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**Explanation of Analytes Included in the  
Total Polycyclic Aromatic Hydrocarbon  
Sums Used by the *Deepwater Horizon*  
Natural Resource Damage Assessment Toxicity Group  
Technical Report**

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Measurements of polycyclic aromatic hydrocarbons (PAHs) and related hydrocarbons are reported by analytical laboratories as concentrations of individual PAH compounds and as concentrations of various groups of compounds (i.e., alkylated homologs). To interpret toxicity testing results for organisms exposed to complex oil mixtures, reported concentrations of certain individual components may be summed to characterize exposures in terms of a total PAH (TPAH) concentration.

This report presents a list of PAHs and related compounds that the *Deepwater Horizon* (DWH) natural resource damage assessment (NRDA) toxicity group has selected for TPAH and discusses the rationale behind this list. We refer to the set of TPAH analytes presented here as the TPAH50 set, to distinguish it from historical sets and from other sets used by DWH Trustees and researchers.

TPAH50 concentrations are an important exposure metric when interpreting and analyzing toxicity testing data; however, DWH researchers did not restrict their analysis of dose-response to TPAH50 concentrations. Depending on the specific test, species, exposure scenario, and toxicological endpoint, researchers may have expressed the toxic effects of oil as a function of an individual PAH, a subset of TPAH, or a mix of PAHs and other analytes not included in the TPAH50 list.

## 1. TPAH50 Set of Analytes

Samples generated as part of the DWH NRDA aquatic toxicity testing program were analyzed according to the *Analytical Quality Assurance Plan: Mississippi Canyon 252* (Deepwater Horizon) *Natural Resource Damage Assessment* (Analytical QAP; NOAA, 2014). Per the Analytical QAP, laboratories analyzed PAH compounds using procedures based on the EPA Method 8270D (NOAA, 2014). The analytes that the analytical laboratory report as part of this analysis include parent PAH compounds, alkyl homologs of parent PAHs, and a handful of related hydrocarbons.

In the calculation of TPAH using the TPAH50 set, some of the analytes reported by the laboratories from the 8270D method analysis are excluded from the sum (Table 1). Analytes are excluded for one of three reasons:

1. The reported analyte is not a PAH and its structure differs distinctly from PAHs (e.g., decalins, benzothiophenes, and biomarkers)
2. The PAH is already captured in another reported group of PAHs, and thus inclusion would effectively constitute double-counting (e.g., retene is a C4-phenanthrene)
3. In non-oil matrices, the potential biogenic source of the PAH in the environment could significantly confound TPAH summations (e.g., perylene).

**Table 1. Categorization of analytes measured by EPA Method 8270D to support the definition of the TPAH50 analyte set**

| Item | Reported analyte   | TPAH50<br>analyte list | Included in another<br>analyte group | Not a PAH | Dominant<br>source biogenic |
|------|--------------------|------------------------|--------------------------------------|-----------|-----------------------------|
| 1    | cis/trans-Decalin  |                        |                                      | X         |                             |
| 2    | C1-Decalins        |                        |                                      | X         |                             |
| 3    | C2-Decalins        |                        |                                      | X         |                             |
| 4    | C3-Decalins        |                        |                                      | X         |                             |
| 5    | C4-Decalins        |                        |                                      | X         |                             |
| 6    | Benzo(b)thiophene  |                        |                                      | X         |                             |
| 7    | C1-Benzothiophenes |                        |                                      | X         |                             |
| 8    | C2-Benzothiophenes |                        |                                      | X         |                             |
| 9    | C3-Benzothiophenes |                        |                                      | X         |                             |
| 10   | C4-Benzothiophenes |                        |                                      | X         |                             |
| 11   | Naphthalene        | X                      |                                      |           |                             |
| 12   | C1-Naphthalenes    | X                      |                                      |           |                             |
| 13   | C2-Naphthalenes    | X                      |                                      |           |                             |
| 14   | C3-Naphthalenes    | X                      |                                      |           |                             |
| 15   | C4-Naphthalenes    | X                      |                                      |           |                             |
| 16   | Biphenyl           | X                      |                                      |           |                             |
| 17   | Dibenzofuran       | X                      |                                      |           |                             |
| 18   | Acenaphthylene     | X                      |                                      |           |                             |
| 19   | Acenaphthene       | X                      |                                      |           |                             |
| 20   | Fluorene           | X                      |                                      |           |                             |
| 21   | C1 – Fluorenes     | X                      |                                      |           |                             |

