilmington, NC), stirring the mixture using a digital, top-loading mixer (model# BDC250, Caframo, Ontario, Canada) at a speed that would mix the oil without aerating it. The oil was stirred on low heat until a mass loss of approximately 33–38% was achieved. This correlated to a BTEX (i.e., sum of benzene, toluene, ethylbenzene, and xylenes) depletion of approximately 99.9% and a total polycyclic aromatic hydrocarbon [TPAH; sum of 50 polycyclic aromatic hydrocarbons (PAHs) – see Forth et al., 2015] depletion of approximately 22% relative to hopane. This process generally required about 20 hours.

2.3 WAF Preparation

In general, researchers used three methods to create WAFs for the DWH NRDA aquatic toxicity testing program: a low-energy mixing procedure (LEWAF); a high-energy mixing procedure (HEWAF); and a medium-energy, chemically enhanced mixing procedure (CEWAF). For testing conducted at the U.S. Geological Survey with sturgeon, researchers used alternative methods to prepare large volumes of WAFs. For these methods see USFWS (2015). In this report, we use the term WAF to refer to all three of these oil/water mixtures.

Our preparation of the LEWAF and the CEWAF followed the protocols described by Singer et al. (2000) and Aurand and Coelho (2005). First, we added water to an aspirator bottle and turned on a magnetic stirrer to a speed that produced no visible vortex. Then, we added the appropriate mass of oil to the aspirator bottle, covering it to prevent loss of volatiles. For the LEWAF, we continued stirring for 18 to 24 hours, draining the resultant WAF from the bottom of the aspirator bottle without disturbing the top layer. For the CEWAF, after adding the oil, we added the appropriate amount of dispersant (see *Dispersant* section, below) and increased the stir rate to achieve a 20–25% vortex relative to the height of the total water column. After continually stirring the mixture for 18 to 24 hours, we allowed the CEWAF to settle for 3 to 6 hours, and then drained the mixture from the bottom of the aspirator bottle, avoiding the top layer. We used both the LEWAF and the CEWAF as soon as possible, but no more than 24 h after preparation.

To prepare a HEWAF, we followed a protocol developed by NOAA and described by Incardona et al. (2013). First, we added the desired amount of oil to a Waring CB15 commercial food blender. Second, we added enough water to fill the blender (~ 3.7 L), and turned it on low for 30 seconds. We then immediately transferred the blended WAF to a separatory funnel and allowed the mixture to settle for 1 hour. After it settled, we drained the WAF from the separatory funnel without disturbing the top layer. We used all HEWAF preparations as soon as possible, but no more than 24 hours after completion.

For WAF preparations, we used a gas-tight syringe to add the Source oil, the Artificially Weathered Source oil, and the dispersant. The viscosity of Slick oils A and B was too high to dispense using a gas-tight syringe, so we weighed them in aluminum weigh boats and transferred them to the mixing vessel using stainless-steel spatulas. We determined the final amount of oil or dispersant gravimetrically by mass difference of syringe or weigh boat before and after transfer.

2.4 Dispersant

Corexit® 9500 was the most common dispersant that responders used during the spill, applied both at the surface and in the sub-surface at the source of the spill (OSAT, 2010). The recommended range of application rates in the field according to the U.S. National Contingency Plan product bulletin for Corexit® 9500 is a ratio of dispersant to oil of 1:50 to 1:10 (U.S. EPA, 2013). In addition, the Chemical Response to Oil Spills: Ecological Effects Research Forum (CROSERF) used a 1:10 dispersant-to-oil loading ratio as their standard ratio (Aurand and Coelho, 2005). Thus, for a majority of the NRDA aquatic toxicity tests, researchers prepared the CEWAFs using Corexit® 9500 at a 1:10 dispersant-to-oil loading ratio.

2.5 Filtration Protocol

During the course of the toxicity testing program, researchers occasionally filtered WAF preparations for the purposes of determining the components in the "dissolved" phase of the WAF, or for use in a toxicity test as a dissolved-only exposure solution. We adopted the original filtration protocol from the procedures described in Payne et al. (1999), which were also the procedures Payne and others used onboard several of the DWH NRDA cruises (Payne and Beegle-Krause, 2011). For this protocol, we initially used a 0.7-µm glass fiber filter to separate particulate and dissolved oil. We subsequently revised the protocol after we determined that significant droplet breakthrough was occurring with the CEWAF preparations. The revised protocol included two stacked 0.3-µm glass fiber filters to better remove oil droplets. During filtration, we kept the vacuum pressure low (no more than 5–10 cm Hg) to avoid pulling droplets through the filters. Also, we used a 90-mm-diameter filter to help reduce filter overload. All filtered chemistry data presented in this paper used the revised method.

2.6 WAF Characterization and Analysis

Much of the data we present here is a synthesis of chemistry collected from toxicity testing activities from the broader DWH NRDA toxicity testing program. These data were obtained from samples collected from bioassays as well as chemistry-only tests conducted by various toxicity testing laboratories. To obtain additional, more-detailed chemical and physical characterizations of our 12 oil/WAF preparation combinations, we also conducted additional chemistry-only studies. These studies included analysis of filtered and unfiltered samples that we collected from dilutions of a 1-g oil/L stock WAF over 96 hours. The nominal concentrations included in this detailed analytical matrix included 1%, 10%, and 100% WAF solutions (i.e., 99, 90, and 0% dilutions of the WAF stock). We also measured droplet size and concentration using a coulter counter (Beckman M4 Coulter Counter, Brea, CA). The physical properties of the four DWH oils were determined by Triton Analytics Corporation based on American Society for Testing and Materials (ASTM) standard methods.

For all chemistry data presented in this report, ALS Environmental (ALS), formerly Columbia Analytical Services (CAS; Kelso, WA), performed the chemical analyses (NOAA, 2014). The analysis of PAHs, alkyl PAH homologues, and related hetero-compounds were conducted using gas chromatography with low-resolution mass spectrometry using selected ion monitoring (GC/MS-SIM) based on U.S. Environmental Protection Agency (EPA) Method 8270D. The GC and MS operating conditions were optimized for separation and sensitivity of the target analytes. Alkyl PAH homologues were quantified using a response factor assigned from the parent PAH compound and the individual analyte; the target method detection limit for aqueous matrices ranged from 1 to 5 ng/L. The analysis of monocyclic aromatic hydrocarbons (i.e., BTEX) was conducted using GC/MS based on EPA Method 8260B with the individual analyte; the target method detection limit for aqueous matrices ranged from 0.05 to 0.5 µg/L. For both BTEX and PAH analyses, an initial calibration with a minimum of five concentration levels was run before every sequence, with continuing calibrations run every 12 hours. In addition, we ran a blank spike and a procedural blank after every 20 field samples; if there were fewer than 20 field samples, we instead ran one blank per batch. Finally, every sample contained spiked surrogates (for surrogate correction) and internal standards.

3. Results and Discussion

3.1 Chemical and Physical Characterization of the Four DWH Oil Samples

The purpose of using four different DWH oils in the toxicity testing program was to capture the broad range of oil compositions that GoM species may have encountered during and after the spill. The chemical analysis of the four DWH oils shows they ranged from the fresh Source oil to highly weathered slick oils. For instance, one of the more significant changes to occur as oil weathers is the preferential removal of the lighter hydrocarbons. This removal occurs as the result of several processes, including dissolution, evaporation, and biodegradation (Jordan and Payne, 1980; NRC, 2003, 2005). Figure 1 shows this characteristic progression in the chemical profiles of the Source oil, the Artificially Weathered Source oil, Slick A oil, and Slick B oil. This progression reveals not only an overall loss of BTEX and PAH compounds in the oil, but also a preferential loss of the lighter analytes (i.e., naphthalenes), with a compositional shift to the heavier compounds.

Furthermore, the extent that an oil sample has weathered in the environment is often represented by total mass loss or the total mass loss of a particular, preferentially removed fraction (i.e., PAHs) compared to the unweathered Source oil. The percent PAH depletion in the Artificially Weathered Source, Slick A, and Slick B oils relative to the Source oil is 27%, 68%, and 85%, respectively. To calculate percent depletion, we compared TPAH and hopane concentrations measured for the three weathered oils to TPAH and hopane concentrations averaged from over 600 GC/MS measurements of the Source oil taken during various DWH Trustee activities (Stout, 2015). In comparison, Aeppli et al. (2012) reported a range of 35–78% depletion of TPAHs for surface slick oils that they collected in the northern GoM from May to June 2010. These percent depletion calculations were conducted relative to a MC252 Source oil sample that was collected independently of the NRDA Source oil collection (Reddy et al., 2011). Additionally, various NRDA sampling efforts measured a range of 11–96% PAH depletion in floating oils collected from May to late August 2010 (Stout, 2015). Overall, these results show that the range of weathering states of the four oils used for the NRDA DWH aquatic toxicity testing program roughly encompass the range of weathering states for surface slicks and floating oils that other researchers observed in the field.

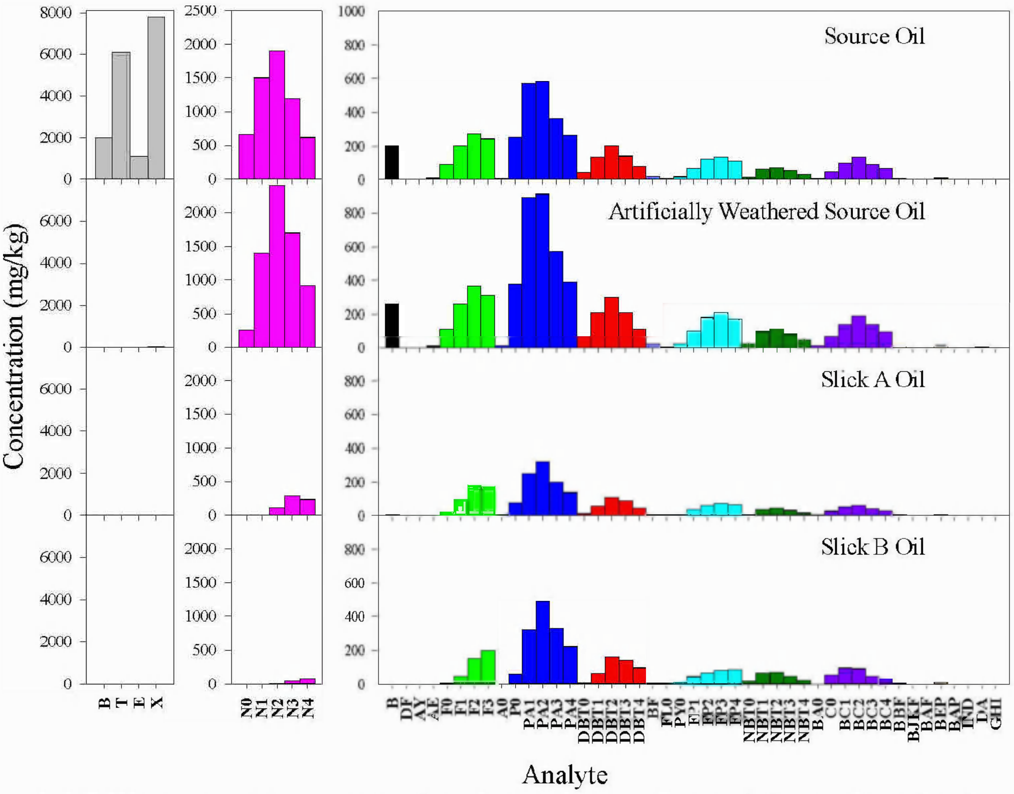

Figure 1. BTEX and PAH concentrations in the four DWH toxicity testing oils. See Supplemental Information, Table S.2, at the end of this report, for definitions of analyte abbreviations.

In addition to differences in the chemical composition of the four oils, the physical properties of these oils also reflect differences in their degree of weathering. For instance, it is expected that the specific gravity, pour point, viscosity, and density all increase as the degree of weathering increases (Jordan and Payne, 1980; Neff and Anderson, 1981; NRC, 2003, 2005). Table 1 shows the relative degree of weathering for the four oils is as follows: Source oil < Artificially Weathered Source oil < Slick A oil < Slick B oil. In addition, the two field-collected slick oils show evidence of substantial water-in-oil emulsification (mousse formation), with sediment and water fractions of 50% and 25% for Slick A and Slick B oil, respectively. Ultimately, the physical properties of an oil sample affect how the oil interacts with both water and dispersants; this fact not only influences the final chemistry of WAF preparations (Girling, 1989; Blenkinsopp et al., 1996), but also has important implications for how and where the oil moves through the environment (MacKay and Leinonen, 1977; Delvigne and Sweeney, 1988; French-McCay, 2004). For the full set of results from the chemical characterization of the four DWH oils, see Table S.1 in the *Supplemental Information* section.

Table 1. Physical characteristics and chemical properties of field-collected and Artificially Weathered oil

		Oil sample			
	Units	**Source**	**Artificially Weathered Source**	**Slick A**	**Slick B**
Gravity/pour point					
API gravity at 60°F (16°C)	API°	36	25	12.6	9.7
Specific gravity at 60°F (16°C)		0.8449	0.9041	0.9819	1.0025
Pour point	°F	-55	55	85	95
Kinematic viscosity					
at 86°F (30°C)	mm^2/s	4.714	53.16	6,387	91,658[a]
at 104°F (40°C)	mm^2/s	3.764	33.22	1,565	24,489
Density					
Density at 60°F (16°C)	g/cm^3	0.8440	0.9032	0.9809	1.0015
Density at 86°F (30°C)	g/cm^3	0.8335	0.8934	0.9719	0.9926
Composition					
Sediment and water	LV%[b]	< 0.025	0.05	50	25
Sulfur	% wt	0.262	0.415	0.222	0.188
TPAH50	mg/kg	10,666	13,808	3,061	3,257
C30-hopane	mg/kg	60	99	50	110
TPAH50 depletion	%[c]	-	27	68	85

a. This is an extrapolated value from an upper instrumental detection limit of 30,000 mm^2/s.
b. % liquid volume.
c. % TPAH depletion relative to hopane.

