



## ***Chemical Fingerprinting Methodology and the Classification of Nearshore Samples Used in the Deepwater Horizon NRDA***

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### ***Abstract***

The *Deepwater Horizon* NOAA NRDA oil spill investigation includes nearshore samples collected between 2010 and 2012 along the coastlines of Louisiana, Mississippi, Alabama, and Florida and proximal waters within 3 nautical miles of the northern Gulf of Mexico shoreline. Nearshore samples consist of soils, sediments, solids, sheens, pom-poms, and tissues. These samples were chemically analyzed and forensically compared to fresh and weathered Macondo oil reference samples. The chemical composition and spatial distribution of hydrocarbon signatures help identify the natural resources that were exposed to Macondo oil as a result of the 2010 *Deepwater Horizon* oil spill. This report describes the chemical fingerprinting methodology used to detect the presence of oil from the failed Macondo well in nearshore samples.

Samples are categorized into five (5) Match Classification Codes (A to E; Table 1) using multiple lines of evidence. The lines of evidence included an evaluation of the dominant hydrocarbon types, a qualitative evaluation of mixing, a quantitative comparison of diagnostic geochemical biomarker source ratios, and spatial proximity to other indicators of Macondo oil impacts. Classification A fingerprints are consistent with weathered Macondo oil. Classification B fingerprints are mostly consistent with weathered Macondo oil with differences attributable to the effects of severe weathering and/or mixing with background hydrocarbons. Classification C fingerprints are dominated by background hydrocarbons although some weathered Macondo oil may be present. Classification D fingerprints are indeterminate. Classification E fingerprints represent elevated oil impacts attributed to non-Macondo oil.

The match categories are similar to published methods for oil spill source identification. However, the published methods match single source samples to single fugitive oil samples without provision for extreme weathering, distant migration, and mixing with ambient media. Hence, the match categories required additional consideration for 1) various states of environmental weathering of the Macondo oil and 2) mixing with ambient hydrocarbon sources in the nearshore environment. Effects of environmental weathering and mixing are recognized in the patterns of saturated hydrocarbons and polycyclic aromatic hydrocarbons while the more recalcitrant biomarkers provide reliable long-term evidence of oil. The natural and anthropogenic hydrocarbons in field samples are evaluated 1) temporally by comparison of samples collected before and after the Macondo oil arrived at specific coastal locations and 2) spatially by comparison of proximal samples with and without Macondo oil signatures. The resulting nearshore match categories provide a means for identifying Macondo impacts in a complex coastal environment.



## ***Introduction***

The *BP Deepwater Horizon* drilling platform exploded on April 20, 2010 and released millions of gallons of crude oil from the Macondo well (Mississippi Canyon Block 252, abbreviated MC252) before the leak was stopped on July 15, 2010 (Crone and Tolstoy 2010). The distribution and character of crude oil from the Macondo well that reached nearshore environments varied along the northern Gulf of Mexico (GOM) shoreline. Nearshore shallow water and benthic habitats are valuable for fisheries. Therefore, characterizing the extent and nature of oil introduced into these habitats is an important step in determining exposure and potential injury resulting from the *Deepwater Horizon* oil spill.

The delineation of Macondo oil impacts began with a shoreline reconnaissance by the Shoreline Cleanup Assessment Technique (SCAT) program, which identified some environments with heavy oiling and others with less obvious impacts. The less obvious impacts often occurred when oil mixed with the sediment and imparted a range of visual and olfactory effects. Field teams also observed neutrally buoyant flocculent material floating at the sediment-water interface believed to contain Macondo crude oil that prompted the use of specialized sampling equipment (Emsbo-Mattingly 2015). Impacts from dissolved oil constituents to nearshore water or biota could not be visually observed, but could be chemically detected. The hydrocarbon fingerprinting methods described herein help identify Macondo oil over a wider range of concentrations than the visual or olfactory methods used by the field teams alone.

Multiple technical working groups (TWGs) investigated the impacts in greater detail with specific interest in ecologically sensitive or economically important receptors. The TWGs developed numerous sampling work plans (WPs) that governed the collection of thousands of multimedia samples (soil, sediment, water, tissue and sorbent material) in order to establish the extent of Macondo oil impacts in the nearshore environment.<sup>1</sup> Each sample was analyzed for a combination of polycyclic aromatic hydrocarbons (PAHs), saturated hydrocarbons (SHC), and/or geochemical biomarkers. The environmental laboratory results from these analyses provided “chemical fingerprinting” data appropriate for identifying samples with Macondo oil impacts. The objective of chemical fingerprinting is to aid other scientists in assessing exposure of natural resources in nearshore environments to Macondo oil. This report discusses the methods used to chemically analyze solid, pom-pom, and tissue nearshore samples and the integration of other lines of evidence used to determine the presence of Macondo oil in various types of nearshore samples. The nearshore water samples are discussed in Driskell and Payne (2015).

## ***Methods***

### ***Analytical Chemistry Methods***

All of the samples in this report were analyzed by Alpha Analytical (Mansfield, Massachusetts) for detailed hydrocarbon composition in accordance with the Analytical Quality Assurance Plan (AQAP) prepared for the Mississippi Canyon 252 (*Deepwater Horizon*) Natural Resource Damage Assessment (NOAA 2014).

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<sup>1</sup> The nearshore environment is functionally defined in this report as the supratidal (above the high tide water), intertidal (between low and high tide water), and subtidal (below the low tide water) environment from which field teams collected samples in accordance with the WPs produced by the applicable TWGs. The nearshore environment generally extends inland to the stormwater reach of GOM seawater and offshore approximately 3 nautical miles.



The forensic methods included:

- (1) *DEM Screening*: Designated samples were analyzed by EPA Methods 1664A (modified) and 9071B (modified) to estimate the concentration of TPH for screening purposes. Approximately 2.5 g to 5 g of sample are mixed with sodium sulfate to remove moisture and shake extracted with dichloromethane (DCM). The DCM extractable material (DEM) can include many organic substances such as petroleum, tar, and detrital organics. Polar hydrocarbons that make up the majority of natural organic material and sewage are removed by adding deactivated silica gel to the extract. The DEM concentration is determined gravimetrically and reported as mg/kg<sub>wet</sub> (ppm).
- (2) *Hydrocarbon Sample Extraction and Preparation*: The sample preparation procedure varied by matrix in accordance with NOAA (1993, 1998, 2002) and Douglas et al. (2015). Oil and tar ball samples were dissolved in DCM, dried with sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>), filtered, and spiked with surrogate. Solid samples (e.g., soil and sediment) were dried with Na<sub>2</sub>SO<sub>4</sub>, spiked with surrogate, serially shake extracted with DCM, concentrated in a Kuderna-Danish (KD) apparatus, eluted through a silica gel column, and concentrated using KD and nitrogen blowdown (NB). Tissue samples are dried with Na<sub>2</sub>SO<sub>4</sub>, spiked with surrogate, and serially extracted using a tissumizer and DCM, concentrated by KD and NB, eluted through an alumina/silica gel column, eluted through a high pressure liquid chromatography (HPLC) column, and concentrated using KD and NB. Selected solid and tissue samples scheduled for the measurement of geochemical biomarkers were eluted through a silica gel column to generate a saturated hydrocarbon fraction with the designation "F1." All of the sample extracts were spiked with internal standards before analysis by one or more gas chromatography (GC) techniques.
- (3) *TPH and Selected Alkane Quantification and Fingerprinting*: Designated sample extracts were analyzed on a gas chromatograph equipped with a flame ionization detector (GC/FID) in accordance with EPA Method 8015B (modified). This method determined the total concentration of extractable hydrocarbons (total petroleum hydrocarbons or TPH) eluting between *n*-nonane and *n*-tetratetracontane (*n*-C<sub>9</sub> to *n*-C<sub>44</sub>). It also measured the concentrations of individual *n*-alkanes (*n*-C<sub>9</sub> to *n*-C<sub>40</sub>) and (*n*-C<sub>15</sub> to *n*-C<sub>20</sub>) acyclic isoprenoids (e.g., pristane and phytane) (Table 2). The concentrations of target compounds are presented herein as ug/kg<sub>dry</sub> for solids (soils and sediments) and mg/kg<sub>oil</sub> for tar balls and oil rinsed from biota and pom-poms. The concentrations are not surrogate corrected. This analysis simultaneously provided a high resolution hydrocarbon fingerprint capable of identifying the dominant hydrocarbons in the sample extract.
- (4) *Parent and Alkylated PAHs*: Designated samples were analyzed for PAHs using a gas chromatograph equipped with a mass spectrometer (GC/MS) operated in selected ion monitoring (SIM) mode in accordance with EPA Method 8270 (modified). This method determined the concentrations of 52 PAHs and alkylated PAHs including sulfur-containing aromatics (Table 3). The concentrations of target compounds are presented herein as ug/kg<sub>dry</sub> for solids (soils and sediments) and ug/kg<sub>oil</sub> for tar balls and oil residues (rinsed from biota and pom-pom samples).

