
Method Reference: D7363-07, ASTM International

1. Scope and Application

Matrices: Sediment Pore Water

Definitions: Refer to Alpha Analytical Quality Manual.

The USEPA narcosis model for benthic organisms in sediments contaminated with polycyclic aromatic hydrocarbons (PAHs) is based on concentrations of dissolved PAHs in the interstitial water of “pore water” in sediment. This test method covers the separation of pore water from PAH-impacted sediment samples, the removal of colloids, and the subsequent measurement of dissolved concentrations of the required 20 parent PAHs and 15 groups of alkylated daughter PAHs in the pore water samples. The “35 PAHs” are determined using solid-phase microextraction (SPME) followed by Gas Chromatography/Mass Spectrometry (GC/MS) analysis in selected ion monitoring (SIM) mode. Isotopically labeled analogs of the target compounds are introduced prior to the extraction, and are used as quantification references.

The data report packages present the documentation of any method modification related to the samples tested. Depending upon the nature of the modification and the extent of intended use, the laboratory may be required to demonstrate that the modifications will produce equivalent results for the matrix. Approval of all method modifications is by one or more of the following laboratory personnel before performing the modification: Area Supervisor, Department Supervisor, Laboratory Director, or Quality Assurance Officer.

This method is restricted to use by or under the supervision of analysts experienced in the operation of the SPME GC/MS and in the interpretation of SPME GC/MS data. Each analyst must demonstrate the ability to generate acceptable results with this method by performing an initial demonstration of capability, analyzing a proficiency test sample and completing the record of training.

After initial demonstration, ongoing demonstration is based on acceptable laboratory performance of at least a quarterly laboratory control sample or acceptable performance from an annual proficiency test sample. A major modification to this procedure requires demonstration of performance. The identification of major method modification requiring performance demonstration is directed by the Quality Assurance Officer and/or Laboratory Director on a case-by-case basis.
2. Summary of Method

Pore water is generated from sediment by use of a centrifuge. It is then transferred to a 2 ml auto sampler vial for analysis. The auto sampler is used to perform SPME extraction and the subsequent injection of collected analytes into the GC/MS. The analytes are separated using a Gas Chromatograph interfaced to a Mass Spectrometer (MS). The MS is setup in single ion monitoring (SIM) to identify and quantitate the above target parameters.

2.1 Method Modifications from Reference

Add C1 – C4 Chrysenes and Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[e]pyrene, Benzo[a]pyrene, Perylene, Indeno(1,2,3-cd)pyrene, Dibenz[ah]anthracene and Benzo[g,h,i]perylene.

Note the RL’s below for 1-methylnaphthalene, 2-methylnaphthalene and homologs have incorporated the SPME factor (see reference #5) in the calculation for the RL.
3. Reporting Limits

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RL (ng/g)</th>
<th>Parameter</th>
<th>RL (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>0.8</td>
<td>Fluoranthene</td>
<td>0.5</td>
</tr>
<tr>
<td>2-Methylnaphthalene</td>
<td>0.45</td>
<td>Pyrene</td>
<td>0.5</td>
</tr>
<tr>
<td>1-Methylnaphthalene</td>
<td>0.57</td>
<td>C1-Fluoranthenes/pyrenes</td>
<td>0.98</td>
</tr>
<tr>
<td>C2-Naphthalene</td>
<td>0.28</td>
<td>Benzo[a]anthracene</td>
<td>0.2</td>
</tr>
<tr>
<td>C3-Naphthalene</td>
<td>0.46</td>
<td>Chrysene</td>
<td>0.1</td>
</tr>
<tr>
<td>C4-Naphthalene</td>
<td>0.7</td>
<td>C1-Chrysenes</td>
<td>0.16</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>0.2</td>
<td>C2-Chrysenes</td>
<td>0.35</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>0.2</td>
<td>C3-Chrysenes</td>
<td>0.87</td>
</tr>
<tr>
<td>Fluorene</td>
<td>0.1</td>
<td>C4-Chrysenes</td>
<td>2.4</td>
</tr>
<tr>
<td>C1-Fluorenes</td>
<td>0.14</td>
<td>Benzo[b]fluoranthene</td>
<td>0.1</td>
</tr>
<tr>
<td>C2-Fluorenes</td>
<td>0.17</td>
<td>Benzo[k]fluoranthene</td>
<td>0.1</td>
</tr>
<tr>
<td>C3-Fluorenes</td>
<td>1.1</td>
<td>Benzo[e]pyrene</td>
<td>0.1</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>0.2</td>
<td>Benzo[a]pyrene</td>
<td>0.1</td>
</tr>
<tr>
<td>Anthracene</td>
<td>0.2</td>
<td>Perylene</td>
<td>0.1</td>
</tr>
<tr>
<td>C1-Phenanthrenes/Anthracenes</td>
<td>0.35</td>
<td>Indeno(1,2,3-cd)pyrene</td>
<td>0.1</td>
</tr>
<tr>
<td>C2-Phenanthrenes/Anthracenes</td>
<td>0.63</td>
<td>Dibenz[ah]anthracene</td>
<td>0.1</td>
</tr>
<tr>
<td>C3-Phenanthrenes/Anthracenes</td>
<td>0.69</td>
<td>Benzo[g,h,i]perylene</td>
<td>0.1</td>
</tr>
<tr>
<td>C4-Phenanthrenes/Anthracenes</td>
<td>1.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. Interferences