3.2 Concentration of Total PAHs in WAF Preparations

Crude oil is a complex mixture with only a fraction of those compounds amenable to current analytical techniques. Consequently, the oil spill community often represents oil contamination by the concentrations of only a particular subset of hydrocarbons in the oil. Out of the several major hydrocarbon groups found in oil, scientists generally consider the aromatic hydrocarbon group (i.e., BTEX and PAH compounds) as one of the most important drivers of oil toxicity (Neff and Anderson, 1981; NRC, 2003; Boehm and Page, 2007). In particular, PAHs are typically more persistent in the environment following an oil spill than their monocyclic counterparts. Because of this, researchers often characterize oil contamination and exposure in terms of individual PAH concentrations, a sum of those individual PAH concentrations, or TPAH (French-McCay and Payne, 2001).

The available list of target analytes to include in the TPAH sum has changed over time as analytical techniques have improved, and additional analytes are more commonly measured. EPA has listed 16 parent PAH compounds on their priority pollutant list (40 CFR Part 423), which many early studies and monitoring programs used to define TPAH values. In 1991, NOAA introduced a list of 24 PAHs (19 parent + 5 alkyl homologs) for their monitoring programs (NOAA, 1991). EPA later introduced a more comprehensive set of 34 PAHs (18 parent + 16 alkyl homologs), which the agency used in its Environmental Monitoring and Assessment Program (Mount et al., 2003), and suggested that as the minimum list to use in establishing equilibrium partitioning sediment benchmarks for PAHs (U.S. EPA, 2003). Many other agencies and researchers have adopted this list of 34 PAHs. In addition to those mentioned above, various independent studies have presented countless other TPAH versions. For the DWH NRDA toxicity testing program, researchers are using a list of 50 analytes (23 parent + 27 alkyl homologs) to calculate TPAH concentrations (hereafter TPAH50). TPAH50 includes all PAH and related hetero-polycyclic compounds that the analytical laboratories reported as part of the standard PAH analysis performed for the DWH NRDA (EPA Method 8270D). The TPAH50 list, along with the other historical PAH lists, are provided in Table S.3 in the *Supplemental Information* section.

Table 2 presents the average TPAH50 concentration for the 12 oil/WAF preparations, each prepared with a nominal oil concentration of 1 g oil/L. Not surprisingly, the PAH concentration directly correlates to the WAF method's energy regime, with HEWAFs producing the highest TPAH50 concentrations and LEWAFs producing the lowest TPAH50 concentrations. In addition to the energy regime, the degree of weathering of the oil used to produce the WAF also strongly influences the WAF TPAH50 concentrations. Between these two variables, the average TPAH50 concentration of a 1 g oil/L stock WAF for the 12 WAF/oil preparations ranged from 4 µg/L to almost 7,000 µg/L.

Table 2. TPAH50 of the three different WAF preparations prepared with the four different DWH toxicity testing oils at a nominal oil loading concentration of 1 g oil/L

WAF preparation	Oil type	Average TPAH50 (µg/L)	1 SD (µg/L)	RSD (%)	Total replicates	Total laboratories[a]
HEWAF	Source	6,006	1,028	17	10	3
	Artificially Weathered Source	6,836	2,454	36	14	5
	Slick A	2,373	422	18	155	5
	Slick B	263	33	13	6	3
CEWAF	Source	1,981	2,855	144	8	3
	Artificially Weathered Source	3,685	1,607	44	9	3
	Slick A	303	202	67	101	6
	Slick B	37	16	44	11	3

Table 2. TPAH50 of the three different WAF preparations prepared with the four different DWH toxicity testing oils at a nominal oil loading concentration of 1 g oil/L

WAF preparation	Oil type	Average TPAH50 (µg/L)	1 SD (µg/L)	RSD (%)	Total replicates	Total laboratories[a]
LEWAF	Source	196	38	19	4	2
	Artificially Weathered Source	167	29	17	3	2
	Slick A	10	10	100	3	2
	Slick B	4	2	59	3	1

RSD = relative standard deviation.

a. Indicates the number of different laboratories represented in the average and standard deviation.

More notably, we saw substantial variability from preparation to preparation, despite our use of the same mixing method and oil type. For example, the data in Table 2 demonstrate that the four most reproducible of the 12 WAF preparations (i.e., the WAF preparation with the smallest RSDs) had RSDs of around 15%, with the Source oil CEWAF preparation showing deviations as high as 144%. Interestingly, these data indicate that the HEWAF preparations were generally as consistent as the LEWAF preparations, with the CEWAF preparation showing considerably more variability with all oils used. Similarly, Gardiner et al. (2013) observed greater variation in their CEWAF preparations (67% RSD, n = 12) compared to their LEWAF preparations (33% RSD, n = 11). In addition, Faksness et al. (2008) reported an RSD of approximately 17% and Hansen et al. (2011) reported an RSD of slightly more than 8% for LEWAFs prepared with different crude oils. The slightly higher deviations we observed compared to other researchers likely reflected the variation across different preparations, as well as variation caused by different mixing vessel sizes, laboratories, technicians, water sources, and temperatures used to produce the WAFs. Overall, the variability we observed across different WAF preparation methods, as well as across WAFs prepared using the same method and same oil, demonstrate the inadequacy of reporting bioassay results in terms of nominal WAF concentrations and highlights the importance of analyzing actual exposure media to the greatest extent possible, a conclusion shared by many previous researchers in the field (NRC, 2003; Aurand and Coelho, 2005).

3.3 Chemical Composition of the WAF Preparations

As described above, the NRDA toxicity testing program included four different DWH oils and three different WAF preparation methods, producing a matrix of 12 distinct WAF/oil preparations with a wide variety of PAH compositions. Comparing the chemical profile of the 12 WAF/oil preparations illustrates that the composition of the oil used to produce the WAF, as well as the mixing energy, influenced the WAF chemistry (Figure 2). For example, both the

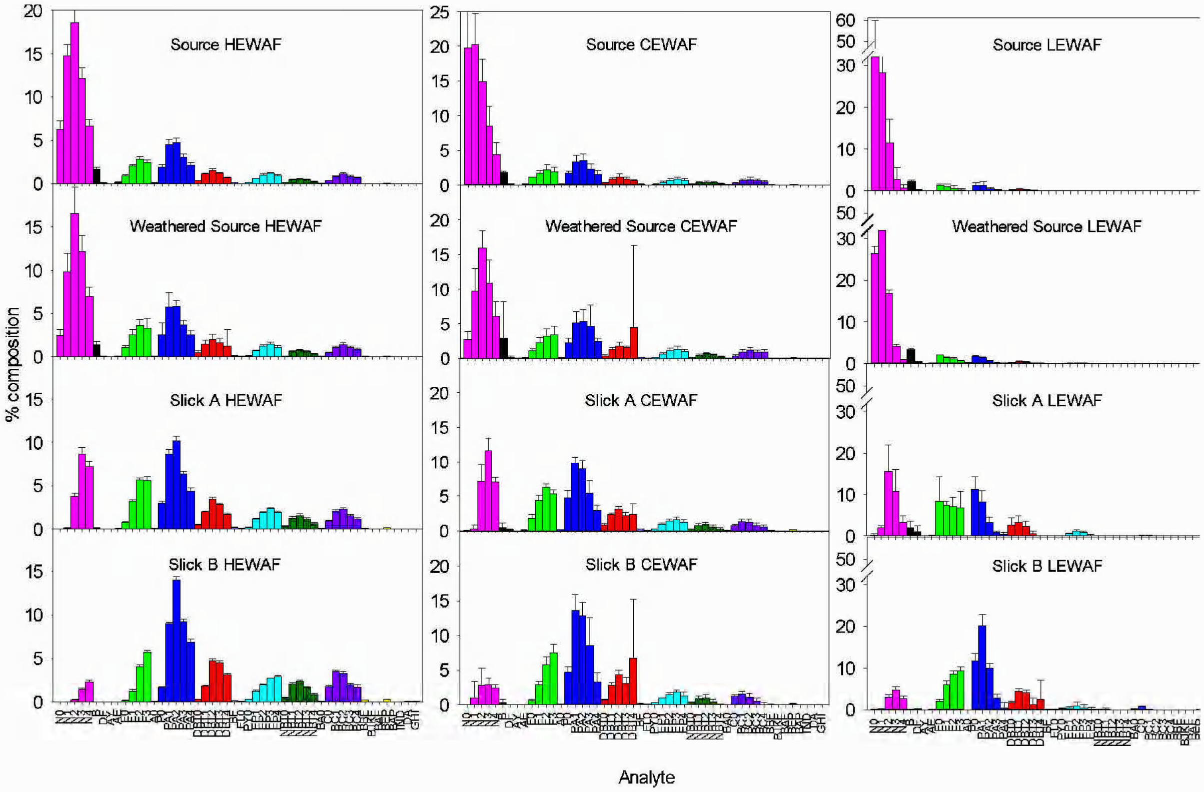

Figure 2. Mean percent composition (+ SD) of HEWAFs, CEWAFs, and LEWAFs prepared with the four different DWH toxicity testing oils.

HEWAF and the CEWAF chemical composition generally mirrored the composition of the starting oil with which the WAF was prepared. This characteristic is indicative of a WAF dominated by particulate oil (Singer et al., 2000; Payne and Driskell, 2003), which correlates with the higher mixing energies used to disperse particulate oil into the HEWAF and CEWAF preparations. Comparing the TPAH concentrations of these two WAF preparation methods reveals the HEWAF preparations, with a much greater mixing energy, dispersed the oil to a much greater extent than the CEWAF preparations, which used a considerably lower mixing energy, but with a much longer contact time with the bulk oil. Consequently, the chemical compositions of the CEWAF preparations showed a slight enrichment of the naphthalenes (the most soluble PAHs) compared to the corresponding HEWAF (Figure 2). As further discussed below, the oil droplets represented a large fraction of the PAH concentration in the final stock WAF, explaining why the PAH composition of both the HEWAF and CEWAF preparations closely resembled the chemical profile of the oil used to produce the WAFs. In contrast, the mixing energy of the LEWAF method specifically minimized the generation of particulate oil (Aurand and Coelho, 2005). Consequently, LEWAF chemical compositions were highly enriched in the more soluble PAHs and were generally devoid of the less-soluble PAHs. Overall, chemical profiles of the three WAF preparations reflect different potential exposure scenarios; one dominated by physically dispersed oil, a second where dispersant and chemically dispersed oil coexist, and a third that consists primarily of soluble oil components.

In addition to the oil, the chemical components that make up the dispersant Corexit® 9500 also contributed to the chemical profile of all CEWAF preparations. While Corexit® 9500 is a mixture of many different surfactants and hydrocarbon-based solvents, our chemical characterization of dispersant in our CEWAF preparations was typically limited to only a few components. One component in particular, dioctyl sodium sulfosuccinate, or DOSS, was often the only chemical used to identify and quantify the presence of the dispersant in the field (Kujawinski et al., 2011; Payne and Driskell, 2015) and in toxicity tests for the DWH NRDA (Morris et al., 2015).

3.4 Oil-to-Water Mixing Ratio

Although an exact oil-to-water mixing ratio was not specified in the WAF preparation protocols for the DWH NRDA toxicity testing program, researchers typically prepared their stock WAFs with no more than 1–2 g oil/L. The reason for this is that there was little appreciable increase in the measured PAH concentration of the stock WAF as the nominal oil-to-water mixing ratio increased above 1 g oil/L (Figure 3). Although highly variable, this general trend was observed across the different WAF preparation methods, and for WAFs prepared with oils of considerably different weathering states (e.g., Source versus Slick A oil). Shiu et al. (1990) observed a similar leveling off in hydrocarbon concentrations when investigating water soluble fractions of various crude oils and petroleum products, which they attributed to the solubility behavior of the oil mixture. The hydrocarbon concentrations in the HEWAF and CEWAF preparations are

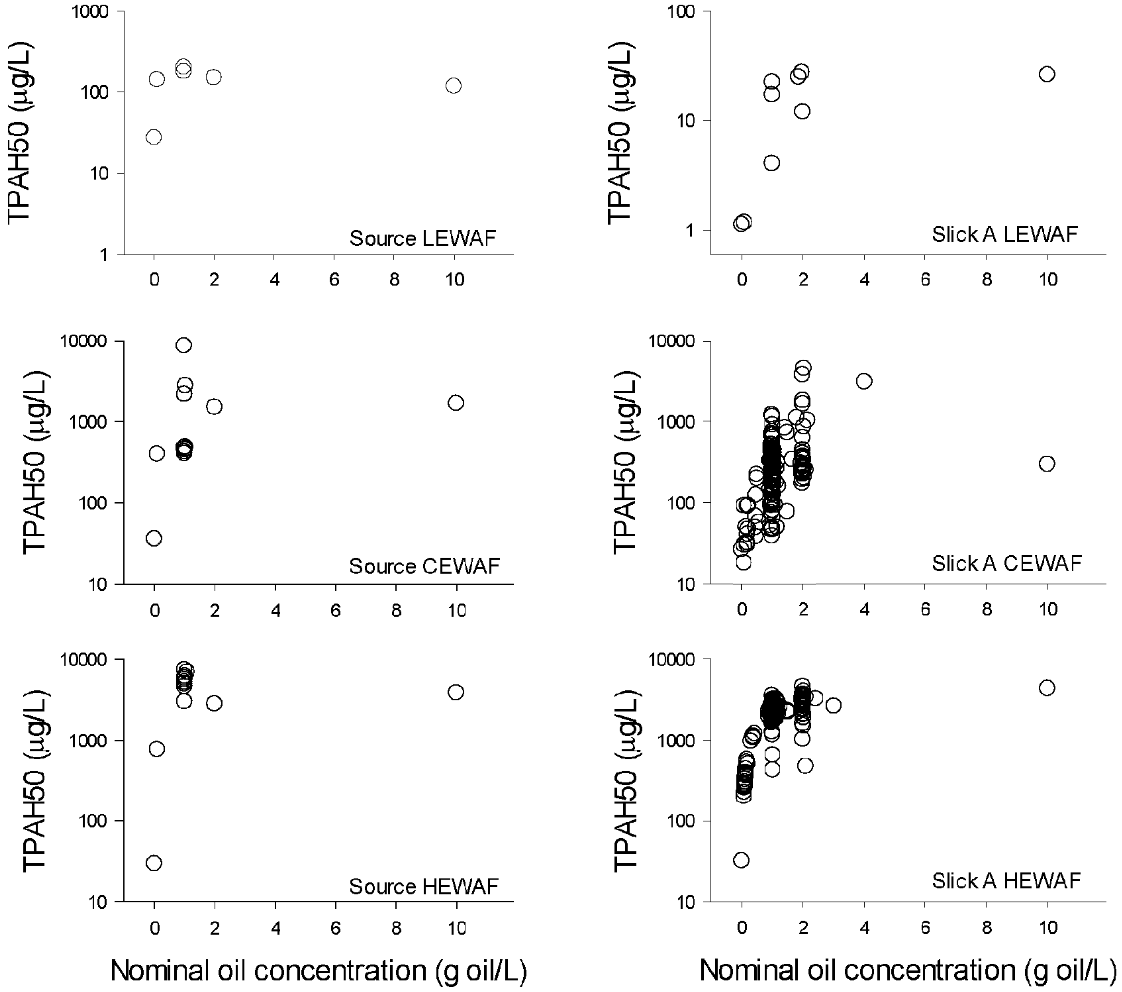

Figure 3. TPAH50 (µg/L) versus nominal oil concentration (g oil/L) for HEWAFs, CEWAFs, and LEWAFs prepared with Source and Slick A oils.