The concentration and distribution of PAHs provided greater detail and specificity about the type of petroleum, creosote, combustion byproducts, urban background, and diagenetic matter in the field samples. For example, petroleum possesses a petrogenic PAH pattern consisting of low parent PAH abundance relative to the alkylated PAHs; e.g., N0 < N1 < N2. By contrast, pyrogenic PAHs form during the partial combustion or



pyrolysis of organic matter. A pyrogenic PAH pattern exhibits high abundance of parent PAHs relative to the alkylated PAHs; e.g.,  $N_0 > N_1 > N_2$ , before weathering. Finally, diagenetic PAHs, like retene and perylene, form naturally in sediments containing specific types of decayed vegetation. Forensic scientists study the distribution and relative abundances of diagnostic PAH assemblages to help identify the presence of PAHs from these various sources (Douglas et al. 2015).

- (5) *Geochemical Biomarkers*: Designated samples were analyzed for geochemical biomarkers using a GC/MS operated in SIM mode in accordance with EPA Method 8270 (modified). This method determined the concentrations of 54 petroleum biomarkers including tricyclic and pentacyclic triterpanes, regular and rearranged steranes, and triaromatic steroids (Table 4). The concentrations of target compounds are presented herein as  $\mu\text{g}/\text{kg}_{\text{dry}}$  for solids (soils and sediments) and  $\mu\text{g}/\text{kg}_{\text{oil}}$  for tar balls and oil residues (rinsed from biota and pom-pom samples).

### *Chemical Fingerprinting Methods*

The chemical fingerprint of the Macondo crude oil is represented by the oil collected on May 21, 2010 through the riser insertion tube on the *Discoverer Enterprise* (Stout 2015a). However, environmental weathering changed the fugitive Macondo crude oil during its migration to the surface water (Stout 2015b) and nearshore environment (Stout 2015c). During its migration to the nearshore environments the Macondo oil acquired additional chemical complexity with dispersant applications and mixing with ambient media, such as, soil, sediment, biota, and water. The most appropriate Macondo reference sample for comparison to nearshore samples varied regionally in recognition of these weathering and mixing effects (Douglas et al. 2015).

The technical approach for recognizing Macondo crude oil in nearshore samples is based on multiple lines of scientific evidence and a systematic interpretation procedure (Figure 1). The source identification procedure begins with a qualitative comparison of GC/FID chromatograms based upon standard ASTM methods (ASTM 2000a and 2000b). During the first step, features, such as the presence of an unresolved hydrocarbon mixture (UCM) and saturated hydrocarbon patterns in the crude oil range provide chemical fingerprinting evidence that Macondo oil may be present. These oil signatures are distinct from the mixtures of natural organic material (NOM) from plant detritus and pyrogenic hydrocarbons from fuel combustion commonly encountered in nearshore environments. If these hydrocarbon signatures appear, the patterns are further investigated to confirm the presence of Macondo oil based on additional information. This screening process helped prioritize the analysis of samples with the highest likelihood of Macondo crude oil impact and identify candidate reference samples in the event that an adequate amount of time was not available for the analysis of all of the samples.

Due to the large influx of samples, employing GC/FID screening results enabled the prioritization of advanced PAH and petroleum biomarker analyses for samples with potential Macondo-range features; for example, normal alkanes, isoprenoid hydrocarbons, or a Macondo-range UCM. It was not possible to analyze the large number of sediment samples with little to no discernable Macondo hydrocarbons based on the GC/FID chromatograms; however, a spatially distributed subset of the samples were approved for advanced analyses (PAHs and petroleum biomarkers) to represent the regional variation in the background hydrocarbon patterns. These results assisted in the confirmation of the presence or absence of Macondo oil in nearshore samples.

The chemical signature(s) of the oil is defined by the assemblage of saturated and aromatic hydrocarbons in the source oil. The source oil signature includes replicate analyses of fresh Macondo reference oil ( $n = 619$ ) plus weathered Macondo oil ( $n = 1,188$ ) identified in the stranded oil samples previously studied



(Stout 2015c). This nearshore forensic fingerprinting method compares the field samples to both the fresh and weathered Macondo oil reference samples through a combination of qualitative and quantitative techniques (Figure 1). The initial tier of analysis determines the potential presence of Macondo Oil in a field sample based on a qualitative comparison of the high resolution hydrocarbon fingerprints from the field and Macondo reference samples. Samples selected for advanced PAH and possibly geochemical biomarker testing are subjected to quantitative analyses consisting of several steps. First, scaling ratios (SRs; Table 5) are used to determine the potential degree of mixing with NOM and other ambient hydrocarbons. Second, diagnostic ratios (DRs; Table 6) are used to determine if the field and Macondo reference samples forensically match quantitatively. SRs and DRs primarily consist of petroleum biomarkers, whose pattern is source-specific and weathering-resistant (Wang et al. 2006). DRs based on diagnostic PAHs are also considered; however, they are frequently excluded due to 1) significant loss of PAHs from weathering, 2) bias resulting from the presence of interferences, or 3) absence because of analyte concentrations below the reporting or detection limits. Consequently, the DRs derived from biomarkers are primarily used to evaluate weathering and mixing among the nearshore soils, sediments, solids, sheens, and pom-poms samples. The PAH pattern plays a more important role for identifying Macondo oil in tissue samples, because many tissue samples were not analyzed for geochemical biomarkers.

The collective results of the qualitative and quantitative comparison of each field sample to the Macondo source samples are used to group the field samples into one of five classifications (Table 1). The nearshore samples with Classification Code A typically demonstrate evaporated and biodegraded patterns of light and middle range hydrocarbons and PAHs; however, the sterane and triterpane biomarker patterns match the Macondo oil closely. The nearshore samples with Classification Code B also demonstrate environmental weathering plus a minor degree of mixing with NOM and other ambient hydrocarbons typically expressed in the high resolution hydrocarbon fingerprints, PAH, and biomarker patterns. The ambient hydrocarbons dominate the nearshore samples with Classification Code C; however, these samples possibly contain Macondo oil based on 1) the close proximity of SCAT oiling or other nearshore samples with A/B Classifications and 2) the occurrence of a Macondo oil pattern that was mixed or diluted within these samples. Many nearshore samples are in Classification Code D, which signifies that the presence of Macondo oil is indeterminate due to the high abundance of ambient hydrocarbons or the high frequency of concentrations below the detection limit of the method. Nearshore samples assigned to Classification Code E contained high concentrations of petroleum likely from an independent spill. With few exceptions, the Classification Code E samples contain sufficient amount of oil to be considered stranded oil samples, which are amenable to oil-oil fingerprinting methods and were discussed elsewhere (Stout 2015c). The systematic nearshore classification system (Figure 1) employs conservative criteria for identifying variably weathered Macondo oil in a wide range of realistic coastal environments.