Non–target hydrocarbons can cause peaks on selected ion current profiles (SICPs) intended for other PAHs. Pattern recognition must be employed for identifying interfering peaks, and peak series that should not be considered for the homolog or target PAH under concentrations. Analysts should be intimately familiar with both parent and alkyl PAH analysis in complex environmental samples. Representative samples having higher PAH concentrations should periodically be analyzed by full scan GC/MS so that pattern recognition of alkylated PAHs (and interfering species) can be verified by their full mass spectra. This procedure is particularly important for newer operators.

5. Health and Safety

The toxicity or carcinogenicity of each reagent and standard used in this method is not fully established; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. A reference file of material safety data sheets is available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available in the Chemical Hygiene Plan.

All personnel handling environmental samples known to contain or to have been in contact with municipal waste must follow safety practices for handling known disease causative agents.

Solvents, reagents, glassware and other sample processing hardware may yield discrete artifacts or elevated baselines that may cause misinterpretation of the chromatographic data. All of the materials must be demonstrated to be free from interferences under the conditions of analysis by performing
laboratory method blanks. Analysts should avoid using PVC gloves, powdered gloves, or gloves with measurable levels of phthalates.

6. Sample Collection, Preservation, Shipping and Handling

6.1 Sample Collection

Collect sample in accordance with Practices D3370 and specification D1192. Prior to shipping, the samples should be mixed well. Sieve the slurry of sediment and site water through a 2-mm screen to remove debris. If the sieved slurry is to be stored or shipped before use, store in 250 mL to 1 L jars with PTFE-lined lids.

6.2 Sample Preservation

Maintain temperature at 6°C

6.3 Sample Shipping

Ship in ice chest at 6°C

6.4 Sample Handling

Store at the laboratory in the dark at 0 to 6°C.

7. Equipment and Supplies

7.1 Centrifuge, capable of sustaining 1000 g with cups for securing 40 ml and 20 ml vials

7.2 SPME Fiber Holder, compatible with 30-um SPME fiber and compatible with either the auto sampler or the manual method

7.3 SPME Fibers, 30-um diameter, polydimethylsiloxane fibers in (PDMS) coating or equivalent

7.4 GC/MS Auto sampler, capable of SPME extraction and injection

7.5 Cleaning Port, capable of purging SPME fibers in a helium-swept atmosphere at 320°C

7.6 Gas Chromatograph, requires a split/split less injection port for capillary column, temperature program with isothermal hold

7.7 GC Column, 60mm x 0.25 mm ID x 25 um film thickness HP5-MS

7.8 Inlet Liner, 2 mm ID Islamized Glass

7.9 GC Inlet, 320°C, split less mode

7.10 Oven Program, Isothermal 5 min hold a 40°C. Ramp at 50°C/min to 110°C, followed by a temperature ramp of 12°C/min to 320°C (hold for 8 min).

7.11 Mass Spectrometer, Electron Impact ionization with the ionization energy optimized for best instrument sensitivity (typically 70 eV), stability and signal to noise ratio. Shall be capable of repetitively selectively monitoring at least 12 m/z during a period of approximately 1 s and shall meet all manufacturers’ specifications.

7.12 GC/MS Interface, The mass spectrometer (MS) shall be interfaced to the GC such that the end of the capillary column terminates within 1 cm of the ion source but does not intercept the electron or ion beam
7.13 Data System, capable of collecting, recording and sorting MS data

7.14 40ml and 20 ml vials with Teflon lined caps

7.15 10 ml Auto sampler vials

8. Reagents and Standards

Purity of Reagents – Reagent grade chemicals must be used in all tests. Unless otherwise indicated, it is intended that all reagents shall confirm to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specification are available.