determined primarily by the concentration of the dispersed particulate oil, and thus should not be limited by the solubility of the oil constituents; however, a similar plateau in these WAFs was observed. Previous research in the area of emulsion formation and stability has shown that as the fraction of the dispersed phase increases, so does the coalescence rate of the dispersed particulates; this is because of an increase in the frequency of droplet-droplet interactions (Danner and Schubert, 2001). This in turn leads to enhanced removal of the dispersed phase because of faster rising of the larger droplets. Although a number of parameters can affect droplet formation and coalescence processes, this basic relationship between the dispersed phase volume fraction and coalescence rate can in part explain why the particulate-dominated HEWAFs and CEWAFs also exhibit an apparent saturation effect with regards to WAF hydrocarbon concentration.

For the LEWAF preparation, many researchers have found that the oil-to-water mixing ratio influences not only the LEWAF concentration, but can also change the LEWAF chemical composition (Shiu et al., 1990; Girling et al., 1994; Blenkinsopp et al., 1996; Singer et al., 2000; Aurand and Coelho, 2005). Specifically, these researchers have found that as oil-to-water mixing ratios increase, so do the relative contributions of the more soluble compounds. In comparing across different oils, Shiu et al. (1990) found that these compositional changes are more pronounced for the more soluble compounds, and that the relative concentrations of the less-soluble compounds, such as the higher molecular weight PAHs, were less affected by changes in the oil-to-water mixing ratio. Similarly, our results for both Source oil and Slick A LEWAFs showed a shift in PAH composition, with changing oil-to-water mixing ratios, with the biggest changes occurring with the lighter, more soluble PAH fractions (Figure 4).

Alternatively, chemical compositions of the HEWAF and CEWAF preparations may be less affected by changes in the oil-to-water ratio because of the presence of particulate oil (Singer et al., 2000). For the Slick A HEWAF and CEWAF preparations, our results corroborate these previous findings, showing very little change in chemical composition across the oil-to-water mixing ratios tested (Figure 4). However, our Source oil HEWAF and CEWAF preparations show a distinct shift in chemical composition as the oil-to-water mixing ratios change. The observed shifts for both the Source oil HEWAF and CEWAF preparations were similar in trend to the shifts observed in the LEWAF preparations with the contribution of the more soluble components increasing as the oil-to-water ratio increased. This suggests that like LEWAF preparations, chemical compositions of the Source oil HEWAF and CEWAF preparations are not just determined by the particulate phase, but are also influenced by the solubility behavior of oil constituents. Data across a range of oil-to-water mixing ratios were not available for WAFs prepared with Artificially Weathered Source and Slick B oils, and thus we were only able to conduct these analyses on Source and Slick A oils.

Ultimately, observed changes in chemical composition for all WAF preparations are generally more pronounced at the lowest oil-to-water mixing ratios tested (0.01–0.1 g oil/L) and when oil-to-water mixing ratios differ substantially. Consequently, for many of the toxicity tests in the DWH NRDA program for which the stock WAF was prepared using a relatively narrow range of mixing ratios (typically 1–2 g oil/L), any shifts in chemical composition would be much less noticeable across different WAF preparations.

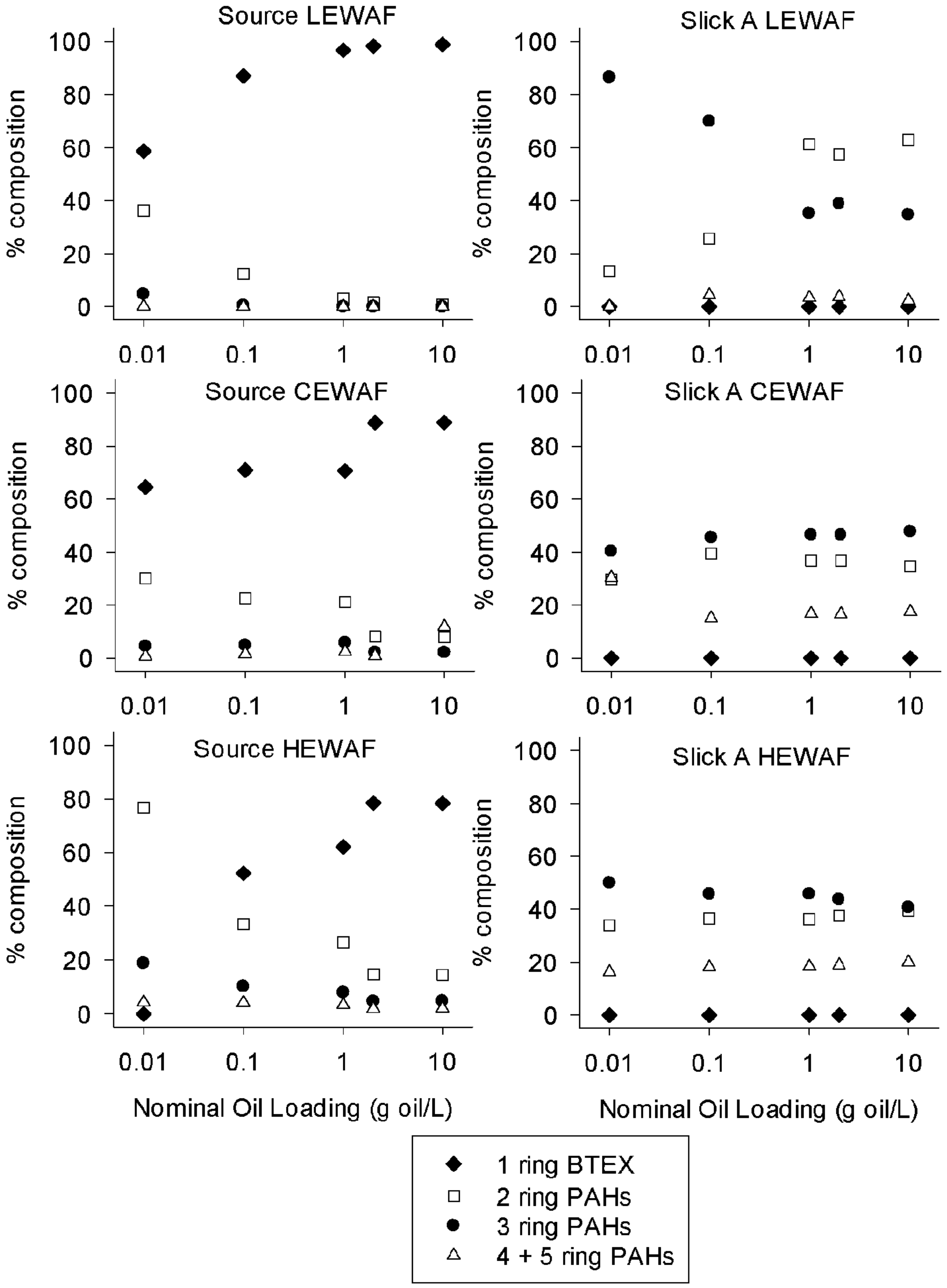

Figure 4. Percent composition of 1-ring BTEX analytes and 2-ring, 3-ring, and 4 + 5-ring PAH analytes versus nominal oil loading (g oil/L) for Source oil and Slick A LEWAFs, CEWAFs, and HEWAFs.

3.5 Preparation of WAF Treatments

For many DWH NRDA toxicity tests, researchers prepared WAF treatments by diluting from a single, high-concentration WAF to different concentrations. This is in contrast to producing each treatment from separately prepared WAFs using different oil-to-water mixing ratios, which CROSERF researchers and others have recommended in the past (Girling et al., 1994; Blenkinsopp et al., 1996; Singer et al., 2000; Aurand and Coelho, 2005). In recent discussions, many researchers have suggested that dilution from a high-concentration WAF may be more appropriate for generating treatments in a toxicity test (Barron and Ka'aihue, 2003; Boehm and Page, 2007; Landrum et al., 2012). The crux of their argument is that different oil-to-water mixing ratios produce WAFs with different concentrations, as well as different chemical compositions (as discussed above), which can complicate interpretation of the dose-response relationship. Furthermore, as Figure 5 demonstrates, dilution from a high-concentration WAF can reproducibly generate treatments in a specific TPAH50 concentration range with the highest uncertainty coming from the TPAH50 concentration of the initial stock WAF. However, treatments prepared from separate WAFs each have their own high uncertainty, as demonstrated by the high RSDs shown in Table 2, which can present challenges when trying to target a narrow range of treatment concentrations. Figure 5 also illustrates that in the event that a sample for a particular treatment is lost or there was an error in the analysis, the initial concentration of treatments prepared by dilution of a WAF can be reliably inferred from the measured concentration of the stock WAF or other surrounding treatments. This would not be the case for treatments produced from separately prepared WAFs. Furthermore, in cases where analytical costs become limiting, estimation of treatment concentrations that have been diluted from a single, measured stock solution has been previously recommended (Barron and Ka'aihue, 2003), and Figure 5 indicates this could be a reasonable alternative. Ultimately, dilution from a high-concentration stock WAF was more appropriate for many of our toxicity tests, where we needed to target a narrow range of TPAH50 concentrations at low µg/L levels.

3.6 WAF Dissolved Fraction

Crude oil is made up of potentially hundreds of thousands of compounds. How these compounds partition into water is a reflection of their solubility, with monocyclic hydrocarbons and lower-molecular-weight PAHs dissolving preferentially over the less-soluble compounds, such as the higher-molecular-weight PAHs and aliphatic hydrocarbons (Jordan and Payne, 1980; NRC, 2003, 2005). The partitioning of oil components between the dissolved and particulate phases, including suspended sediment, live and dead cells, and dispersed oil droplets, influences their bioavailability, which can ultimately affect the oil's toxicity. For example, truly dissolved compounds are generally considered more bioavailable (i.e., more accessible to biological receptors) than particulate forms, with a number of studies demonstrating that the dissolved fraction is the primary driver of oil toxicity (Carls et al., 2008; McGrath and DiToro, 2009;

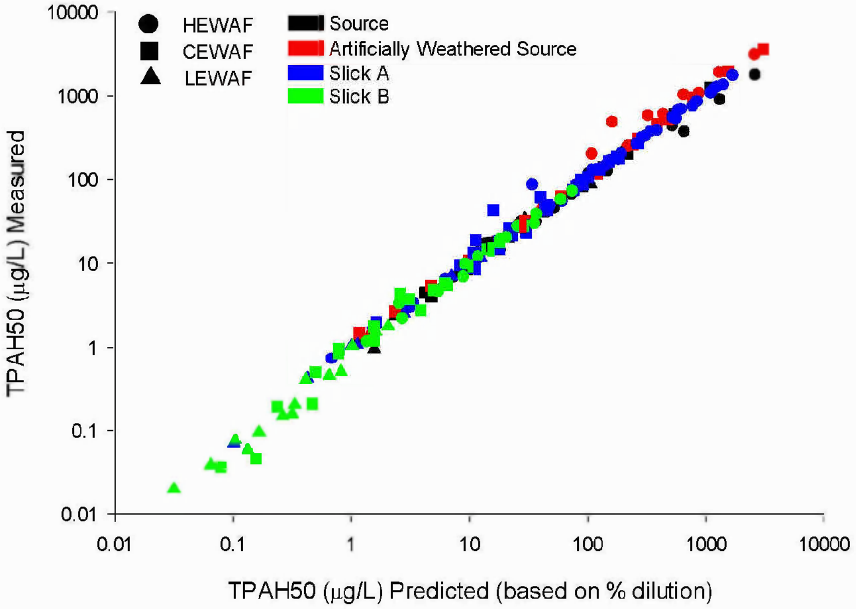

Figure 5. Measured TPAH50 versus predicted (i.e., nominal) TPAH50, based on measured stock TPAH50 and percent dilution of treatment. Examples from each of the 12 oil/WAF preparations are included. Total number of samples = 110, total number of laboratories = 9, total number of tests = 30.