### *PAH Depletion*

The degree of weathering in field samples with petroleum that matched Macondo oil is based upon the loss of a target analyte relative to a conservative internal marker within the oil, *viz.*, 17 $\alpha$ (H),21 $\beta$ (H)-hopane (hopane) (Prince et al 1994). To ensure the source oil is unweathered and representative of fresh Macondo oil, target analyte concentrations of six samples collected directly from the riser insertion tube are averaged and used in all weathering calculations (Stout 2015a). These six Macondo oil samples are the freshest source samples within the database. This approach is used to estimate the percent total mass loss of the liquid oil (C<sub>1</sub>-C<sub>4</sub> gases excluded) based on the following formula:

$$\% \text{Total Mass Loss} = (1 - (H_i/H_s)) \times 100 \quad \text{Equation (1)}$$



where  $H_0$  and  $H_s$  are the concentrations of hopane in the average fresh MC252 source oil and field sample, respectively. The percent mass loss of any given fraction (e.g., PetPAH<sub>27</sub>) or individual chemical (e.g., naphthalene) in the nearshore samples is estimated using the following formula:

$$\% \text{Mass Loss A} = [(A_0/H_0) - (A_s/H_s)] / (A_0/H_0) \times 100 \quad \text{Equation (2)}$$

Where  $A_s$  and  $H_s$  are the concentrations of the target analyte and hopane in the field sample, respectively, and  $A_0$  and  $H_0$  are the average concentrations of the target analyte and hopane in fresh Macondo source oil. As is common practice, and in order to eliminate the effects of varying surrogate recoveries on the % depletion calculations, non-surrogate corrected concentrations are used.<sup>2</sup> Total and individual mass losses calculated by these methods account only for mass loss from the liquid oil, i.e., they do not account for mass losses of gases (C<sub>1</sub>-C<sub>4</sub>) originally present in the Macondo oil.

## ***Characterization of Macondo Oil***

The composition of the fresh Macondo oil changed as it migrated away from the wellhead, contacted the shoreline, and mixed with ambient media (Figure 2). A summary of these compositional changes is important for understanding the transformation and migration of the oil throughout the nearshore environments of the northern GOM.

### ***Fresh Macondo Oil***

Fresh Macondo oil contains a wide range of semivolatile hydrocarbons, which are the focus of this chemical fingerprinting investigation because some of these compounds demonstrate the weathering processes and others contain source signature patterns (Stout 2015a). The high resolution hydrocarbon fingerprint demonstrates the prominent character of the normal alkanes eluting from *n*-nonane (*n*-C<sub>9</sub>) to *n*-tetratetracontane (*n*-C<sub>44</sub>) (Table 2) with high proportions of the lightest *n*-alkanes in this range (Figure 2a). The saturated hydrocarbon pattern features the *n*-alkanes and acyclic isoprenoid hydrocarbons (i.e., isoprenoids) eluting between *n*-nonane (*n*-C<sub>9</sub>) and *n*-tetracontane (*n*-C<sub>40</sub>) because these compounds demonstrate the oil weathering process well. The fresh Macondo oil exhibits declining concentrations of *n*-alkanes with increasing molecular weight. In addition, the isoprenoids are found in lower concentrations than corresponding *n*-alkanes (e.g., *n*-heptadecane is higher in concentration than the isoprenoid pristane [*n*-C<sub>17</sub>/Pr > 1] and *n*-octadecane is higher in concentration than the isoprenoid phytane [*n*-C<sub>18</sub>/Ph > 1]). The fresh Macondo source oil also contains a full range of 2- to 4-ring PAHs (Table 3) with high proportions of the lightest compounds (i.e., naphthalenes) in this range. The high proportions of alkylated PAHs in every homolog group serve as a petrogenic signature used to identify the presence of petroleum. The sum of the alkylated PAHs (Table 3; PetPAH<sub>27</sub>) is a bulk PAH measurement that is sensitive to fresh and weathered Macondo oil and particularly useful for evaluating environmental weathering (see % depletion discussion). The fresh source oil also contains geochemical biomarkers (Table 4). The pattern of geochemical biomarkers in Macondo oil (e.g., the black line demonstrating the proportion of triterpanes and steranes biomarkers to one another in Figure 2) resists the effects of environmental weathering and serves as the most reliable chemical fingerprint for identifying weathered Macondo residues in the nearshore samples.

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<sup>2</sup> Data within the queryable NOAA DIVER database are surrogate corrected. Data downloads by matrix of non-surrogate corrected results are also available from NOAA DIVER.



## *Stranded Oils*

Stout (2015c) identifies 1,188 stranded oil samples that contain Macondo oil residues along 500 miles of shoreline from western Terrebonne Bay, Louisiana to Apalachicola Bay, Florida using oil spill source identification procedures (Stout 2015d). The stranded oil samples attributable to Macondo oil exhibit a large loss of mass primarily due to evaporation. The transformation of the hydrocarbon composition due to weathering is significant (Figure 2b). The high resolution hydrocarbon fingerprint demonstrates a dramatic reduction of hydrocarbons eluting before *n*-octadecane (*n*-C<sub>18</sub>), which produces high proportions of the middle weight *n*-alkanes eluting around *n*-eicosane (*n*-C<sub>20</sub>) in the MC252 residue. Due to very similar volatilities, the isoprenoids remain at lower concentrations than corresponding *n*-alkanes (e.g., *n*-C<sub>17</sub>/Pr > 1 and *n*-C<sub>18</sub>/Ph > 1), which demonstrates that the loss of mass is primarily due to evaporation as opposed to biodegradation.<sup>3</sup> The stranded Macondo oil residues contain 2- to 4-ring PAHs with high proportions of alkylated phenanthrenes and lower proportions of alkylated naphthalenes and alkylated fluorenes. The reduction of these 2- and 3-ring PAHs is attributed to evaporation and dissolution (Stout 2015c). The pattern of triterpanes and steranes in fresh and stranded Macondo oil samples match closely and exhibit little to no change. The proportion of triaromatic steroids (TAS) relative to steranes is lower in the stranded oil than in the fresh Macondo source oil likely due to photo-oxidation (Aeppli et al. 2014; Stout 2015c).

## *Recognizing the Macondo Source Signature in Nearshore Solid and Pom-Pom Samples*

The identification of weathered Macondo oil in the nearshore environment requires the use of multiple lines of evidence, especially in samples that contain high proportions of ambient background hydrocarbons. The primary lines of evidence that support the identification of Macondo oil include 1) the presence of UCMs in the *n*-C<sub>18</sub> to *n*-C<sub>40</sub> range, 2) the pattern of alkylated 3- and 4-ring PAHs, 3) the patterns of selected triterpanes and steranes, 4) the degree of mixing, 5) the diagnostic ratios, and 6) other factors, such as the spatial proximity to stranded Macondo oil samples and SCAT oiling levels. The following discussion presents the technical framework for considering and interpreting the variable hydrocarbon patterns in mixtures of Macondo residues and ambient background hydrocarbons.

The nearshore samples reveal additional hydrocarbon transformations beyond those expressed among the stranded oils (Figure 2c). The high resolution hydrocarbon fingerprint of Macondo-impacted samples exhibit prominent unresolved complex mixtures (UCMs) in the *n*-C<sub>18</sub> to *n*-C<sub>40</sub> range. The saturated hydrocarbon pattern demonstrates dramatic reductions in the *n*-alkanes attributable to biodegradation (i.e., *n*-C<sub>17</sub>/Pr << 1 and *n*-C<sub>18</sub>/Ph << 1), reductions which were not largely evident in “pure” stranded oils. More advanced degree of weathering also appears in the form of depleted mono-, di-, and tri-alkylated PAHs and TAS compounds. The predominance of petrogenic 4-ring PAHs is consistent with heavily weathered Macondo oil. Despite depletions of the *n*-alkanes and PAHs, the pattern of triterpanes and steranes in many nearshore samples matches the Macondo source oil well.