**Table 1. Categorization of analytes measured by EPA Method 8270D to support the definition of the TPAH50 analyte set (cont.)**

| Item | Reported analyte             | TPAH50 analyte list | Included in another analyte group | Not a PAH | Dominant source biogenic |
|------|------------------------------|---------------------|-----------------------------------|-----------|--------------------------|
| 22   | C2 – Fluorenes               | X                   |                                   |           |                          |
| 23   | C3 – Fluorenes               | X                   |                                   |           |                          |
| 24   | Anthracene                   | X                   |                                   |           |                          |
| 25   | Phenanthrene                 | X                   |                                   |           |                          |
| 26   | C1-Phenanthrenes/Anthracenes | X                   |                                   |           |                          |
| 27   | C2-Phenanthrenes/Anthracenes | X                   |                                   |           |                          |
| 28   | C3-Phenanthrenes/Anthracenes | X                   |                                   |           |                          |
| 29   | C4-Phenanthrenes/Anthracenes | X                   |                                   |           |                          |
| 30   | Retene <sup>a</sup>          |                     | X                                 |           |                          |
| 31   | Dibenzothiophene             | X                   |                                   |           |                          |
| 32   | C1 – Dibenzothiophenes       | X                   |                                   |           |                          |
| 33   | C2 – Dibenzothiophenes       | X                   |                                   |           |                          |
| 34   | C3 – Dibenzothiophenes       | X                   |                                   |           |                          |
| 35   | C4 – Dibenzothiophenes       | X                   |                                   |           |                          |
| 36   | Benzo(b)fluorene             | X                   |                                   |           |                          |
| 37   | Fluoranthene                 | X                   |                                   |           |                          |
| 38   | Pyrene                       | X                   |                                   |           |                          |
| 39   | C1 – Fluoranthenes/Pyrenes   | X                   |                                   |           |                          |
| 40   | C2 – Fluoranthenes/Pyrenes   | X                   |                                   |           |                          |
| 41   | C3 – Fluoranthenes/Pyrenes   | X                   |                                   |           |                          |

**Table 1. Categorization of analytes measured by EPA Method 8270D to support the definition of the TPAH50 analyte set (cont.)**

| Item | Reported analyte                      | TPAH50 analyte list | Included in another analyte group | Not a PAH | Dominant source biogenic |
|------|---------------------------------------|---------------------|-----------------------------------|-----------|--------------------------|
| 42   | C4-Fluoranthenes/Pyrenes              | X                   |                                   |           |                          |
| 43   | Naphthobenzothiophene                 | X                   |                                   |           |                          |
| 44   | C1-Naphthobenzothiophenes             | X                   |                                   |           |                          |
| 45   | C2-Naphthobenzothiophenes             | X                   |                                   |           |                          |
| 46   | C3-Naphthobenzothiophenes             | X                   |                                   |           |                          |
| 47   | C4-Naphthobenzothiophenes             | X                   |                                   |           |                          |
| 48   | Benz(a)anthracene                     | X                   |                                   |           |                          |
| 49a  | Chrysene <sup>b</sup>                 | X                   |                                   |           |                          |
| 49b  | Chrysene + Triphenylene <sup>b</sup>  | X                   |                                   |           |                          |
| 50   | C1 – Chrysenes                        | X                   |                                   |           |                          |
| 51   | C2 – Chrysenes                        | X                   |                                   |           |                          |
| 52   | C3 – Chrysenes                        | X                   |                                   |           |                          |
| 53   | C4 – Chrysenes                        | X                   |                                   |           |                          |
| 54a  | Benzo(b)fluoranthene <sup>b</sup>     | X                   |                                   |           |                          |
| 54b  | Benzo(b + j)fluoranthene <sup>b</sup> | X                   |                                   |           |                          |
| 55a  | Benzo(j + k)fluoranthene <sup>b</sup> | X                   |                                   |           |                          |
| 55b  | Benzo(k)fluoranthene <sup>b</sup>     | X                   |                                   |           |                          |
| 56   | Benzo(a)fluoranthene                  | X                   |                                   |           |                          |
| 57   | Benzo(e)pyrene                        | X                   |                                   |           |                          |
| 58   | C30-Hopane                            |                     |                                   | X         |                          |