Nordtug et al., 2011). On the other hand, other studies have shown that for various routes of exposure, such as physical fouling and ingestion, particulate oil can be the main contributor to the oil's uptake, and thus is the primary fraction of oil interacting with biological receptors (Payne and Driskell, 2003; NRC, 2005). Ultimately, characterizing the dissolved and particulate phases of a toxicity testing media is important for understanding and defining the nature of the exposure, and subsequent effects from both physically and chemically dispersed oil.

Above, we demonstrated that different WAF preparations produced considerably different unfiltered TPAH50 concentrations. Not surprisingly, TPAH concentrations were directly related to the energy regime of the WAF method and inversely related to the degree of weathering of the starting oil. Similarly, when looking at just the dissolved fraction of the WAF concentration, there is an inverse relationship between the dissolved TPAH50 concentration and the degree of weathering of the starting oil (Table 3). However, energy regime does not appear to affect the dissolved concentration in the WAF. For example, Table 3 shows no substantial difference in the dissolved (i.e., filtered) TPAH50 concentrations of a 1 g/L stock HEWAF, CEWAF, or LEWAF prepared with the same oil (except for Slick B, which is discussed below). Furthermore, the chemical profiles of the dissolved fraction across these three WAF preparation methods are nearly identical when comparing the same oil (Supplemental Figures S.1 to S.4). Interestingly,

Table 3. Filtered ("dissolved") TPAH50 concentrations of the three different WAF preparations prepared with the four different toxicity testing oils at a nominal oil loading of 1 g oil/L sea water

Oil type	WAF preparation	Average filtered TPAH50 (µg/L)	SD	Total number	Number laboratories	% dissolved
Source	HEWAF	213	41	5	2	4
	CEWAF	250	43	5	2	13
	LEWAF	193	33	3	2	99
Artificially Weathered Source	HEWAF	156	36	5	2	2
	CEWAF	175	60	5	2	5
	LEWAF	153	12	2	1	91
Slick A	HEWAF	23	6	8	3	1
	CEWAF	20	12	8	3	7
	LEWAF	16	6	4	2	100[a]
Slick B	HEWAF	7	1	4	2	3
	CEWAF	5	2	5	2	13
	LEWAF	3	1	2	1	81

a. Occasionally the measured dissolved (i.e., filtered) concentrations were higher than the corresponding total (i.e., unfiltered) concentrations for a sample as a result of analytical variability. If this occurred, we rounded down to 100%.

these data show that despite the differences in mixing energy among these three methods, the mixing method does not considerably affect the final dissolved concentration or composition of a stock WAF when prepared with the same oil and oil-to-water mixing ratio. These results suggest equilibrium is being reached between the oil and the water irrespective of whether the oil is physically dispersed as small droplets by a high mixing energy, chemically dispersed using a medium mixing energy, or mixed slowly to minimize dispersion of oil droplets. For the CEWAF and LEWAF preparations, this is not surprising, given that those methods were both designed with at least 21 hours of contact time between oil and water so that equilibrium could be reached (Singer et al., 2000). However, the HEWAF method provides only one hour of contact time, yet given the similar dissolved concentrations between the HEWAF preparations and the other two WAF preparations, equilibrium does appear to have been reached in this short time. Likely this is because the oil is sufficiently entrained and the oil droplets are small enough in size such that diffusion control in the oil phase is no longer a factor.

One exception to the above finding is Slick B oil, which shows a small, but notable difference between the filtered TPAH50 concentrations of the HEWAF preparation compared to the CEWAF and LEWAF preparations. This is likely a result of the thick and viscous nature of Slick B oil, which not only reduces diffusion rates, but also prevents the oil from spreading on the water's surface. Instead, the oil remains concentrated in the center as a thick, bulk-oil globule, increasing the diffusion path length of PAH analytes out of the bulk oil and limiting the partitioning of the oil components out of the bulk oil.

Note that WAFs prepared with oil-to-water ratios higher than 1 g oil/L showed a considerable reduction in the mass entrainment rates (Figure 3). This change in entrainment would likely affect the rate at which the WAF would come to equilibrium. Thus, the similarities seen in Table 3 may not translate to WAFs prepared with higher oil-to-water mixing ratios. For the toxicity tests performed as part of the DWH NRDA, this issue is minor given that these toxicity tests were for the most part conducted using stock WAFs prepared with 1–2 g oil/L oil-to-water.

The concentration and composition of the dissolved fraction in the stock WAF appear to be similar across mixing methods, given a certain oil-to-water mixing ratio; however, when the stock WAF is diluted to make subsequent treatments, the concentration and composition do not remain the same across WAF preparation methods. This divergence is a consequence of the HEWAF, CEWAF, and LEWAF preparations containing different fractions of particulate oil from which additional oil components partition out of and into the dissolved phase as the WAF is diluted. For instance, Figure 6 shows that for a 100% Slick A HEWAF, only 1% of the unfiltered TPAH50 was in the dissolved phase; however, when that HEWAF was diluted to 0.1% WAF (which is about 2 µg/L unfiltered TPAH50), the dissolved concentration represents as much as 92% of the unfiltered TPAH50 concentration. The concentration of the dissolved phase TPAH50 does not change proportionally with dilution. Instead, the fraction of the unfiltered TPAH50 that is in the dissolved phase increases as the WAF is diluted, which indicates that additional oil components from the particulate phase are continuing to partition into the dissolved phase as the WAF is diluted. All HEWAF and CEWAF preparations show similar trends. This is the result of differential partitioning of PAH analytes, with lower-molecular-weight PAHs partitioning faster and to a greater extent than the higher-molecular-weight PAHs (French-McCay, 2004; Redman et al., 2012). On the other hand, the dissolved fraction of the LEWAF preparations, being nearly 100% dissolved to begin with, change proportionally with dilution.

3.7 Oil Droplet Size Distributions

Both the HEWAF and the CEWAF preparations generate dispersed oil droplets, which, as shown in Table 3, dominate the hydrocarbon concentrations measured in a stock WAF. Figure 7 shows the droplet size distributions of the particulate phase of 1 g oil/L stock HEWAF and CEWAF preparations for all four DWH oils. The LEWAF preparations all demonstrated droplet distributions and concentrations near background levels for the coulter counter, and thus the particulate phase in the LEWAF preparations was considered minimal. In general, the droplet sizes in all eight HEWAF and CEWAF preparations were generally less than 30 µm, with volume mean diameters ranging from 5 to 10 µm (Figure 8). However, within this narrow range,

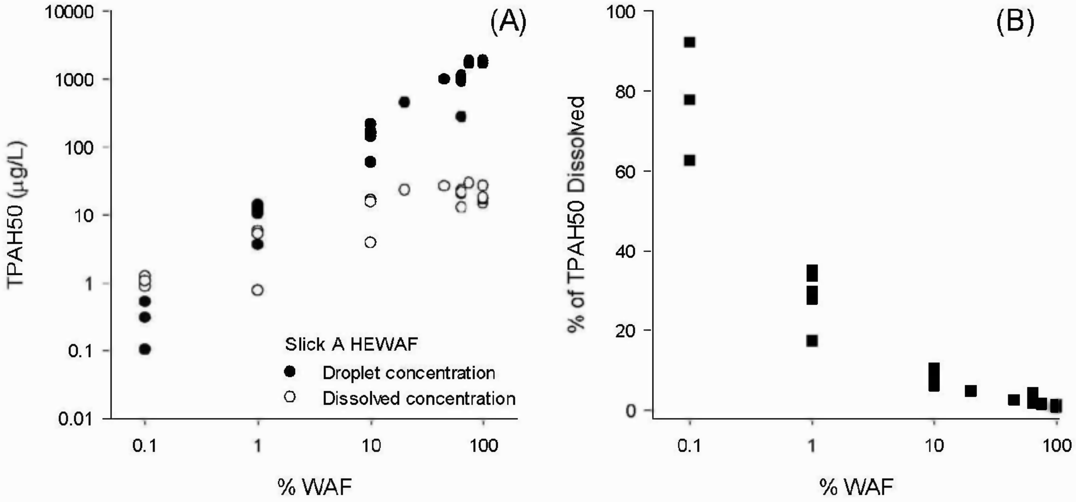

Figure 6. TPAH50 concentration of dissolved (i.e., filtered) and droplet (i.e., unfiltered minus filtered) phases of the Slick A HEWAF preparation across different dilutions of 1 g oil/L stock WAFs (A); and the corresponding percent dissolved for those different dilutions (B).

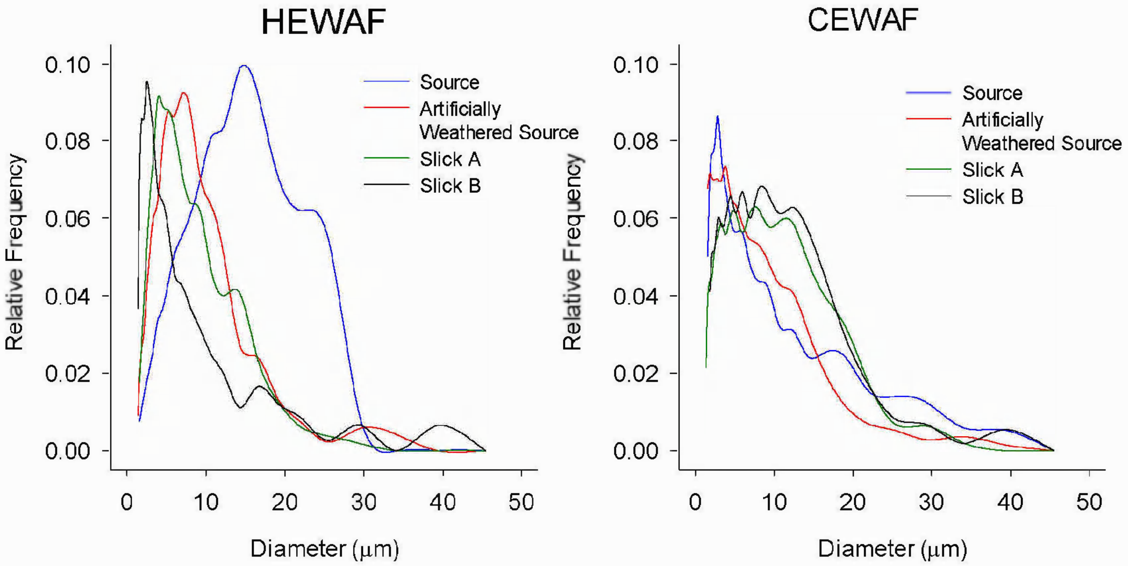

Figure 7. Oil droplet size distributions for HEWAFs and CEWAFs prepared with Source oil, Artificially Weathered Source oil, Slick A oil, and Slick B oil.

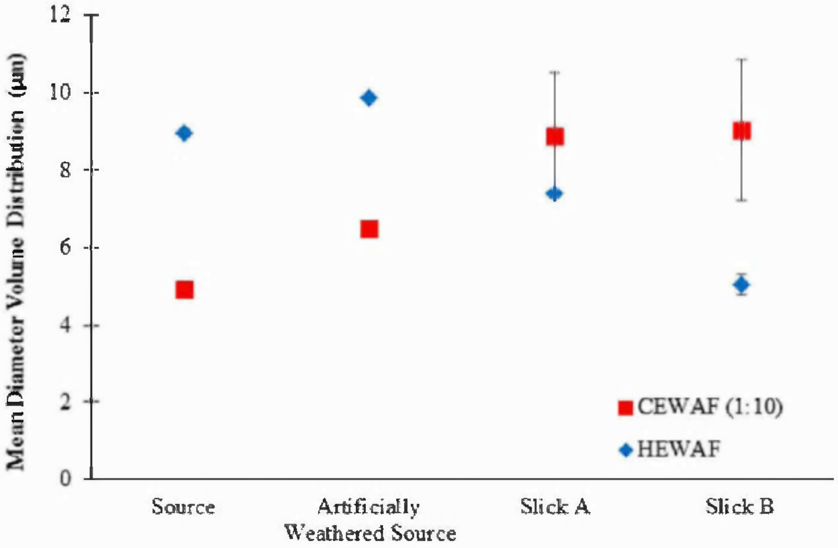

Figure 8. Volume mean oil droplet diameters (µm) of HEWAFs and CEWAFs prepared with Source oil, Artificially Weathered Source oil, Slick A oil, and Slick B oil.

there were differences in the mean droplet size across oil types (Figure 8). For instance, the mean droplet size in the HEWAF preparations decreased with more weathered oils. Alternatively, for the CEWAF preparations, we observed the opposite trend: the mean droplet size increased with less weathered oils. Additionally, we found that a Source oil HEWAF produced larger droplets than a Source oil CEWAF, while a Slick B HEWAF produced smaller droplets than a Slick B CEWAF.

In the field, Lunel (1995) found that 80–90% of the volume of dispersed oil droplets from oil slicks was less than 70 µm in diameter, with median droplet sizes ranging from 35 to 50 µm under the different oil slicks studied. This was true for both physically and chemically dispersed oil slicks made from different oils under a variety of wind conditions. Furthermore, theoretical calculations show that droplets greater than 50 to 70 µm will resurface, with only smaller droplet sizes remaining dispersed in the water column (NRC, 2005; North et al., 2011).

Similarly, in a toxicity testing exposure system, both physically and chemically dispersed oil droplets above a certain size will tend to settle out (i.e., rise to the surface) quickly, and thus not be part of the exposure. However, unlike field conditions, for a static toxicity exposure, energy input into the system is generally minimal and the water column depth of the exposure tank or dish is often relatively shallow. Consequently, after the initial mixing of treatments, there is little to no energy input to re-disperse droplets, and a relatively short distance for droplets to travel to leave the water column. Thus, as we observed, droplet sizes that are expected to remain dispersed in a laboratory setting are expected to be smaller than those in the field.