Two sets of biomarker ratios, scaling ratios (SRs) and diagnostic ratios (DRs), help recognize Macondo oil impacts. SRs are comprised of the more abundant triterpane and sterane biomarkers in the Macondo

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<sup>3</sup> Normal alkanes (e.g., heptadecane or *n*-C<sub>17</sub>) are present at higher concentrations than comparable isoprenoid hydrocarbons (e.g., pristane or Pr) in middle range petroleum products. Microbes degrade normal alkanes much faster than isoprenoid hydrocarbons. Middle range petroleum with little to no biodegradation typically exhibits higher abundances of normal alkanes relative to isoprenoids (*n*-C<sub>17</sub>/Pr > 1). As microbes biodegrade the middle range petroleum hydrocarbons, they preferentially metabolize the normal alkanes while isoprenoid hydrocarbons are degraded much more slowly. Light biodegradation is recognized as *n*-C<sub>17</sub>/Pr approaches 1. Moderate biodegradation is recognized when *n*-C<sub>17</sub>/Pr < 1. Heavy biodegradation is recognized when *n*-C<sub>17</sub>/Pr approaches 0.



oil normalized to hopane (Table 5). The triterpane and sterane biomarkers relied on most heavily for the scaling of nearshore samples include T15, T19, S12, S17, S20, S23, S24, S25, S26, S27, and S28. These biomarkers exhibit 1) the least degree of environmental weathering, 2) the least degree of interferences, and 3) high detection frequencies. By contrast, some geochemical biomarkers prove less useful, because they possess lower natural abundances and detection frequencies in Macondo oil (e.g., X, T17, T18, T20, T21, T22, T22a, T26, T27, T30, T31, T32, T33, T34, T35, and S19) or fall below the method detection limit due to extreme environmental weathering (e.g., T4, T5, T6, T6a, T6b, T6c, T7, T8, T9, T10, T11, T12, T14a, T14b, S4, S5, S14, and S15).

DRs focus on the signature of Macondo oil and are comprised of ratios frequently used in forensic investigations that assist in matching contamination to a suspected source (Table 6). The primary DRs used for matching field and Macondo oil samples are composed of triterpane and sterane biomarkers, while the DRs composed of PAHs help identify weathering and mixing. Classification Code A signifies field sample DRs that compare very well with fresh or weathered Macondo oil reference samples ( $r^2 \geq 0.9$ ). Classification Code B signifies field samples that possess geochemical biomarker patterns of Macondo oil after mixing and interferences from ambient hydrocarbons are considered; specifically, the biomarker DRs in the field sample are at least as great as the fresh or weathered Macondo oil reference samples after a minor degree (between approximately 0% and 50%) of mixing or dilution (see SR discussion below). Classification Code C considers 1) the potential effect of interferences from ambient hydrocarbons when the primary DRs in the sample are at least as great as the fresh or weathered Macondo oil reference samples after a major degree (see SR discussion below) of mixing or dilution and 2) the close proximity ( $< 100\text{m}$ ) of Match A/B samples or SCAT observations. These additional considerations provide a realistic and systematic procedure for recognizing Macondo oil when environmental weathering and mixing occurs.

SRs estimate the maximum amount of Macondo oil in a field sample that potentially contains ambient hydrocarbons. The degree of mixing is estimated by multiplying the SRs by a mixing factor ranging from 0 and 1 for samples that fail the criteria associated with Classification Code A (above). The scaling factor is effectively the maximum decimal percentage of the geochemical biomarkers attributable to Macondo oil. Procedurally, the scaling factor is reduced from 1 until the SRs in the Macondo oil align with the field sample. Once the SRs are aligned, the field sample DRs are recalculated after multiplying the analyte concentrations by the scaling factor. Once the DRs in the field sample are effectively “un-mixed”, the regression with the DRs in the Macondo source oil is re-run. Field samples are determined to be in Classification Code B when 1) a scaling factor greater than approximately 0.5 (*i.e.*, more than 50% of the geochemical biomarkers are attributed to Macondo oil) produces field sample DRs that match Macondo oil ( $r^2 \geq 0.9$ ) and 2) the residual pattern is consistent with the biomarkers in the ambient sediment. The field sample is determined to be in Classification Code C when 1) a scaling factor between 0.3 and 0.5 (*i.e.*, between 30% and 50% of the geochemical biomarkers are attributed to Macondo oil) produces field sample DRs that match Macondo oil ( $r^2 \geq 0.9$ ), and 2) the sample is within 100 m of a SCAT oiled shoreline or a Match A/B stranded oil sample. Collectively, this “un-mixing” process minimizes the influence of non-Macondo oil and other ambient interferences within the geochemical biomarker pattern.

### *Hydrocarbon Interferences*

Many samples contain a UCM in the  $n\text{-C}_{18}$  to  $n\text{-C}_{40}$  range (Figure 2d); however, the presence of natural organic matter (NOM) sometimes obscures the UCM in approximately the  $n\text{-C}_{25}$  to  $n\text{-C}_{40}$  range (Figure 2e). The NOM exhibits several features. The chemical signature of plant waxes (*i.e.*,  $n$ -alkanes in the  $n\text{-C}_{21}$  to  $n\text{-C}_{37}$  range with higher proportions of odd carbon numbers) appears “saw-toothed” (Tissot and Welte 1978; Kennicutt and Comet 1992). Samples with high proportions of Macondo oil typically exhibit petrogenic 3- to 4- ring PAHs. Some samples demonstrate the presence of NOM in the form of



enriched concentrations of diagenetic PAHs, such as RET and PER (Figure 2e). Background material can also appear in the form of 2- to 6-ring pyrogenic PAHs. As the Macondo oil mixes with ambient sediment, the resulting patterns of normal alkanes and PAHs exhibit progressively greater influence from the plant waxes, diagenetic, and pyrogenic PAHs attributed to NOM and combustion byproducts. For this reason, the forensic role of *n*-alkane and PAH signatures in the nearshore sediments is primarily used to evaluate weathering and mixing when the potential Macondo oil signatures are obscured by the hydrocarbons in the ambient sediment.

### ***Geochemical Biomarker Interferences***

The pattern of triterpanes and steranes provides the primary means for recognizing Macondo oil in samples with heavily weathered hydrocarbons and mixed origins, because these compounds resist weathering and exhibit minimal interferences (Peters et al. 2005; Wang et al 2006), as seen in Figure 2d. The Macondo oil biomarker signature is characterized by enriched steranes relative to triterpanes plus many distinct DR patterns. However, some nearshore sediment samples containing high proportions of NOM and background hydrocarbons can contain triterpenoid interferences (Figures 2e). The biomarkers that exhibit the most variability from interferences include T14b, T16, T17, T18, T20, T26, T32, T33, and S18. Some of the interferences are likely associated with the diagenesis of detrital vegetation and other organic material, while other interferences are likely associated with ambient petroleum residues (Yunker et al 2011; Wang et al 2009; Simoneit 1986; Dembicki 2010).