**Table 1. Categorization of analytes measured by EPA Method 8270D to support the definition of the TPAH50 analyte set (cont.)**

| Item | Reported analyte                         | TPAH50 analyte list | Included in another analyte group | Not a PAH | Dominant source biogenic |
|------|--|---------------------|-----------------------------------|-----------|--------------------------|
| 59   | Benzo(a)pyrene                           | X                   |                                   |           |                          |
| 60   | Perylene                                 |                     |                                   |           | X                        |
| 61   | Indeno(1,2,3-cd)pyrene                   | X                   |                                   |           |                          |
| 62a  | Dibenz(a,h)anthracene <sup>b</sup>       | X                   |                                   |           |                          |
| 62b  | Dibenz(a,h + a,c)anthracene <sup>b</sup> | X                   |                                   |           |                          |
| 63   | Benzo(g,h,i)perylene                     | X                   |                                   |           |                          |
| 64   | 4-Methyldibenzothiophene                 |                     | X                                 |           |                          |
| 65   | 2/3-Methyldibenzothiophene               |                     | X                                 |           |                          |
| 66   | 1-Methyldibenzothiophene                 |                     | X                                 |           |                          |
| 67   | 3-Methylphenanthrene                     |                     | X                                 |           |                          |
| 68   | 2-Methylphenanthrene                     |                     | X                                 |           |                          |
| 69   | 2-Methylanthracene                       |                     | X                                 |           |                          |
| 70   | 4/9-Methylphenanthrene                   |                     | X                                 |           |                          |
| 71   | 1-Methylphenanthrene                     |                     | X                                 |           |                          |
| 72   | 2-Methylnaphthalene                      |                     | X                                 |           |                          |
| 73   | 1-Methylnaphthalene                      |                     | X                                 |           |                          |
| 74   | 2,6-Dimethylnaphthalene                  |                     | X                                 |           |                          |
| 75   | 2,3,5-Trimethylnaphthalene               |                     | X                                 |           |                          |
| 76   | Carbazole                                |                     |                                   | X         |                          |

a. Retene is a component of measurements of C4-Phenanthrenes/Anthracenes.

b. Analytes numbered as "a" and "b" pairs are subject to co-elution, and while reported differently by different analytical laboratories, are typically synonymous. Only one concentration from the "a" and "b" pairs should be reported for any individual sample.

## 1.1 What Are PAHs

While oil is composed of thousands of organic compounds, PAHs are of particular interest when evaluating the impact of oil as they are typically considered the more toxic fraction (NRC, 2005). A true PAH compound is defined as a hydrocarbon with at least two fused aromatic rings (Figure 1); however, researchers assessing oil toxicity often include closely related heterocyclic compounds (i.e., hydrocarbons where there have been one or more sulfur, nitrogen, or oxygen substitutions for carbon in the aromatic rings) with true PAHs (NRC, 2005).

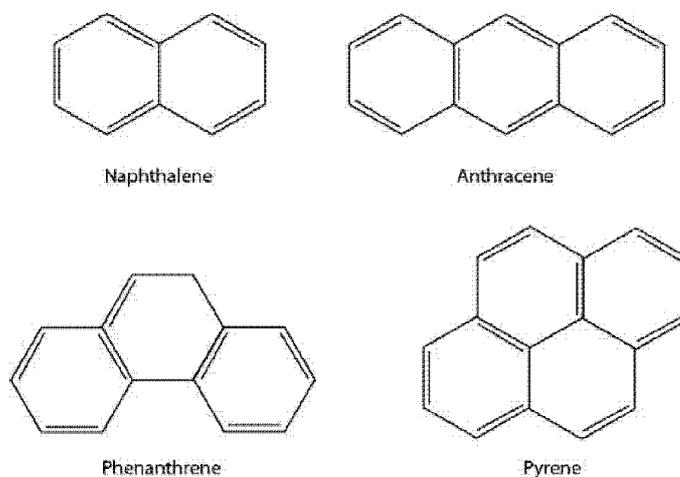


Figure 1. The chemical structures of four PAHs.

## 1.2 Related Hydrocarbons

A number of the laboratory-reported analytes were excluded from the TPAH50 set because they are not PAHs – a hydrocarbon with at least two fused aromatic rings. Hydrocarbons that we excluded were decalins (parent + alkyl homologs), benzothiophenes (parent + alkyl homologs), and C30-hopane (and other biomarkers, if reported). We included biphenyl, dibenzofuran, dibenzothiophene compounds, and naphthobenzothiophene compounds; although they are arguably not true PAHs, their structures are similar to PAHs, and therefore their modes of toxicity are likely to be similar to PAHs (NRC, 2005).