The rate at which a dispersed oil droplet settles is called its rise velocity, and can be calculated using the difference in densities between the oil droplet and the water (Lunel, 1995). Given the densities of the four DWH oils (see Table 1), the density of 30 ppt seawater at 30°C, and assuming most exposure tanks or dishes are no deeper than 30 cm, we estimated that droplets larger than approximately 20 μm will rise out of the water column within the first few hours, and only droplets of sizes below approximately 5 μm will remain dispersed for four or more days (the length of a typical acute toxicity test).

3.8 Changes in WAF Chemistry over Time

When reporting results of a toxicity test, researchers often define exposure using concentrations measured at the beginning of the test. However, over a typical acute test (24–96 hours), this convention may not accurately capture changes in concentration that occur throughout the study. For instance, many previous studies have reported decreases in TPAH concentrations over time (NRC, 2005). Similarly, we observed a decrease in the TPAH50 concentrations during many of our typical acute, static tests. We found that the changes in concentration were primarily associated with changes in the droplet phase, with the dissolved concentrations generally remaining steady across a full 96 hours for all WAF preparations (Figure 9). Consequently, for LEWAF preparations, which are dominated by the dissolved phase, we observed minimal change in TPAH50 concentrations with time (Figure 9). For HEWAF and CEWAF preparations, we observed that the TPAH50 concentration in the HEWAF preparation generally decreased more rapidly than the CEWAF preparation. Furthermore, TPAH50 concentrations did not decrease as rapidly in WAFs produced with more weathered oils compared to those produced with less weathered oils. Finally, we have observed that decreases in the unfiltered TPAH50 concentrations were most rapid in the first 24 hours and mostly level out after 48 hours. All of these trends have one common theme: the rate of change in the unfiltered TPAH50 concentration increases as the starting TPAH50 concentration increased. One explanation for this observed trend is that as the fraction of dispersed oil increases, so does the coalescence rate of the particulate oil; this is because of an increase in the frequency of droplet-droplet interactions. This in turn leads to the enhanced removal of the dispersed oil because of a faster rise velocity of the larger oil droplets. In CEWAFs, droplet coalescence is also affected by the presence of dispersant, which can form a surfactant mono-layer around the droplets, thus reducing the potential for coalescence (Danner and Schubert, 2001). Reduced coalescence rates will tend to decrease the rise rates of the dispersed phase, as smaller droplets resist coalescing into larger droplets. Ultimately the TPAH50 concentration of CEWAFs demonstrated considerably greater stability than HEWAFs, which could be the combined result of lower starting concentrations (i.e., CEWAFs generally had lower dispersion rates than the corresponding HEWAF preparation) and reduced coalescence rates because of the presence of dispersants that served to stabilize the particulate phase.

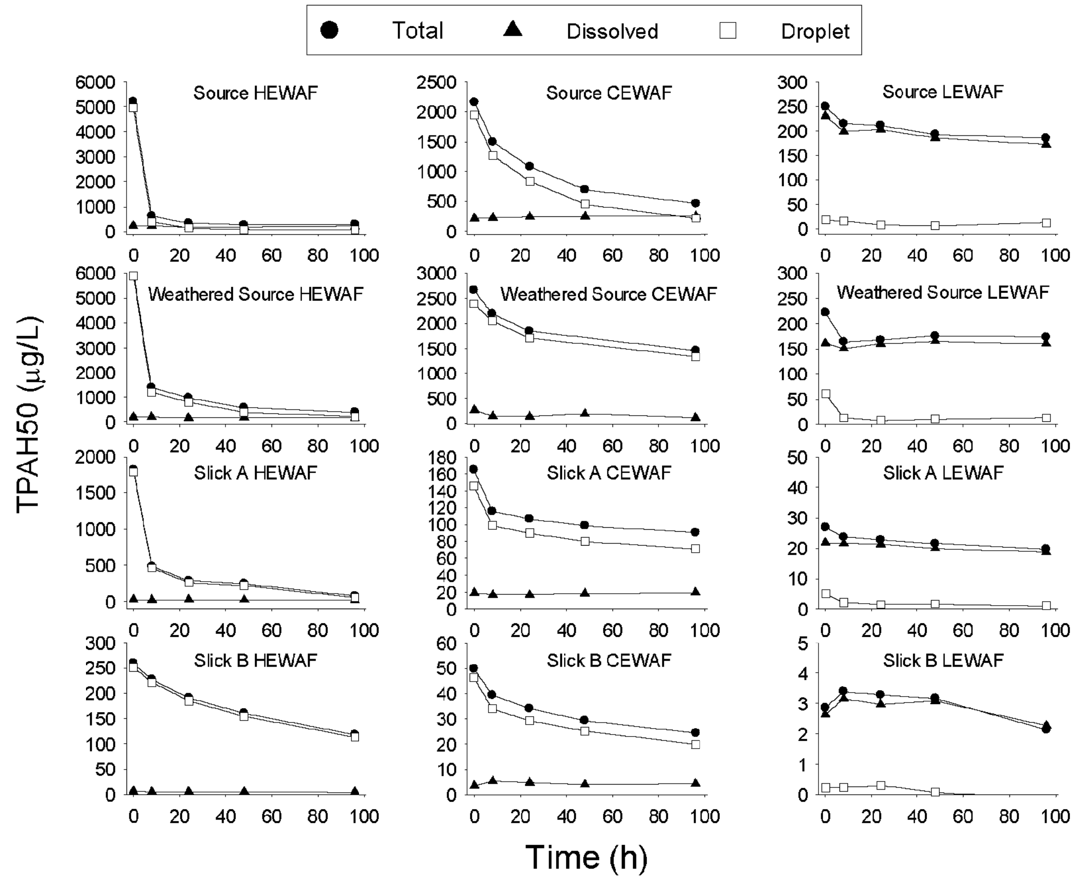

Figure 9. Total (unfiltered), dissolved (filtered), and droplet (unfiltered minus filtered) TPAH50 concentrations (µg/L) for 1 g oil/L stock WAFs over 96 hours in static test conditions.

3.9 Laboratory-Prepared WAFs versus Field-Sample Chemistry

Finally, to compare PAH compositions of the various WAFs to potential exposures in the field, we examined the chemical composition of water samples collected from the field during and immediately after the DWH oil spill (Figure 10). We obtained the field-collected water sample data for this analysis from the DIVER website (DIVER, 2015), downloaded on March 6, 2015. For the purposes of this analysis, we only included whole water PAH samples collected from 0–20-m depth in 2010 with TPAH50 concentrations equal to or greater than 0.5 µg/L, because this concentration represents the lower spectrum of exposure concentrations sufficient to cause adverse effects to sensitive biota (Morris et al., 2015). For a more detailed description of the data included this analysis, see the *Supplemental Information* section.

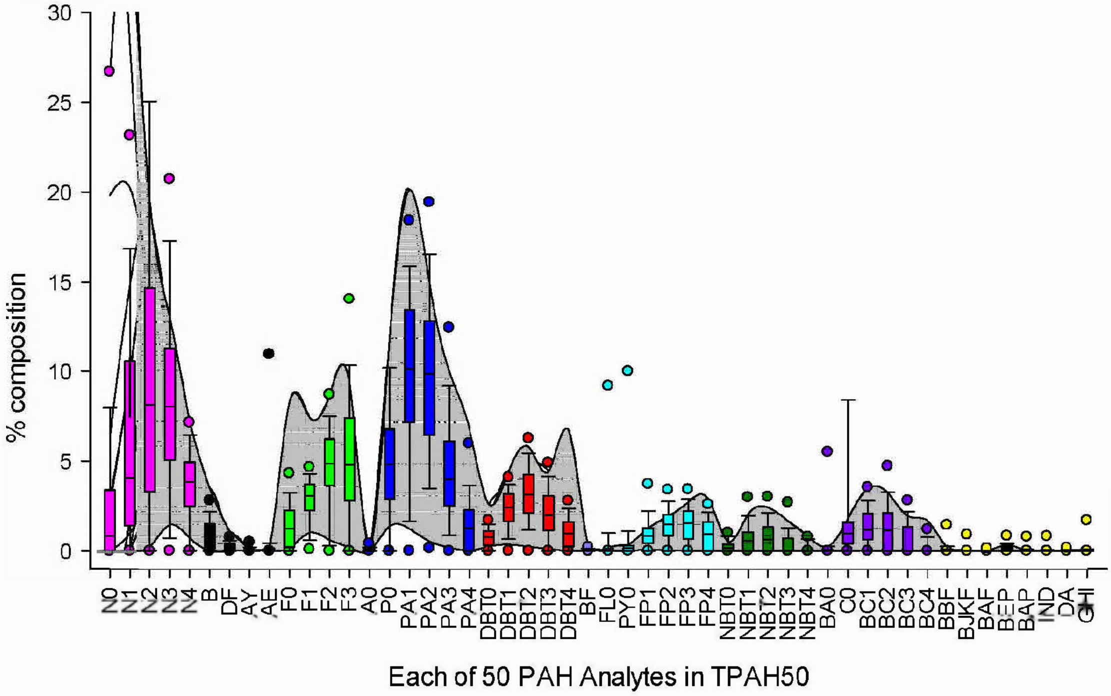

Figure 10. Summary box and whisker plot of PAH chemical profiles for water samples collected from the GoM (0–20 m; TPAH ≥ 0.5 µg/L) during and immediately after spill (n = 394 total samples). The top and bottom of the box show the 25th and 75th percentiles and the middle line dividing the box is the median. Whiskers represent 10th and 90th percentiles and dots represent 5th and 95th percentiles. Field-collected water sample data were obtained on March 6, 2015, from the DIVER website (DIVER, 2015). For comparison, thin grey lines behind the box and whisker plot show the average chemical profiles of the 12 DWH NRDA WAF preparations, with the area between the highest and lowest percent composition for each analyte shaded grey to highlight the range of chemical profiles produced by the 12 WAFs.

Not surprisingly, we found that the chemical compositions of the field data are variable. Therefore, no single profile best describes the oil exposure that aquatic organisms experienced in the field. Instead, a range of oil weathering states and WAF preparation methods are needed to span the myriad of different exposures experienced in the field. Consequently, the DWH NRDA toxicity testing program often included multiple oils and WAF preparations for each species tested.

Figure 10 also reveals that a considerable number of water samples from the field showed evidence of particulate oil. For example, many of the most insoluble PAH analytes (i.e., C4 fluoranthenes/pyrenes, C2/C3-napthobenzothiophenes, C2-chrysenes) were detected in more than 50% of the water samples (as evident from the median lines in the boxplots for these analytes). Furthermore, the general range in the percent composition of these insoluble PAH analytes were congruent with the percent composition of the analytes found in the fresh and weathered DWH oils. Given that the chemical profile of the water samples indicate that they contain particulate oil, as opposed to purely dissolved constituents, [i.e., they mimic the chemical profile of the bulk oil (Payne and Driskell, 2003)], these results indicate the presence of particulate oil droplets in many of the water samples collected during and immediately after the spill. This supports the use of multiple WAF preparation methods, which allows us to investigate dissolved-only exposures as well as exposures with physically and/or chemically dispersed oil.

3.10 Implications for Interpretation of Toxicity Tests

The DWH disaster released unprecedented quantities of oil into the environment, from the deep sea to the surface, and from offshore to nearshore and onshore environments, all over the course of several months. Because of this span through space and time, the Trustees designed a toxicity testing matrix that included a wide range of species, life stages, endpoints, exposure scenarios, and toxicant mixtures. This report presents a detailed characterization of the four oils and the range of WAFs prepared with these oils in the DWH NRDA toxicity testing program. Our results show how various WAF preparation parameters, such as mixing energy, composition of the starting oil, and the oil-to-water mixing ratio, ultimately affect the concentrations, compositions, and forms of the oil-in-water mixtures. In addition, the results highlight the complexity and inconsistency of WAF preparations, and demonstrate the importance of reporting measured chemistry when defining exposure in a toxicity test. Ultimately, these results will help support the interpretation and comparison of more than 300 waterborne toxicity tests conducted as part of the DWH NRDA; they will also inform the design and implementation of future oil toxicology research.

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Certificate of Analysis from Triton Analytics Corporation.

Description of the water sample data compilation from DIVER.

Chemical and Physical Characterization of DWH Oil Samples

Table S.1. Chemical characterization of four DWH NRDA toxicity testing oils

Analyte name	Units	Source oil	Qualifier[a]	Artificially Weathered Source oil	Qualifier[a]	Slick A	Qualifier[a]	Slick B	Qualifier[a]
Semivolatile organic compounds, including PAHs and homologs (8270D SIM)									
Cis/trans-Decalin	mg/kg	530	J	84	J	0.18	U	0.19	U
C1 – Decalins	mg/kg	950	J	270	J	0.47	J	0.19	U
C2 – Decalins	mg/kg	1,200	J	720	J	1.2	J	0.19	U
C3 – Decalins	mg/kg	1,100	J	970	J	12	J	0.19	U
C4 – Decalins	mg/kg	860	J	940	J	58	J	2.4	J
Benzo(b)thiophene	mg/kg	7.1	J	4.5	J	0.18	U	0.19	U
C1 – Benzothiophenes	mg/kg	28	J	27	J	0.39	J	0.19	U
C2 – Benzothiophenes	mg/kg	25	J	32	J	1.7	J	0.19	U
C3 – Benzothiophenes	mg/kg	27	J	42	J	5.6	J	0.61	J
C4 – Benzothiophenes	mg/kg	26	J	37	J	12	J	4.2	J
Naphthalene	mg/kg	670	J	260	J	0.18	U	0.19	U
C1 – Naphthalenes	mg/kg	1,500	J	1,400	J	3.2	J	0.2	J
C2 – Naphthalenes	mg/kg	1,900	J	2,400	J	120	J	6.8	J
C3 – Naphthalenes	mg/kg	1,200	J	1,700	J	290	J	45	J
C4 – Naphthalenes	mg/kg	620	J	920	J	240	J	76	J
Biphenyl	mg/kg	200	J	260	J	2.6	J	0.26	U
Dibenzofuran	mg/kg	27	U	33	U	6.1	U	1.6	U
Acenaphthylene	mg/kg	6	U	7.6	U	0.66	U	0.19	U
Acenaphthene	mg/kg	11	J	13	J	1.5	J	0.19	U
Fluorene	mg/kg	88	J	110	J	22	J	4.2	J
C1 – Fluorenes	mg/kg	200	J	260	J	99	J	46	J

Table S.1. Chemical characterization of four DWH NRDA toxicity testing oils (cont.)