### ***Pom-Pom Sampling Artifacts***

Pom-poms are composed of polyesters, plasticizers, and other synthetic materials that are hydrophobic, buoyant, and well suited for adsorbing petroleum droplets within the water column. Unfortunately, the pom-pom matrix dissolves in organic solvents (such as DCM) and produces complex patterns of organic interferences as shown in Figure 3. Pom-pom samples containing weathered Macondo oil demonstrate hydrocarbon patterns consistent with those observed in other matrices; however, there are some abnormalities that need to be considered. First, the hydrocarbon fingerprint does not reveal a distinct UCM as is evident in the SHC profile of sediments and other matrices. Instead, the extractable pom-pom hydrocarbons produce a chromatogram with a broad range of interferences eluting throughout the light, middle, and heavy hydrocarbon ranges (Figures 3a and 3b). As a result, high resolution hydrocarbon fingerprints generated by GC/FID are not used as a line of evidence for identifying Macondo oil in pom-pom samples. Second, interferences also appear in the triterpane pattern, specifically, the homohopanes from T26 to T35 (Figures 3c and 3d). Third, minor interferences occurred in the sterane pattern near S12 and S17 (Figure 3e and 3f). However, the nearshore samples containing petroleum exhibit abundances of S12 and S17 several orders of magnitude greater than the trace interferences in the unspiked pom-pom sample. Therefore, most hopane and sterane biomarkers are not affected by the pom-pom interferences and serve well for identifying Macondo oil in pom-pom samples.

### ***Pre-Oil Samples and Ambient Baseline Conditions***

Field teams collected samples from the nearshore environment to establish baseline conditions before the impact of Macondo oil. The unexpected nature of the spill dictated that the field teams did not have perfect knowledge about the exact timing of Macondo oil impacts in the nearshore environment. It was difficult to determine the first incidence of Macondo oil impacts aside from the obvious visual or olfactory evidence of gross contamination at the time of sample collection. Consequently, other methods for determining the first contact of Macondo oil in the nearshore environment were needed to identify a population of nearshore samples that represent the pre-Macondo oil condition.



One of the more reliable methods for estimating the first arrival of Macondo oil is Synthetic Aperture Radar (SAR). It provides high resolution imagery of large surface areas, regardless of inclement weather or time of day. SAR involves several components. First, a radar antenna attached to an aircraft emits electromagnetic energy towards Earth where it is reflected off of objects on the Earth back to the aircraft antenna. This data is then transmitted again to another antenna on Earth, where it is processed into a depiction of the oil footprint. Oil appears as a dark area on the SAR image, because it masks the backscatter from the ocean's surface (Alpers 2008; Brekke and Solberg 2005). In this way, the SAR data is used to determine when and where the Macondo oil reached land with a high level of confidence.

Federal and state field teams mobilized quickly in an attempt to collect samples representative of the ambient conditions before the arrival of Macondo oil. The SAR results demonstrated that some of these samples were likely collected before the oil first impacted the shoreline, but other samples were possibly collected after exposure to Macondo oil. As a conservative measure, field observations and SAR data are used to identify the Pre-Oil samples for the purpose of this investigation. The field teams collected a total of 176 Pre-Oil sediments in Louisiana, Mississippi, Alabama and Florida. These samples were initially subjected to the chemical fingerprinting methods discussed herein without prior knowledge that these samples were in the Pre-Oil category.

These samples provided a range of ambient hydrocarbon signatures that pre-date the arrival of the Macondo oil. The fingerprinting method described herein determined that all 176 of the Pre-Oil samples were associated with Classification Code D. These findings confirmed that the Pre-Oil samples were unaffected by Macondo oil at the time of the sample collection.

The Pre-Oil samples were collected between April 29 and July 15, 2010 and were from a combination of studies:

- FLDEP—Baseline—Early May 2010/FLDEP—Baseline—Late May 2010
- MDEQ Preassessment Early May 2010/MDEQ Preassessment Late April 2010
- Nearshore Sed&Water—Baseline—Early July 2010/Nearshore Sed&Water—Baseline—Late June 2010
- SAV—Baseline-Tier 1—2010
- Shoreline—Baseline—2010

These Pre-Oil samples exemplify the hydrocarbon mixtures in sediments before the spill occurred. They offer “baseline” fingerprints that are considered when assigning match codes to potentially oiled samples (Emsbo-Mattingly and Martin 2015). The SHC fingerprint often exhibits pyrogenic PAHs and a late eluting UCM that is consistent with urban runoff (Stout et al. 2004). In remote and wetland areas, the SHC fingerprint often exhibits NOM in sediments (Stout et al. 2007). The saturated hydrocarbon chromatogram (m/z 85) from a typical background sample features a dominant pattern of NOM with little UCM. The PAH fingerprints of the baseline samples are frequently enriched in pyrogenic PAHs with lower proportions of petrogenic PAHs (Stout et al. 2004). Geochemical biomarkers are either present at very low levels or largely not detected. These results and patterns are used when interpreting samples collected after oil may have reached the shoreline. They both help identify other likely background samples (e.g., Match D samples greater than 100m from a SCAT oiled shoreline or a Match A/B stranded oil sample). They also help account for residual biomarkers and PAHs that might otherwise obscure the chemical fingerprint of Macondo oil.



### *Recognizing the Macondo Source Signature in Nearshore Tissues*

The procedure for identifying Macondo oil in benthic tissue samples is described in (Douglas and Liu 2015). This procedure is very similar to the nearshore classification method for solid and pom-pom samples described previously. The DRs from the field sample are compared to reference samples of fresh and weathered Macondo oil (in the case of benthic tissue, to weathered oil in deep-sea sediments). The procedure for benthic tissue samples also considers possible changes in the source signature and DRs attributed to metabolic processes as evidenced in tissue samples with known exposure to Macondo oil; e.g., the red crab tissue samples collected near the well-head. Additional flexibility is also required, because the laboratories generated less forensic data for tissue samples. For example, PAH concentrations are determined for all of the designated tissue samples; however, only a limited number of tissue samples are fractionated and analyzed for geochemical biomarker analysis. In addition, high resolution GC/FID fingerprints are not generated for tissue samples because the biogenic interferences (e.g., natural fats and oils) often obscure the hydrocarbon signature of Macondo oil. Recognizing the potential of metabolic changes and the limited availability of forensic data, the match classification procedure for nearshore tissues requires several modifications.

The match determination procedure for nearshore tissue samples employs quantitative and qualitative criteria. A summary of the modified nearshore tissue classification codes follow:

- Classification Code A – The geochemical biomarker pattern of the tissue sample matches a Macondo oil reference sample (e.g., fresh, weathered, or exposed tissue);
- Classification Code B – The geochemical biomarker pattern of the field sample matches a Macondo oil reference sample (e.g., fresh, weathered, or exposed tissue) with consideration for environmental weathering and mixing or there are no geochemical biomarker results, but the PAH pattern of the field sample matches a Macondo oil reference sample (e.g., fresh, weathered, or exposed tissue) with consideration for environmental weathering and mixing;
- Classification Code C – The geochemical biomarker or PAH patterns possibly match a Macondo oil reference sample (e.g., fresh, weathered, or exposed tissue) with consideration for environmental weathering and mixing;
- Classification Code D – It is not possible to match the field and Macondo oil reference samples due to numerous non-detects or interferences; and
- Classification Code E – the geochemical biomarker pattern strongly indicates the presence of petroleum from non-Macondo oil.



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**Table 1. Nearshore Forensic Classifications Codes.**

Nearshore Classification Code	Description
A	Chemical fingerprints are consistent with fresh or weathered Macondo oil.
B	Chemical fingerprints are mostly consistent with fresh or weathered Macondo oil with differences being attributable to the effects of severe weathering and/or mixing with background hydrocarbons.
C	Chemical fingerprints are dominated by background hydrocarbons although some Macondo oil may be present.
D	Chemical fingerprints are indeterminate due to: 1) the high proportion of background or 2) the high frequency of target analyte non-detects
E	Chemical fingerprints are inconsistent with fresh or weathered Macondo oil, but enriched petroleum from a non-Macondo oil is likely present.