## 1.3 Treatment of Grouped Analytes

For several alkylated PAHs, the analytical laboratory reports concentrations for both the individual PAH and the grouped alkyl homolog PAH concentrations. For example, the laboratory

reports concentrations for 1-methylnaphthalene and 2-methylnaphthalene individually, as well as for the entire C1-naphthalenes group (which includes 1-methylnaphthalene and 2-methylnaphthalene). In our TPAH50 summation, we have included the PAH homolog groups (such as C1-naphthalenes) and excluded the individual alkylated PAHs to avoid double-counting (Table 1).

Although C1-naphthalenes should represent the sum of methylnaphthalene concentrations, reported C1-naphthalene concentrations do not always equal the sum of the individual concentrations of 1- and 2-methylnaphthalene. The reason for this is the grouped alkylates (C1–C4) are measured using a response factor based on the peak of the parent PAH compound (as per the Analytical QAP). In contrast, the individual compounds are measured by comparing them to a calibration curve from standards for each individual compound. Given that all other alkylated compounds are measured using the parent response factors, the grouped naphthalene alkylated homologs were included with the TPAH50 set instead of with the individual naphthalene alkylates for consistency [see the Analytical QAP (NOAA, 2014) for further discussion on the quantification of these analytes].

## 1.4 Biogenic PAHs

Perylene can be produced through natural biogenic processes (Burgess et al., 2003; Silliman et al., 2001) and thus, at times, it can be the dominant PAH found in an environmental sample (particularly sediments). On the other hand, perylene only makes up approximately 0.02% of the PAHs found in Mississippi Canyon Block 252 (MC252) source oil (Stout, 2015). Because elevated perylene in an environmental sample may not arise from any oil present in the sample, and since perylene from the oil is expected to have minimal impact on TPAH50 sums, we excluded perylene from the TPAH50 list.

## 1.5 Comparison of TPAH50 List to Other DWH TPAH Lists

The list of analytes included in various TPAH sums has evolved over time and has been adapted to fit the needs of different disciplines. Consequently, the TPAH50 list is not the only list that DWH researchers have used to report total PAH concentrations. For example, some toxicity testing data have historically been reported using a sum of 40 PAH analytes. To compare DWH toxicity results to this previous research, some investigators have reported their results using both the TPAH50 list and the TPAH40 list (Incardona et al., 2014). In addition, for fingerprinting and weathering analyses of oils where background PAHs are not a factor, DWH researchers used a TPAH51 list that includes the same 50 analytes in TPAH50, plus the addition of perylene (Stout, 2015). Finally, in some cases samples were not analyzed for the full suite of PAH analytes described in the Analytical QAP (NOAA, 2014), and thus TPAH sums for these samples included a smaller list of analytes. For example, the U.S. Department of the Interior's sturgeon

work used an in-house laboratory that analyzed for 33 PAHs (USFWS, 2015). Table 2 contains a complete list of the analytes in TPAH50, TPAH51, TPAH40, and TPAH33.