Analyte name	Units	Source oil	Qualifier[a]	Artificially Weathered Source oil	Qualifier[a]	Slick A	Qualifier[a]	Slick B	Qualifier[a]
C2 – Fluorenes	mg/kg	270	J	370	J	180	J	150	J
C3 – Fluorenes	mg/kg	240	J	310	J	170	J	200	J
Anthracene	mg/kg	9.3	J	13	J	2.8	J	1	J
Phenanthrene	mg/kg	250	J	380	J	75	J	57	J
C1 – Phenanthrenes/Anthracenes	mg/kg	570	J	890	J	250	J	320	J
C2 – Phenanthrenes/Anthracenes	mg/kg	580	J	910	J	320	J	490	J
C3 – Phenanthrenes/Anthracenes	mg/kg	360	J	570	J	200	J	330	J
C4 – Phenanthrenes/Anthracenes	mg/kg	260	J	390	J	140	J	220	J
Retene	mg/kg	9.9	J	15	J	5.9	J	9.4	J
Dibenzothiophene	mg/kg	40	J	65	J	13	J	6.9	J
C1 – Dibenzothiophenes	mg/kg	130	J	210	J	60	J	59	J
C2 – Dibenzothiophenes	mg/kg	200	J	300	J	110	J	160	J
C3 – Dibenzothiophenes	mg/kg	140	J	210	J	90	J	140	J
C4 – Dibenzothiophenes	mg/kg	76	J	110	J	48	J	94	J
Benzo(b)fluorene	mg/kg	18	J	24	J	6.8	J	5.1	J
Fluoranthene	mg/kg	4.5	J	6.7	J	2.7	J	4.6	J
Pyrene	mg/kg	17	J	26	J	8	J	11	J
C1 – Fluoranthenes/Pyrenes	mg/kg	66	J	100	J	36	J	44	J
C2 – Fluoranthenes/Pyrenes	mg/kg	120	J	180	J	62	J	63	J
C3 – Fluoranthenes/Pyrenes	mg/kg	130	J	210	J	74	J	82	J
C4 – Fluoranthenes/Pyrenes	mg/kg	110	J	170	J	65	J	84	J
Naphthobenzothiophene	mg/kg	15	J	23	J	9	J	16	J
C1 – Naphthobenzothiophenes	mg/kg	62	J	97	J	36	J	64	J
C2 – Naphthobenzothiophenes	mg/kg	71	J	110	J	43	J	71	J

Table S.1. Chemical characterization of four DWH NRDA toxicity testing oils (cont.)

Analyte name	Units	Source oil	Qualifier[a]	Artificially Weathered Source oil	Qualifier[a]	Slick A	Qualifier[a]	Slick B	Qualifier[a]
C3 – Naphthobenzothiophenes	mg/kg	56	J	83	J	31	J	45	J
C4 – Naphthobenzothiophenes	mg/kg	31	J	49	J	17	J	25	J
Benzo(a)anthracene	mg/kg	6.6	J	12	J	2.6	J	2.1	U
Chrysene + Triphenylene	mg/kg	45	J	68	J	28	J	53	J
C1 – Chrysenes	mg/kg	97	J	140	J	56	J	94	J
C2 – Chrysenes	mg/kg	130	J	190	J	63	J	91	J
C3 – Chrysenes	mg/kg	86	J	140	J	41	J	47	J
C4 – Chrysenes	mg/kg	67	J	95	J	30	J	31	J
Benzo(b)fluoranthene	mg/kg	5.1	J	8.1	J	3.9	J	7.3	J
Benzo(j + k)fluoranthene	mg/kg	0.38	U	0.33	U	0.18	U	0.19	U
Benzo(a)fluoranthene	mg/kg	0.38	U	1.5	J	0.71	U	2.2	U
Benzo(e)pyrene	mg/kg	9.9	J	15	J	5.8	J	9.9	J
C30-Hopane	mg/kg	60	J	99	J	50	J	110	J
Benzo(a)pyrene	mg/kg	0.91	J	2.4	J	0.8	J	0.63	J
Perylene	mg/kg	0.38	U	0.33	U	0.18	U	0.19	U
Indeno(1,2,3-cd)pyrene	mg/kg	0.38	U	0.33	U	0.18	U	0.19	U
Dibenzo(a,h)anthracene	mg/kg	2.2	J	3.9	J	1.1	J	1.1	J
Benzo(g,h,i)perylene	mg/kg	1.6	J	2.8	J	0.98	J	1.6	J
4-Methyldibenzothiophene	mg/kg	68	J	110	J	33	J	31	J
2/3-Methyldibenzothiophene	mg/kg	32	J	50	J	14	J	14	J
1-Methyldibenzothiophene	mg/kg	21	J	34	J	10	J	9.7	J
3-Methylphenanthrene	mg/kg	140	J	210	J	59	J	71	J
2-Methylphenanthrene	mg/kg	140	J	220	J	59	J	82	J
2-Methylanthracene	mg/kg	6.3	J	10	J	0.32	J	0.19	U

Table S.1. Chemical characterization of four DWH NRDA toxicity testing oils (cont.)

Analyte name	Units	Source oil	Qualifier[a]	Artificially Weathered Source oil	Qualifier[a]	Slick A	Qualifier[a]	Slick B	Qualifier[a]
4/9-Methylphenanthrene	mg/kg	160	J	260	J	72	J	91	J
1-Methylphenanthrene	mg/kg	120	J	180	J	57	J	65	J
2-Methylnaphthalene	mg/kg	1,400	J	1,300	J	2.1	J	0.19	U
1-Methylnaphthalene	mg/kg	1,000	J	950	J	2.7	J	0.19	U
2,6-Dimethylnaphthalene	mg/kg	970	J	1,200	J	49	J	2.4	J
2,3,5-Trimethylnaphthalene	mg/kg	350	J	550	J	100	J	17	J
Carbazole	mg/kg	2.8	U	4.7	U	0.18	U	0.19	U
Volatile organic compounds, including BTEX (8260C)									
Benzene	mg/kg	2,000	J	0.33	J	0.13	J	0.14	J
Toluene	mg/kg	6,100	J	3		1.2	U	1.2	U
Ethylbenzene	mg/kg	1,100	J	2.9		1.2	U	1.2	U
m,p-Xylenes	mg/kg	5,700	J	21		1.2	U	1.2	U
o-Xylene	mg/kg	2,100	J	12		1.2	U	1.2	U
Styrene	mg/kg	9.7	U	0.97	U	1.2	U	1.2	U
Isopropylbenzene	mg/kg	340	J	3.3	J	4.5	U	4.6	U
n-Propylbenzene	mg/kg	480	J	6.5		4.5	U	4.6	U
1,3,5-Trimethylbenzene	mg/kg	990	J	30		4.5	U	4.6	U
tert-Butylbenzene	mg/kg	15	J	0.63	J	4.5	U	4.6	U
1,2,4-Trimethylbenzene	mg/kg	2,100	J	99		0.2	J	4.6	U
sec-Butylbenzene	mg/kg	150	J	7.6		4.5	U	4.6	U
4-Isopropyltoluene	mg/kg	170	J	11		4.5	U	4.6	U
n-Butylbenzene	mg/kg	190	J	20		4.5	U	4.6	U
Naphthalene	mg/kg	1,000	J	390		4.5	U	4.6	U

Table S.1. Chemical characterization of four DWH NRDA toxicity testing oils (cont.)

Analyte name	Units	Source oil	Qualifier[a]	Artificially Weathered Source oil	Qualifier[a]	Slick A	Qualifier[a]	Slick B	Qualifier[a]
Saturates and TPH (8015C)									
n-Nonane	mg/kg	12,300	J	106	J	50	U	50	U
n-Decane	mg/kg	10,300	J	543	J	50	U	50	U
n-Undecane	mg/kg	11,200	J	2,320	J	50	U	50	U
n-Dodecane	mg/kg	8,730	J	5,200	J	50	U	50	U
n-Tridecane	mg/kg	11,000	J	10,200	J	166		50	U
2,6,10-Trimethyldodecane	mg/kg	1,830	J	2,160	J	131	J	50	U
n-Tetradecane	mg/kg	8,760	J	11,000	J	1140		50	U
2,6,10-Trimethyltridecane	mg/kg	2,460	J	3,360	J	474	J	50	U
n-Pentadecane	mg/kg	7,510	J	10,600	J	2,670		270	
n-Hexadecane	mg/kg	6,610	J	9,800	J	3,380		841	
Norpristane	mg/kg	2,780	J	4,090	J	1,700	J	460	J
n-Heptadecane	mg/kg	5,650	J	8,280	J	3,700		1,660	
Pristane	mg/kg	3,900	J	5,160	J	2,190		949	
n-Octadecane	mg/kg	4,550	J	6,740	J	3,420		2,270	
Phytane	mg/kg	2,310	J	3,100	J	1,510		917	
n-Nonadecane	mg/kg	4,590	J	6,770	J	3,950		3,160	
n-Eicosane	mg/kg	3,560	J	4,900	J	2,860		2,670	
n-Heneicosane	mg/kg	2,880	J	4,410	J	2,660		2,510	
n-Docosane	mg/kg	2,420	J	3,740	J	2,300		2,240	
n-Tricosane	mg/kg	2,190	J	3,380	J	1,960		1,940	
n-Tetracosane	mg/kg	2,160	J	3,160	J	2,160		1,940	
n-Pentacosane	mg/kg	2,040	J	3,180	J	1,900		1,820	
n-Hexacosane	mg/kg	1,720	J	2,650	J	1,530		1,620	

Table S.1. Chemical characterization of four DWH NRDA toxicity testing oils (cont.)

Analyte name	Units	Source oil	Qualifier[a]	Artificially Weathered Source oil	Qualifier[a]	Slick A	Qualifier[a]	Slick B	Qualifier[a]
n-Heptacosane	mg/kg	1,450	J	2,380	J	1,420		1,150	
n-Octacosane	mg/kg	1,130	J	1,720	J	1,030		1,130	
n-Nonacosane	mg/kg	1,170	J	1,840	J	1,070		1,110	
n-Triacontane	mg/kg	779	J	1,410	J	871		656	
n-Hentriacontane	mg/kg	932	J	1,570	J	907		845	
n-Dotriacontane	mg/kg	776	J	1,110	J	703		721	
n-Tritriacontane	mg/kg	757	J	1,060	J	731		622	
n-Tetratriacontane	mg/kg	558	J	714	J	389		419	
n-Pentatriacontane	mg/kg	411	J	391	J	126		279	
n-Hexatriacontane	mg/kg	310	J	50	U	275		208	
n-Heptatriacontane	mg/kg	201	J	50	U	50	U	171	
n-Octatriacontane	mg/kg	50	R	50	U	121		50	U
n-Nonatriacontane	mg/kg	50	U	50	U	50	U	50	U
n-Tetracontane	mg/kg	50	UJ	50	U	50	U	50	U
Total extractable hydrocarbons (TEHs)	mg/kg	663,000	J	735,000	J	337,000		268,000	
Total resolvable hydrocarbons (TRHs)	mg/kg	281,000	D	216,000	D	47,300	D	57,600	D
Unresolved complex mixture (UCM)	mg/kg	382,000	JD	519,000	JD	290,000	JD	210,000	JD
Metals (6010C, 6020A, 7471B)									
Aluminum	mg/kg	0.8	U	3.8	U	11.7		24.7	
Antimony	mg/kg	0.05	UJ	0.02	UJ	0.03	UJ	0.02	UJ
Arsenic	mg/kg	0.49	U	0.5	U	0.12	J	0.13	J
Barium	mg/kg	0.093		0.483		9.38		21.5	

Table S.1. Chemical characterization of four DWH NRDA toxicity testing oils (cont.)

Analyte name	Units	Source oil	Qualifier[a]	Artificially Weathered Source oil	Qualifier[a]	Slick A	Qualifier[a]	Slick B	Qualifier[a]
Beryllium	mg/kg	0.003	J	0.02	U	0.011	J	0.02	U
Cadmium	mg/kg	0.013	J	0.05	J	0.289	J	0.026	J
Calcium	mg/kg	2.3	U	3.9	U	911		1860	
Chromium	mg/kg	1.3	J	1.36	J	0.88	J	0.9	J
Cobalt	mg/kg	0.363	J	1.96	J	0.059	J	0.028	J
Copper	mg/kg	0.11	U	1.65		1		1.38	
Iron	mg/kg	0.5	J	14.3		59.2		27.1	
Lead	mg/kg	5	U	5	U	4.9	U	5	U
Magnesium	mg/kg	0.5	U	1.6	U	657		1,440	
Manganese	mg/kg	0.06		0.15		0.84		0.57	
Mercury	mg/kg	0.018	U	0.004	J	0.013	U	0.012	U
Nickel	mg/kg	1.99	J	3.04	J	1.69	J	1.76	J
Potassium	mg/kg	39.8	U	39.8	U	162		316	
Selenium	mg/kg	0.98	U	0.99	U	0.32	J	0.38	J
Silver	mg/kg	0.02	U	0.02	U	0.02	U	0.02	U
Sodium	mg/kg	2.7	U	7.3	U	3,610		7,480	
Thallium	mg/kg	0.008	U	0.016	U	0.034	J	0.005	U
Vanadium	mg/kg	1.15		1.55		0.76		0.73	
Zinc	mg/kg	0.48	J	0.92	J	0.79	J	0.65	J
DOSS (CAS SOP) and pH									
DOSS	µg/kg	910	UJ	930	U	860	UJ	830	U
pH		6.4		6.69		7.79		6.57	

a. Definition of qualifiers can be found in NOAA's analytical quality assurance plan (NOAA, 2014).