**Table 2. Saturated Hydrocarbon Analytes (GC/FID).**

Analytes	Abbrev	Normal Alkane	Acyclic Isoprenoid	Petroleum Wax Range	Plant Wax Range
n-Nonane	C9	X			
n-Decane	C10	X			
n-Undecane	C11	X			
n-Dodecane	C12	X			
n-Tridecane	C13	X			
n-Tetradecane	C14	X			
n-Pentadecane	C15	X			
n-Hexadecane	C16	X			
n-Heptadecane	C17	X			
n-Octadecane	C18	X			
n-Nonadecane	C19	X			
n-Eicosane	C20	X			
n-Heneicosane	C21	X		X	X
n-Docosane	C22	X		X	
n-Tricosane	C23	X		X	X
n-Tetracosane	C24	X		X	
n-Pentacosane	C25	X		X	X
n-Hexacosane	C26	X		X	
n-Heptacosane	C27	X		X	X
n-Octacosane	C28	X		X	
n-Nonacosane	C29	X		X	X
n-Triacontane	C30	X		X	
n-Hentriacontane	C31	X		X	X
n-Dotriacontane	C32	X		X	
n-Tritriacontane	C33	X		X	X
n-Tetracontane	C34	X		X	
n-Pentatriacontane	C35	X		X	X
n-Hexatriacontane	C36	X		X	
n-Heptatriacontane	C37	X		X	X
n-Octatriacontane	C38	X		X	
n-Nonatriacontane	C39	X		X	X
n-Tetracontane	C40	X		X	
n-Hentetracontane	C41	X		X	
n-Dotetracontane	C42	X		X	
n-Tritetracontane	C43	X		X	
n-Tetratetracontane	C44	X		X	
n-Pentatetracontane	C45	X		X	
2,6,10 Trimethyldodecane	1380		X		
2,6,10 Trimethyltridecane	1470		X		
Norpristane	1650		X		
Pristane	Pr		X		
Phytane	Ph		X		
Total Petroleum Hydrocarbons (C <sub>9</sub> -C <sub>44</sub> )	TPH				
Total	43	37	5	25	10

**Table 3. Polycyclic Aromatic Hydrocarbon Analytes (GC/MS SIM).**

Analytes	Abbrev	Rings	TPAH50	PetPAH27	EPAPAH16	Parent	Alkylated	Diagenetic
Naphthalene	N0	2	X		X	X		
C1-Naphthalenes	N1	2	X	X			X	
C2-Naphthalenes	N2	2	X	X			X	
C3-Naphthalenes	N3	2	X	X			X	
C4-Naphthalenes	N4	2	X	X			X	
Biphenyl	B	2	X			X		
Acenaphthylene	AY	3	X		X	X		
Acenaphthene	AE	3	X		X	X		
Fluorene	F0	3	X		X	X		
Dibenzofuran	DF	3	X			X		
C1-Fluorenes	F1	3	X	X			X	
C2-Fluorenes	F2	3	X	X			X	
C3-Fluorenes	F3	3	X	X			X	
Anthracene	A0	3	X		X	X		
Phenanthrene	P0	3	X		X	X		
C1-Phenanthrenes/Anthracenes	PA1	3	X	X			X	
C2-Phenanthrenes/Anthracenes	PA2	3	X	X			X	
C3-Phenanthrenes/Anthracenes	PA3	3	X	X			X	
C4-Phenanthrenes/Anthracenes	PA4	3	X	X			X	
Retene	RET	3						X
Dibenzothiophene	DBT0	3	X			X		
C1-Dibenzothiophenes	DBT1	3	X	X			X	
C2-Dibenzothiophenes	DBT2	3	X	X			X	
C3-Dibenzothiophenes	DBT3	3	X	X			X	
C4-Dibenzothiophenes	DBT4	3	X	X			X	
Benzo(b)fluorene	BF	4				X		
Fluoranthene	FL0	4	X		X	X		
Pyrene	PY0	4	X		X	X		
C1-Fluoranthenes/Pyrenes	FP1	4	X	X			X	
C2-Fluoranthenes/Pyrenes	FP2	4	X	X			X	
C3-Fluoranthenes/Pyrenes	FP3	4	X	X			X	
C4-Fluoranthenes/Pyrenes	FP4	4	X	X			X	
Naphthobenzothiophenes	NBT0	4	X			X		
C1-Naphthobenzothiophenes	NBT1	4	X	X			X	
C2-Naphthobenzothiophenes	NBT2	4	X	X			X	
C3-Naphthobenzothiophenes	NBT3	4	X	X			X	
C4-Naphthobenzothiophenes	NBT4	4	X	X			X	
Benz[a]anthracene	BA0	4	X		X	X		
Chrysene/Triphenylene	C0	4	X		X	X		
C1-Chrysenes	BC1	4	X	X			X	
C2-Chrysenes	BC2	4	X	X			X	
C3-Chrysenes	BC3	4	X	X			X	
C4-Chrysenes	BC4	4	X	X			X	
Benzo[b]fluoranthene	BBF	5	X		X	X		
Benzo[k]fluoranthene	BJKF	5	X		X	X		
Benzo[a]fluoranthene	BAF	5	X			X		
Benzo[e]pyrene	BEP	5	X			X		
Benzo[a]pyrene	BAP	5	X		X	X		
Perylene	PER	5						X
Dibenz[a,h]anthracene	DA	5	X		X	X		
Indeno[1,2,3-cd]pyrene	IND	6	X		X	X		
Benzo[g,h,i]perylene	GHI	6	X		X	X		
Total		52	49	27	16	23	27	2

**Table 4. Geochemical Biomarker Analytes (GC/MS SIM).**

Analyte	Abbrev	Tricyclic Triterpane	Tetracyclic Triterpane	Pentacyclic Triterpane	Sterane	Triaromatic Steroids
C23 Tricyclic Terpane	T4	X				
C24 Tricyclic Terpane	T5	X				
C25 Tricyclic Terpane	T6	X				
C24 Tetracyclic Terpane	T6a		X			
C26 Tricyclic Terpane-22S	T6b	X				
C26 Tricyclic Terpane-22R	T6c	X				
C28 Tricyclic Terpane-22S	T7	X				
C28 Tricyclic Terpane-22R	T8	X				
C29 Tricyclic Terpane-22S	T9	X				
C29 Tricyclic Terpane-22R	T10	X				
C30 Tricyclic Terpane-22S	T11a	X				
C30 Tricyclic Terpane-22R	T11b	X				
18 $\alpha$ -22,29,30-Trisnorneohopane (Ts)	T11			X		
17 $\alpha$ (H)-22,29,30-Trisnorhopane (Tm)	T12			X		
17 $\alpha$ / $\beta$ ,21 $\beta$ / $\alpha$ 28,30-Bisnorhopane	T14a			X		
17 $\alpha$ (H),21 $\beta$ (H)-25-Norhopane	T14b			X		
30-Norhopane (H29)	T15			X		
18 $\alpha$ (H)-30-Norneohopane (H29Ts)	T16			X		
17 $\alpha$ (H)-Diahopane	X			X		
30-Normoretane	T17			X		
18 $\alpha$ (H)&18 $\beta$ (H)-Oleananes	T18			X		
Hopane (H)	T19			X		
Moretane (M)	T20			X		
30-Homohopane-22S	T21			X		
30-Homohopane-22R	T22			X		
T22 $\alpha$ -Gammacerane/C32-diahopane	T22A			X		
30,31-Bishomohopane-22S	T26			X		
30,31-Bishomohopane-22R	T27			X		
30,31-Trishomohopane-22S	T30			X		
30,31-Trishomohopane-22R	T31			X		
Tetrakishomohopane-22S	T32			X		
Tetrakishomohopane-22R	T33			X		
Pentakishomohopane-22S	T34			X		
Pentakishomohopane-22R	T35			X		
13 $\beta$ (H),17 $\alpha$ (H)-20S-Diacholestane	S4				X	
13 $\beta$ (H),17 $\alpha$ (H)-20R-Diacholestane	S5				X	
13 $\beta$ ,17 $\alpha$ -20S-Methyldiacholestane	S8				X	
14 $\alpha$ (H),17 $\alpha$ (H)-20S-Cholestane	S12				X	
14 $\alpha$ (H),17 $\alpha$ (H)-20R-Cholestane	S17				X	
13 $\beta$ ,17 $\alpha$ -20R-Ethyldiacholestane	S18				X	
13 $\alpha$ ,17 $\beta$ -20S-Ethyldiacholestane	S19				X	
14 $\alpha$ ,17 $\alpha$ -20S-Methylcholestane	S20				X	
14 $\alpha$ ,17 $\alpha$ -20R-Methylcholestane	S24				X	
14 $\alpha$ (H),17 $\alpha$ (H)-20S-Ethylcholestane	S25				X	
14 $\alpha$ (H),17 $\alpha$ (H)-20R-Ethylcholestane	S28				X	
14 $\beta$ (H),17 $\beta$ (H)-20R-Cholestane	S14				X	
14 $\beta$ (H),17 $\beta$ (H)-20S-Cholestane	S15				X	
14 $\beta$ ,17 $\beta$ -20R-Methylcholestane	S22				X	
14 $\beta$ ,17 $\beta$ -20S-Methylcholestane	S23				X	
14 $\beta$ (H),17 $\beta$ (H)-20R-Ethylcholestane	S26				X	
14 $\beta$ (H),17 $\beta$ (H)-20S-Ethylcholestane	S27				X	
C26,20R- +C27,20S- triaromatic steroid	RC26/SC27TA					X
C28,20S-triaromatic steroid	SC28TA					X
C27,20R-triaromatic steroid	RC27TA					X
C28,20R-triaromatic steroid	RC28TA					X
Total	55	11	1	22	17	4