**Table 2. List of measured PAH analytes included in different TPAH sums**

| TPAH50/TPAH51                | TPAH40                       | TPAH33                |
|------------------------------|------------------------------|-----------------------|
| Naphthalene                  | Naphthalene                  | Naphthalene           |
| C1-Naphthalenes              | C1-Naphthalenes              | a                     |
| C2-Naphthalenes              | C2-Naphthalenes              | a                     |
| C3-Naphthalenes              | C3-Naphthalenes              | a                     |
| C4-Naphthalenes              | C4-Naphthalenes              | a                     |
| Biphenyl                     | Biphenyl                     | Biphenyl              |
| Dibenzofuran                 | a                            | a                     |
| Acenaphthylene               | Acenaphthylene               | Acenaphthylene        |
| Acenaphthene                 | Acenaphthene                 | Acenaphthene          |
| Fluorene                     | Fluorene                     | Fluorene              |
| C1 – Fluorenes               | C1 – Fluorenes               | a                     |
| C2 – Fluorenes               | C2 – Fluorenes               | a                     |
| C3 – Fluorenes               | C3 – Fluorenes               | a                     |
| Anthracene                   | Anthracene                   | Anthracene            |
| Phenanthrene                 | Phenanthrene                 | Phenanthrene          |
| C1-Phenanthrenes/Anthracenes | C1-Phenanthrenes/Anthracenes | a                     |
| C2-Phenanthrenes/Anthracenes | C2-Phenanthrenes/Anthracenes | a                     |
| C3-Phenanthrenes/Anthracenes | C3-Phenanthrenes/Anthracenes | a                     |
| C4-Phenanthrenes/Anthracenes | C4-Phenanthrenes/Anthracenes | a                     |
| Dibenzothiophene             | Dibenzothiophene             | Dibenzothiophene      |
| C1-Dibenzothiophenes         | C1-Dibenzothiophenes         | a                     |
| C2-Dibenzothiophenes         | C2-Dibenzothiophenes         | a                     |
| C3-Dibenzothiophenes         | C3-Dibenzothiophenes         | a                     |
| C4-Dibenzothiophenes         | C4-Dibenzothiophenes         | a                     |
| Benzo(b)fluorene             | a                            | a                     |
| Fluoranthene                 | Fluoranthene                 | Fluoranthene          |
| Pyrene                       | Pyrene                       | Pyrene                |
| C1-Fluoranthenes/Pyrenes     | C1-Fluoranthenes/Pyrenes     | a                     |
| C2-Fluoranthenes/Pyrenes     | a                            | a                     |
| C3-Fluoranthenes/Pyrenes     | a                            | a                     |
| C4-Fluoranthenes/Pyrenes     | a                            | a                     |
| Naphthobenzothiophene        | a                            | Naphthobenzothiophene |

**Table 2. List of measured PAH analytes included in different TPAH sums (cont.)**

| TPAH50/TPAH51             | TPAH40                   | TPAH33                     |
|---------------------------|--------------------------|----------------------------|
| C1-Naphthobenzothiophenes | a                        | a                          |
| C2-Naphthobenzothiophenes | a                        | a                          |
| C3-Naphthobenzothiophenes | a                        | a                          |
| C4-Naphthobenzothiophenes | a                        | a                          |
| Benz(a)anthracene         | Benz(a)anthracene        | Benz(a)anthracene          |
| Chrysene + Triphenylene   | Chrysene + Triphenylene  | Chrysene + Triphenylene    |
| C1-Chrysenes              | C1-Chrysenes             | a                          |
| C2-Chrysenes              | C2-Chrysenes             | a                          |
| C3-Chrysenes              | C3-Chrysenes             | a                          |
| C4-Chrysenes              | C4-Chrysenes             | a                          |
| Benzo(b)fluoranthene      | Benzo(b)fluoranthene     | Benzo(b)fluoranthene       |
| Benzo(j + k)fluoranthene  | Benzo(j + k)fluoranthene | Benzo(j + k)fluoranthene   |
| Benzo(a)fluoranthene      | a                        | a                          |
| Benzo(e)pyrene            | Benzo(e)pyrene           | Benzo(e)pyrene             |
| Benzo(a)pyrene            | Benzo(a)pyrene           | Benzo(a)pyrene             |
| Indeno(1,2,3-cd)pyrene    | Indeno(1,2,3-cd)pyrene   | Indeno(1,2,3-cd)pyrene     |
| Dibenz(a,h)anthracene     | Dibenz(a,h)anthracene    | Dibenz(a,h)anthracene      |
| Benzo(g,h,i)perylene      | Benzo(g,h,i)perylene     | Benzo(g,h,i)perylene       |
| b                         | Perylene                 | Perylene                   |
| a                         | a                        | Benzo(b)thiophene          |
| a                         | a                        | 1-methylnaphthalene        |
| a                         | a                        | 2-methylnaphthalene        |
| a                         | a                        | 1-ethylnaphthalene         |
| a                         | a                        | 1,2-dimethylnaphthalene    |
| a                         | a                        | 2,3,5-trimethylnaphthalene |
| a                         | a                        | 1-methylfluorene           |
| a                         | a                        | 2-methylphenanthrene       |
| a                         | a                        | 9-methylanthracene         |
| a                         | a                        | 3,6-dimethylphenanthrene   |
| a                         | a                        | 2-methylfluoranthene       |
| a                         | a                        | 4-methylbiphenyl           |

a. Not included in TPAH sum.

b. Included in TPAH51 sum, but not in TPAH50 sum.

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