Chemical and Physical Characterization of DWH Oil Samples

Table S.2. List of analyte abbreviations

Analyte name	Analyte abbreviation
Benzene	B
Toluene	T
Ethylbenzene	E
Xylenes	X
Naphthalene	N0
C1 – Naphthalenes	N1
C2 – Naphthalenes	N2
C3 – Naphthalenes	N3
C4 – Naphthalenes	N4
Biphenyl	B
Dibenzofuran	DF
Acenaphthylene	AY
Acenaphthene	AE
Fluorene	F0
C1 – Fluorenes	F1
C2 – Fluorenes	F2
C3 – Fluorenes	F3
Anthracene	A0
Phenanthrene	P0
C1 – Phenanthrenes/Anthracenes	PA1
C2 – Phenanthrenes/Anthracenes	PA2
C3 – Phenanthrenes/Anthracenes	PA3
C4 – Phenanthrenes/Anthracenes	PA4
Dibenzothiophene	DBT0
C1 – Dibenzothiophenes	DBT1
C2 – Dibenzothiophenes	DBT2
C3 – Dibenzothiophenes	DBT3
C4 – Dibenzothiophenes	DBT4
Benzo(b)fluorene	BF
Fluoranthene	FL0
Pyrene	PY0
C1 – Fluoranthenes/Pyrenes	FP1

Table S.2. List of analyte abbreviations (cont.)

Analyte name	**Analyte abbreviation**
C2 – Fluoranthenes/Pyrenes	FP2
C3 – Fluoranthenes/Pyrenes	FP3
C4 – Fluoranthenes/Pyrenes	FP4
Naphthobenzothiophene	NBT0
C1 – Naphthobenzothiophenes	NBT1
C2 – Naphthobenzothiophenes	NBT2
C3 – Naphthobenzothiophenes	NBT3
C4 – Naphthobenzothiophenes	NBT4
Benzo(a)anthracene	BA0
Chrysene + Triphenylene	C0
C1 – Chrysenes	BC1
C2 – Chrysenes	BC2
C3 – Chrysenes	BC3
C4 – Chrysenes	BC4
Benzo(b)fluoranthene	BBF
Benzo(j + k)fluoranthene	BJKF
Benzo(a)fluoranthene	BAF
Benzo(e)pyrene	BEP
Benzo(a)pyrene	BAP
Indeno(1,2,3-cd)pyrene	IND
Dibenzo(a,h)anthracene	DA
Benzo(g,h,i)perylene	GHI

WAF Concentration

Table S.3. List of PAH analytes included in the TPAH sum used in this report and four other historical target analyte lists

Analyte	EPA 16	NOAA 24	EPA 34	TPAH50
Naphthalene	X	X	X	X
C1-Naphthalenes			X	X
C2-Naphthalenes			X	X
C3-Naphthalenes			X	X
C4-Naphthalenes			X	X
Biphenyl		X		X
Dibenzofuran				X
Acenaphthylene	X	X	X	X
Acenaphthene	X	X	X	X
Fluorene	X	X	X	X
C1-Fluorenes			X	X
C2-Fluorenes			X	X
C3-Fluorenes			X	X
Anthracene	X	X	X	X
Phenanthrene	X	X	X	X
C1-Phenanthrenes/anthracenes			X	X
C2-Phenanthrenes/anthracenes			X	X
C3-Phenanthrenes/anthracenes			X	X
C4-Phenanthrenes/anthracenes			X	X
Dibenzothiophene				X
C1-Dibenzothiophenes				X
C2-Dibenzothiophenes				X
C3-Dibenzothiophenes				X
C4-Dibenzothiophenes				X
Benzo(b)fluorene				X
Fluoranthene	X	X	X	X
Pyrene	X	X	X	X
C1-Fluoranthenes/pyrenes			X	X
C2-Fluoranthenes/pyrenes				X
C3-Fluoranthenes/pyrenes				X
C4-Fluoranthenes/pyrenes				X

Table S.3. List of PAH analytes included in the TPAH sum used in this report and four other historical target analyte lists (cont.)

Analyte	EPA 16	NOAA 24	EPA 34	TPAH50
Naphthobenzothiophene				X
C1-Naphthobenzothiophenes				X
C2-Naphthobenzothiophenes				X
C3-Naphthobenzothiophenes				X
C4-Naphthobenzothiophenes				X
Benzo(a)anthracene	X	X	X	X
Chrysene + Triphenylene	X	X	X	X
C1-Chrysenes			X	X
C2-Chrysenes			X	X
C3-Chrysenes			X	X
C4-Chrysenes			X	X
Benzo(b)fluoranthene	X	X	X	X
Benzo(j+k)fluoranthene	X	X	X	X
Benzo(a)fluoranthene				X
Benzo(e)pyrene		X	X	X
Benzo(a)pyrene	X	X	X	X
Indeno(1,2,3-c,d)pyrene	X	X	X	X
Dibenzo(a,h)anthracene	X	X	X	X
Benzo(g,h,i)perylene	X	X	X	X
1-methylnaphthalene		X		
2-methylnaphthalene		X		
2,6-dimethylnaphthalene		X		
1,6,7-trimethylnaphthalene		X		
1-methylphenanthrene		X		
Perylene		X	X	

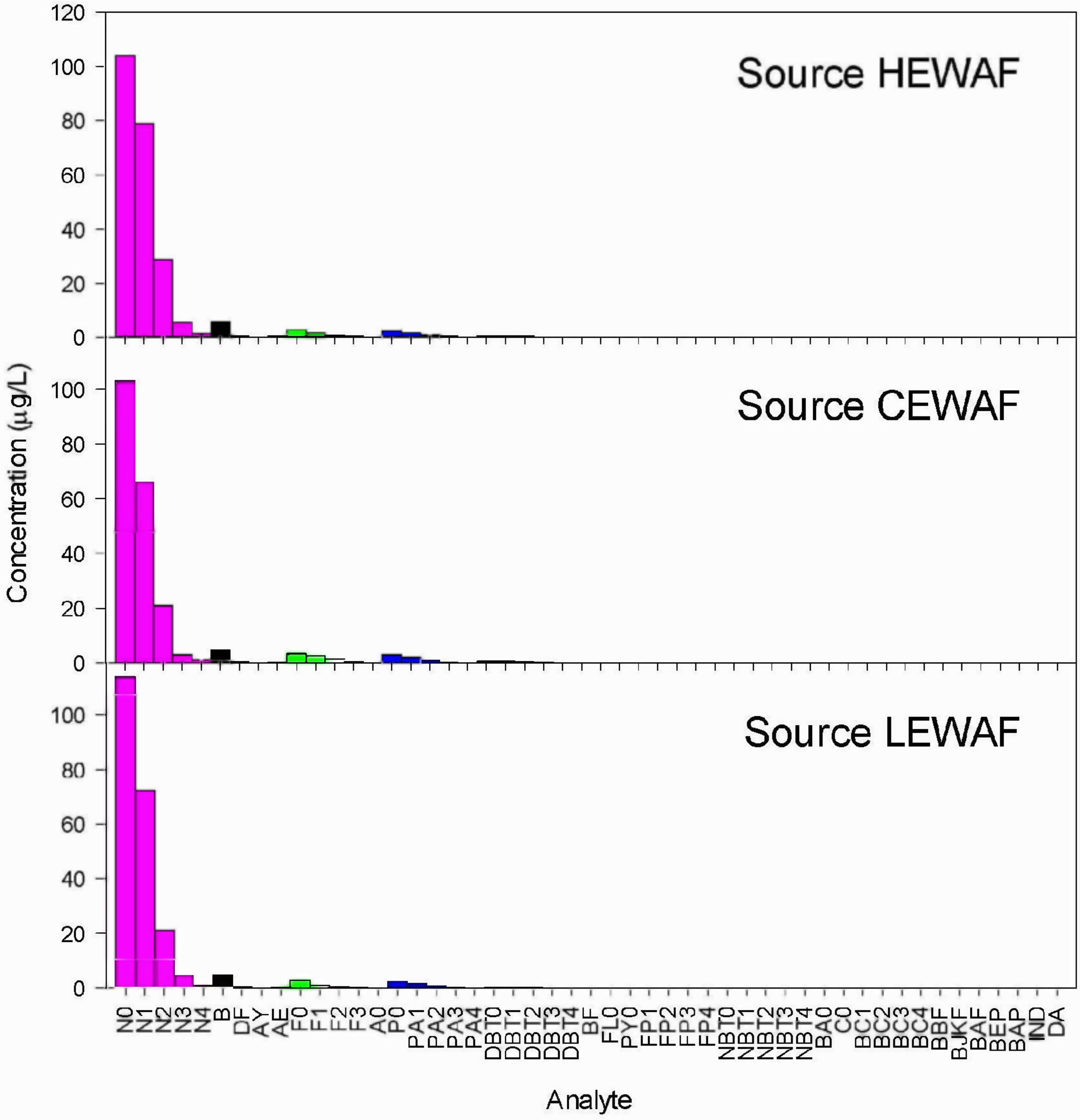


Figure S.1. Concentration of PAH analytes of filtered 1 g oil/L Source oil stock HEWAFs, CEWAFs, and LEWAFs.

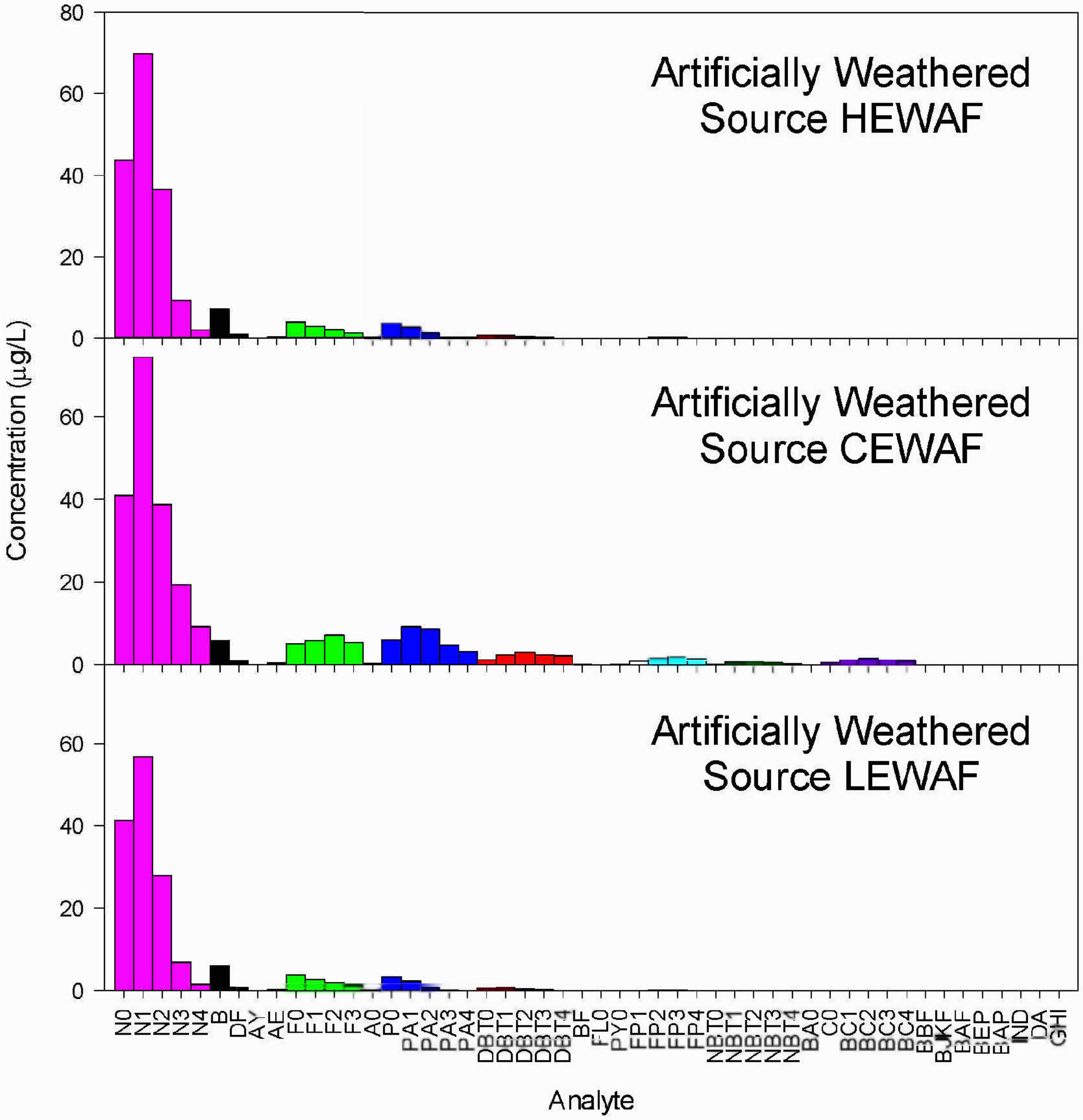


Figure S.2. Concentration of PAH analytes of filtered 1 g oil/L Artificially Weathered Source oil stock HEWAFs, CEWAFs, and LEWAFs.