**Table 5. Scaling Ratios (SRs).**

Scaling Ratio	Description	Indicator Class
$\frac{T15}{(T15+T19)}$	30-norhopane relative to hopane	Biomarker-Triterpane
T19	hopane-Normalization Analyte	Biomarker-Triterpane
$\frac{S12}{(S12+T19)}$	14 $\alpha$ (H), 17 $\alpha$ (H)-20S-Cholestane relative to hopane	Biomarker-Sterane
$\frac{S17}{(S17+T19)}$	14 $\alpha$ (H), 17 $\alpha$ (H)-20R-Cholestane relative to hopane	Biomarker-Sterane
$\frac{S18}{(S18+T19)}$	13 $\beta$ , 17 $\alpha$ -20R-Ethyldiacholestane relative to hopane	Biomarker-Sterane
$\frac{S20}{(S20+T19)}$	14 $\alpha$ , 17 $\alpha$ -20S-Methyldiacholestane relative to hopane	Biomarker-Sterane
$\frac{S22}{(S22+T19)}$	14 $\beta$ , 17 $\beta$ -20R-Methylcholestane relative to hopane	Biomarker-Sterane
$\frac{S23}{(S23+T19)}$	14 $\beta$ , 17 $\beta$ -20S-Methylcholestane relative to hopane	Biomarker-Sterane
$\frac{S24}{(S24+T19)}$	14 $\alpha$ , 17 $\alpha$ -20R-Methylcholestane relative to hopane	Biomarker-Sterane
$\frac{S25}{(S25+T19)}$	14 $\alpha$ (H), 17 $\alpha$ (H)-20S-Ethylcholestane relative to hopane	Biomarker-Sterane
$\frac{S26}{(S26+T19)}$	14 $\beta$ (H), 17 $\beta$ (H)-20R-Ethylcholestane relative to hopane	Biomarker-Sterane
$\frac{S27}{(S27+T19)}$	14 $\beta$ (H), 17 $\beta$ (H)-20S-Ethylcholestane relative to hopane	Biomarker-Sterane
$\frac{S28}{(S28+T19)}$	14 $\alpha$ (H), 17 $\alpha$ (H)-20R-Ethylcholestane relative to hopane	Biomarker-Sterane

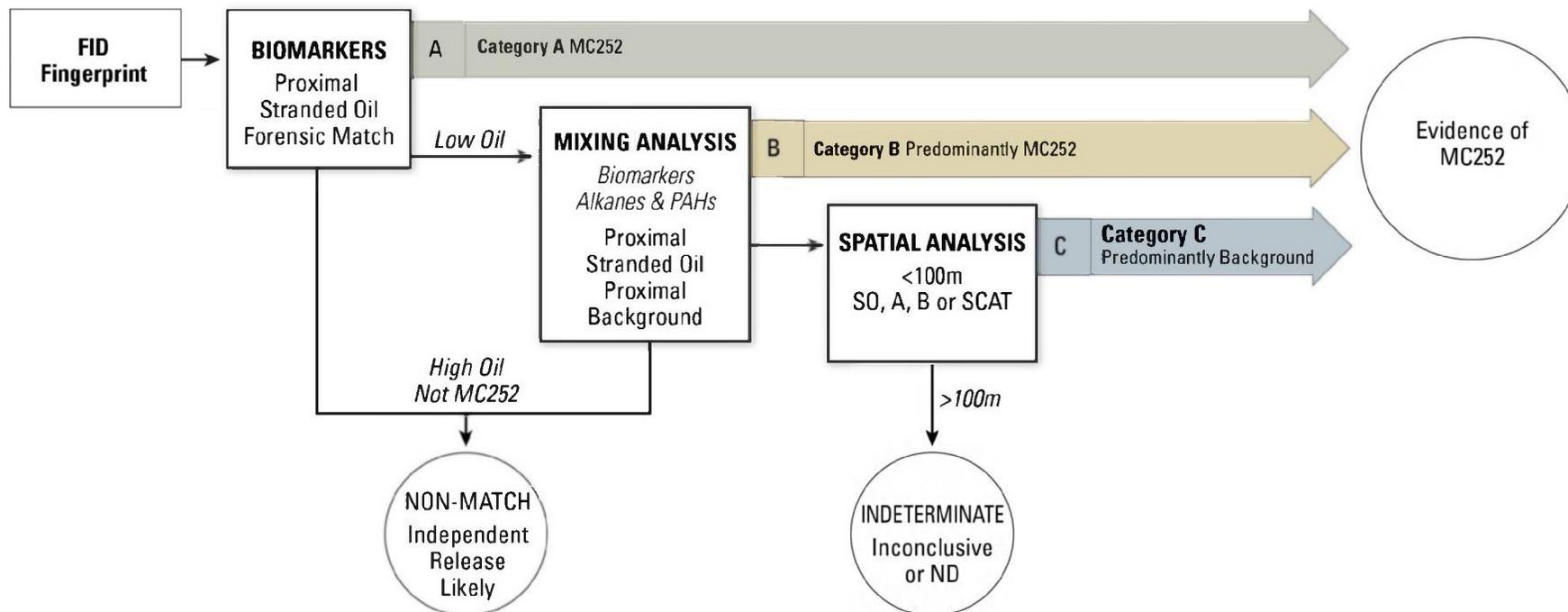
**Table 6. Diagnostic Ratios (DRs).**

Diagnostic Ratio	Description	Indicator Class
$\frac{T11}{(T11+T12)}$	18 $\alpha$ -22,29,30-trisnorneohopane (T <sub>s</sub> ) relative to 17 $\alpha$ (H)-22,29,30-trisnorhopane (T <sub>m</sub> )	Biomarker
$\frac{T16}{(T16+T15)}$	18 $\alpha$ (H)-30-norneohopane (H29Ts) relative to 30-norhopane (H29)	Biomarker
$\frac{X}{(X+T19)}$	17 $\alpha$ (H)-diahopane relative to hopane	Biomarker
$\frac{(T7+T8+T9+T10)}{(T7+T8+T9+T10+T19)}$	C28+C29 tricyclic terpanes relative to hopane	Biomarker
$\frac{T15}{(T15+T19)}$	30-norhopane relative to hopane	Biomarker
$\frac{T18}{(T18+T19)}$	oleanane relative to hopane	Biomarker
$\frac{T20}{(T20+T19)}$	moretane relative to hopane	Biomarker
$\frac{(T32+T33)}{(T32+T33+T19)}$	tetrakishomohopanes relative to hopane	Biomarker
$\frac{\text{Ster}}{(\text{Ster}+\text{Hop})}$	sum of selected steranes (S12+S17+S20+S22+S23+S24+S25+S26+S27+S28) relative to sum of selected hopanes (T11+T12+T19+T22+T27+T31+T32+T33+T34+T35)	Biomarker
$\frac{(S4+S5)}{(S4+S5+S12+S17)}$	C27-diacholestanes relative to C27-cholestanes	Biomarker
$\frac{(S14+S15)}{S14+S15+S22+S23+S26+S27}$	14 $\beta$ (H), 17 $\beta$ (H)-cholestanes relative to 14 $\beta$ (H), 17 $\beta$ (H)-methyl and ethylcholestanes	Biomarker
$\frac{(S22+S23)}{S22+S23+S26+S27}$	14 $\beta$ (H), 17 $\beta$ (H)-methylcholestanes relative to 14 $\beta$ (H), 17 $\beta$ (H)-ethylcholestanes	Biomarker
$\frac{DBT2}{(DBT2+PA2)}$	C2-dibenzothiophenes relative to C2-phenanthrenes	Petrogenic PAHs
$\frac{DBT3}{(DBT3+PA3)}$	C3-dibenzothiophenes relative to C3-phenanthrenes	Petrogenic PAHs
$\frac{NBT2}{(NBT2+BC2)}$	C2-naphthabenzothiophenes relative to C2-benz(a)anthracenes/chrysenes	Petrogenic PAHs
$\frac{NBT3}{(NBT3+BC3)}$	C3-naphthabenzothiophenes relative to C3-benz(a)anthracenes/chrysenes	Petrogenic PAHs
$\frac{BEP}{(BEP+BAP)}$	benzo(e)pyrene relative to benzo(a)pyrene	Petrogenic PAHs
$\frac{BC0}{(BC0+BC2+BC3)}$	benz(a)anthracene and chrysene relative to C2- and C3-benz(a)anthracenes/chrysenes	Pyrogenic PAHs
$\frac{(FLO+PY0)}{(FP0+FP2+FP3)}$	fluoranthene and pyrene relative to C2- and C3-fluoranthene/pyrenes	Pyrogenic PAHs
$\frac{FL0}{(FL0+PY0)}$	fluoranthene relative to pyrene	Pyrogenic PAHs
$\frac{BF}{(BF+FP1)}$	benzofluorene relative to C1-fluoranthene/pyrenes	Pyrogenic PAHs
$\frac{RET}{(RET+PA4)}$	retene relative to C4-phenanthrene/anthracenes	Diagenetic PAHs
$\frac{PER}{(PER+BAP)}$	perylene relative to benzo(a)pyrene	Diagenetic PAHs



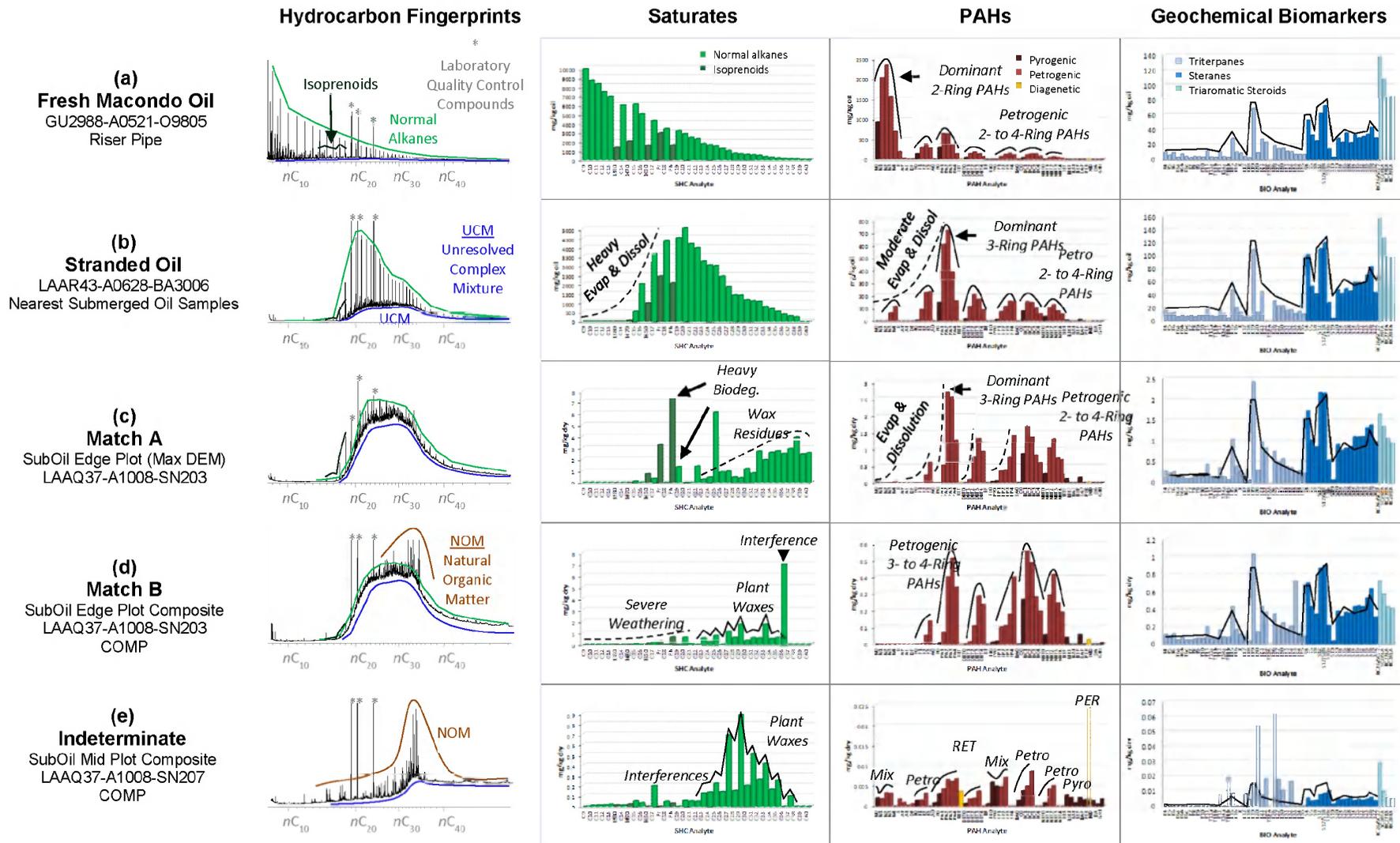
**Figure 1. Nearshore Forensic Classification Flowchart.**

Category	Description
A	Fingerprints consistent with MC252
B	Fingerprints probably MC252 with some background
C	Fingerprints predominantly background with some MC252 possible
Indeterminate	Fingerprints inconclusive (high background or NDs)
Non-Match	Fingerprints consistent with non-MC252 petroleum





**Figure 2. Macondo Oil Hydrocarbon Signatures.**





**Figure 3. Pom-Pom Interferences.**