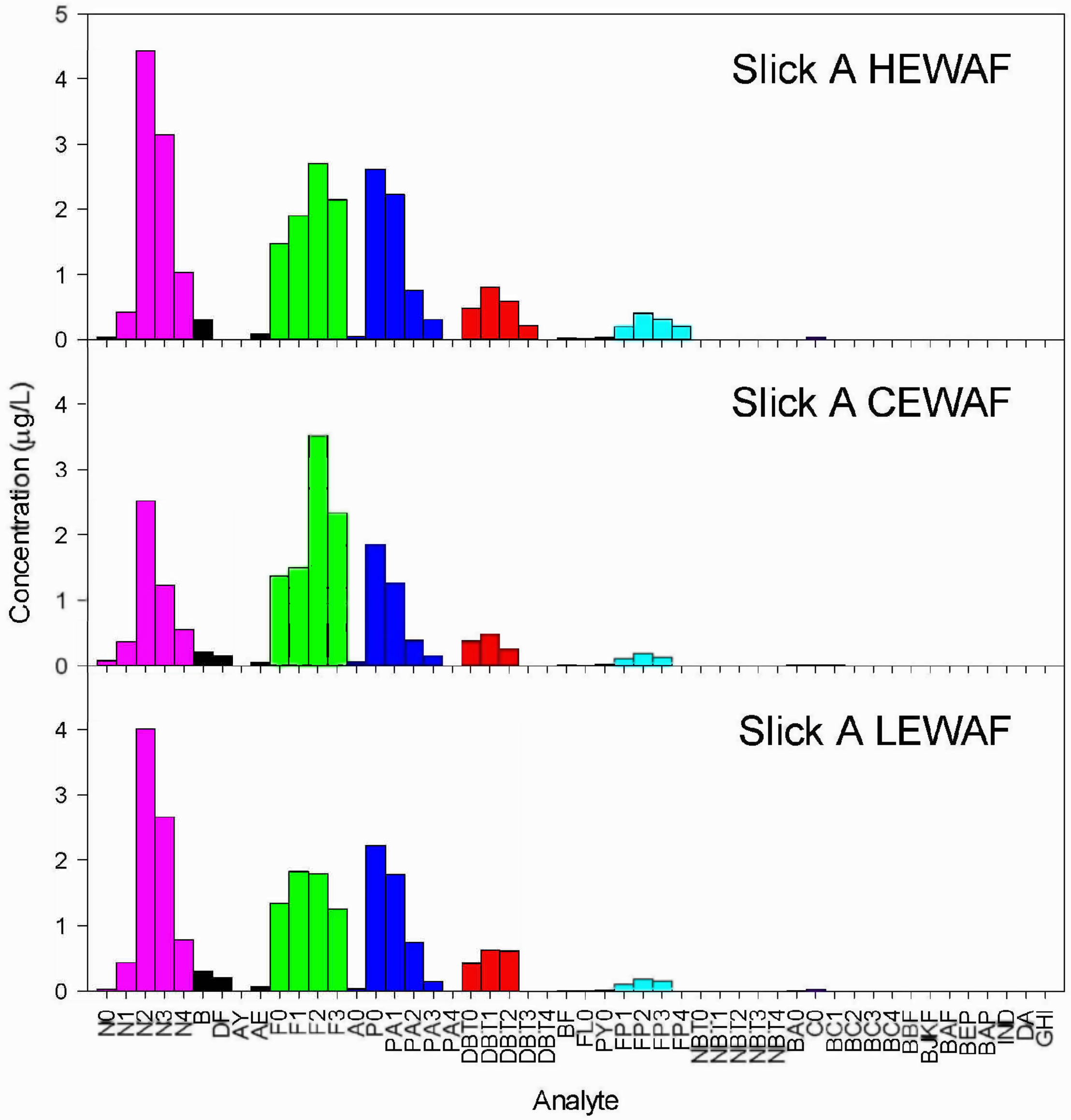


Figure S.3. Concentration of PAH analytes of filtered 1 g oil/L Slick A oil stock HEWAFs, CEWAFs, and LEWAFs.

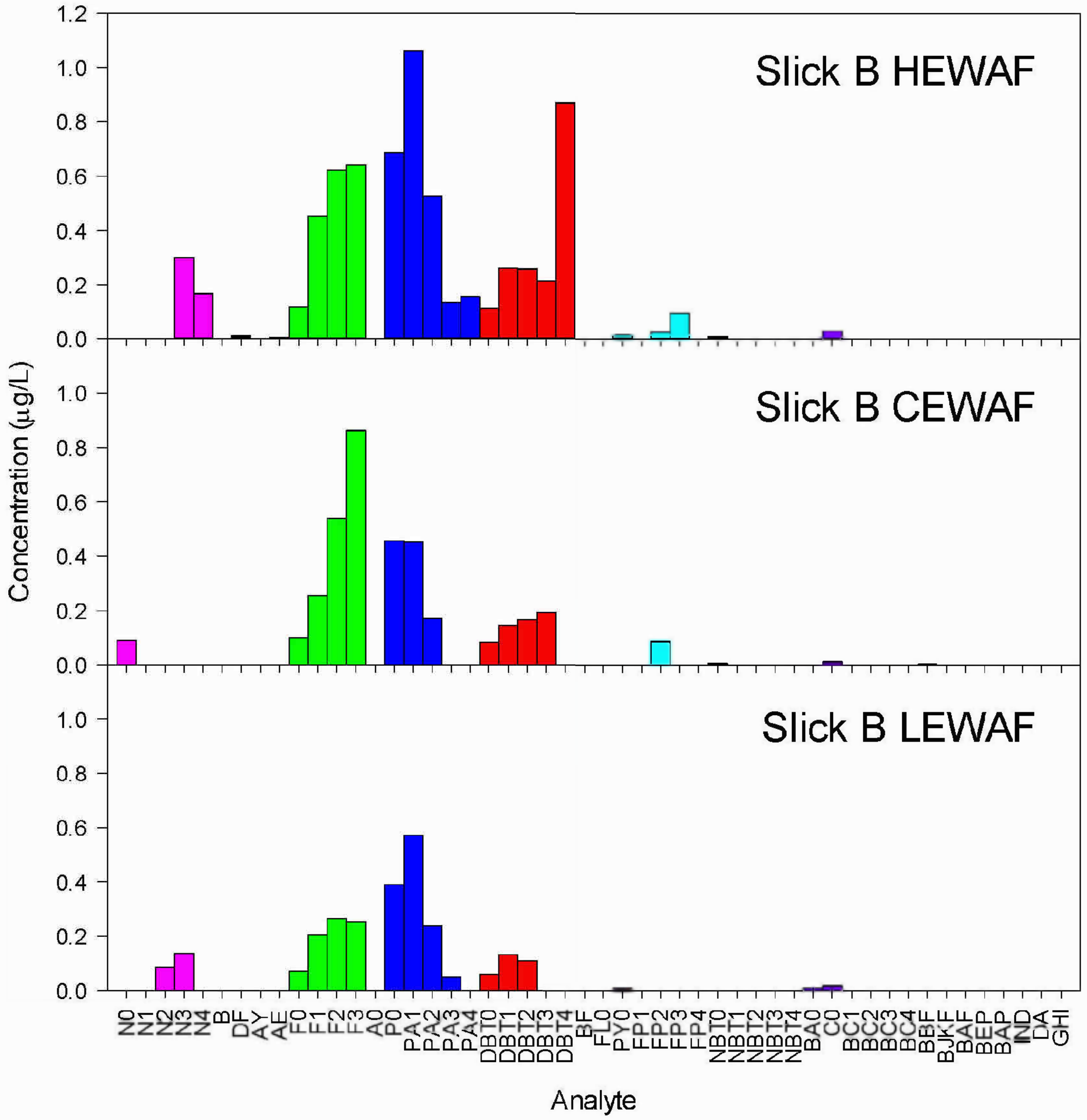
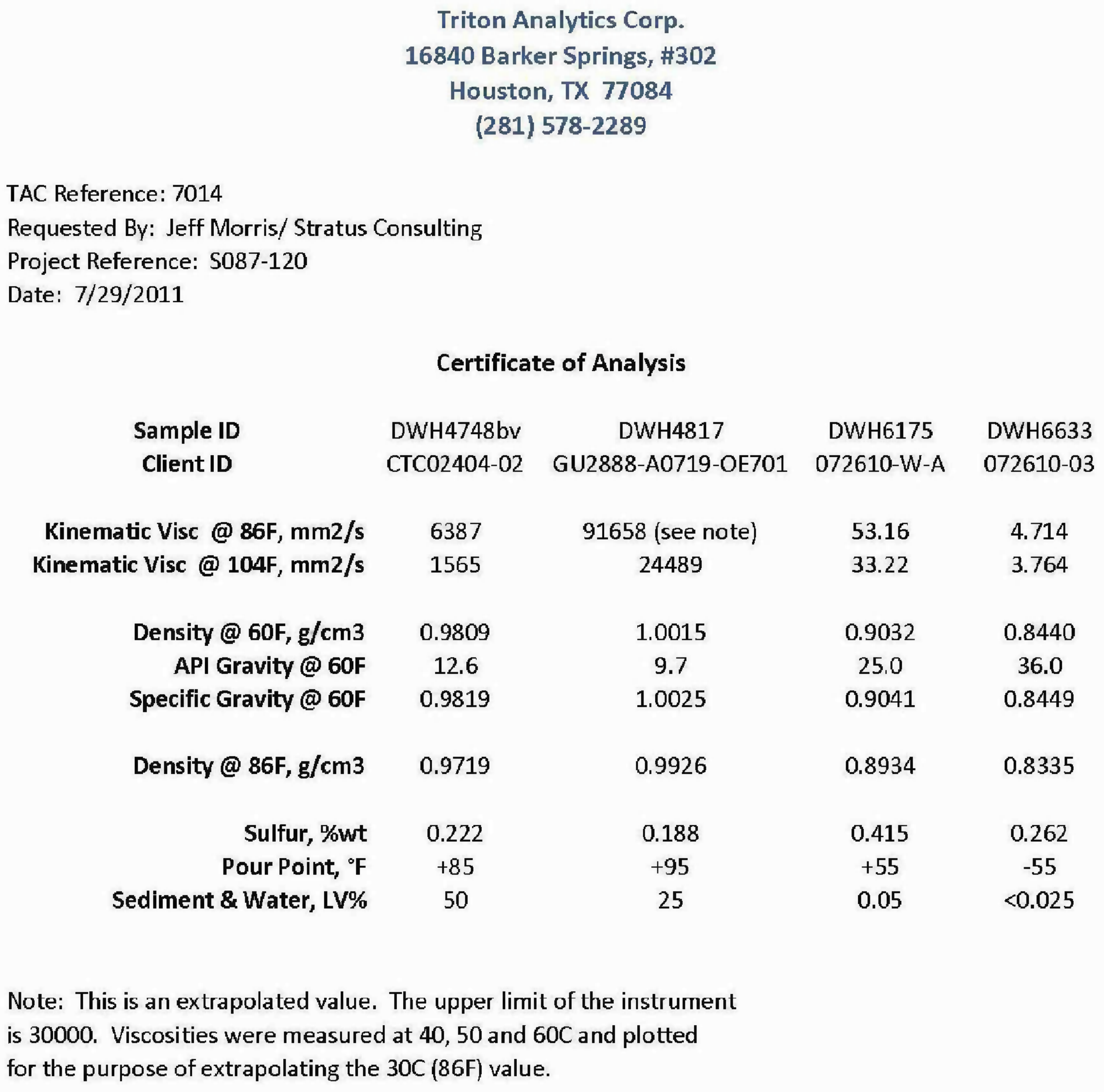


Figure S.4. Concentration of PAH analytes of filtered 1 g oil/L Slick B oil stock HEWAFs, CEWAFs, and LEWAFs.

Certificate of Analysis from Triton Analytics Corporation



Triton Analytics Corp.
16840 Barker Springs, #302
Houston, TX 77084
(281) 578-2289

TAC Reference: 7014
Requested By: Jeff Morris/ Stratus Consulting
Project Reference: S087-120
Date: 7/29/2011

Certificate of Analysis

Sample ID	DWH4748bv	DWH4817	DWH6175	DWH6633
Client ID	CTC02404-02	GU2888-A0719-OE701	072610-W-A	072610-03
Kinematic Visc @ 86F, mm2/s	6387	91658 (see note)	53.16	4.714
Kinematic Visc @ 104F, mm2/s	1565	24489	33.22	3.764
Density @ 60F, g/cm3	0.9809	1.0015	0.9032	0.8440
API Gravity @ 60F	12.6	9.7	25.0	36.0
Specific Gravity @ 60F	0.9819	1.0025	0.9041	0.8449
Density @ 86F, g/cm3	0.9719	0.9926	0.8934	0.8335
Sulfur, %wt	0.222	0.188	0.415	0.262
Pour Point, °F	+85	+95	+55	-55
Sediment & Water, LV%	50	25	0.05	<0.025

Note: This is an extrapolated value. The upper limit of the instrument is 30000. Viscosities were measured at 40, 50 and 60C and plotted for the purpose of extrapolating the 30C (86F) value.

Water Sample Data Compilation from DIVER

For our analysis in Figure 9 of the main report, we downloaded field water column data on March 6, 2015 from DIVER, a data management tool developed by NOAA (DIVER, 2015). To obtain our dataset we first downloaded the entire set of water column data from DIVER (this was a standard export available under the "Download Data" tab on the main DIVER Explorer page). We then applied the filters in Table S.4 to select for whole-water, PAH samples collected from a depth of 0–20 m in 2010. Next, we queried this dataset to select analyte concentrations for the TPAH50 set and removed all samples with TPAH50 concentrations ≥ 0.5 μg/L. Finally, we calculated a percent composition for each PAH analyte and plotted as boxplots. Since this dataset was downloaded in early 2015, the BP Public dataset, which was not yet available through DIVER, was not included. Our final dataset included 394 total samples.

Table S.4. Selection criteria for identifying whole-water, PAH samples in DIVER

Field name	Whole-water sample selection criteria
Lab Replicate	"1," "1a,"
Sample_Type	"Sample"
Upper_Sample_Depth	< 20 (this includes -9 samples)[a]
Anaysis_Method	"*8270*"
Qualifier_Code	Not "*R*"
Sample_Lower_Depth	< 20
Sample_Date	< 12/31/2010
Lab-Result_Matrix_Detailed	"Water (whole)"

a. In DIVER, -9 indicates that the sample was collected at an unspecified depth.