Purity of Water – Unless otherwise indicated, references to water shall be understood to mean reagent water that meets the purity specifications of Type I and Type II water, presented in Specifications D1193

8.1 Internal Standard Stock Solution – A dichloromethane solution of d-PAH internal standards used for preparing spiking solutions by dilution into Acetone. Restek Cat#566783 – variable concentration (500 ug/ml – 3.3 ug/ml)

8.2 Internal Standard Spiking Solution – A dilution of the Internal Standard Stock Solution in acetone used to spike d-PAH internal standards into all samples, calibrations and blank water vials

8.3 Calibration Stock Solution – A dichloromethane solution of PAHs used for prepping calibration standards

PAH1 - Restek Cat#566782 – variable concentration (41.5 mg/ml – 0.03 mg/ml)

PAH2 – Restek Cat#567080 – 1.5 mg/ml

8.4 Calibration Spiking Solution – A series of solutions prepared by diluting the calibration stock solutions with acetone.

8.5 Calibration Working Standards – Prepared by adding internal standards, surrogates and calibration spiking solutions in reagent water

8.6 Surrogate Stock Solution – A dichloromethane solution of d-PAH used to prepare surrogate spiking solution.

8.7 Acetone

8.8 Dichloromethane

8.9 Sodium Hydroxide (NaOH) – Use a 1 M solution in reagent water

8.10 Aluminum Potassium Sulfate Dodecahydrate (AlK(SO4)2 12 H2O)

8.11 Alum Solution – 10 wt. % (wt/vol) of alum in reagent grade water
9. Quality Control

The laboratory must maintain records to document the quality of data that is generated. Ongoing data quality checks are compared with established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

9.1 Blank(s)

9.1.1 Flocculation Blank – (considered the Method Blank) Prepared as needed to assess contamination from flocculation reagents and handling. Target analytes must not be detected above 1/3 of the target performance limits (Table 2 in method) or > 20% of the associated sample result.

If contamination is detected above these levels, re-extract and re-analysis associated samples that are less than ten times the level of the contamination present in the blank.

9.1.2 Extraction and Analytical Blanks – Analyze between every sample to monitor the baseline. Target analytes must not be detected above 1/3 of the target performance limits (Table 2 in method) or > 20% of the associated sample results.

If contamination is above these levels, re-extract and re-analyze associated samples that are less than ten times the level of the contaminants in the method blank.

J Flags will be reported from RL to EDL when requested.

B Flags will be reported if analyte is detected in method blank 1/3 above performance limit.

9.2 Laboratory Control Sample (LCS)

Not applicable as per method.

9.3 Initial Calibration Verification (ICV)

Curve is validated with an independent standard run a various concentration. The ICV is run at three concentrations: 0.5 ng/ml, 1.0 ng/ml and 100 ng/ml. This is necessary to have concentrations in the curve dynamic range of all parent PAH's. The criterion is 70% - 130%.

Continuing calibration is performed daily at the beginning of a 24-h period. The injection of the first continuing calibration begins the 24-h window; within all pore water samples must be injected. Duplicate daily standards (CS3 Standard) are analyzed. Both CCVs need to pass criteria to begin analysis.

Initial curve criteria – Linearity of each PAH standard curve should be r squared > 0.99, and the relative response factor for each target compound should show a %RSD <25% for two and three ring PAHs and <30% for four ring PAHs

9.4 Continuing Calibration Verification (CCV)

1. Must be analyzed every 24 hrs
2. % D < 20% for two/three ring PAHs and < 25% for four ring PAHs
3. If either CC fails it is necessary to stop the instrument and address the reason for failure.

9.5 Matrix Spike

Not applicable.

9.6 Laboratory Duplicate
Samples and blanks run in duplicate.

9.7 Method-specific Quality Control Samples

9.7.1 Surrogates

2-Methylnaphthalene-d10 – 250 ng/ml
Benzo[a]anthracene-d12 – 2.3 ng/ml
Limits 50% - 150%

9.8 Method Sequence

The following sequence is required for analysis:

- Standard
- Standard
- Blank
- Blank
- Flocculation Blank
- Flocculation Blank
- Blank
- Blank
- Sample
- Sample
- Blank
- Blank
- Sample
- Sample
- Blank
- Blank
- Sample
- Sample
- Blank
- Blank

Note: Samples are run in duplicate

10. Procedure

10.1 Equipment Set-up

GC system setup using the following parameters:

a. GC Column Agilent HP5-MS column (0.25 um film thickness, 0.25 mm ID
b. Inlet liner 2-mm silanized glass
c. GC Inlet 320 °C splitless mode
d. Oven Program – Isothermal 5 min hold at 40 °C. Ramp at 50°C/min to 110°C, followed by a temperature ramp of 12 °C/min to 320°C. (hold for 8 min).
e. Set up SIM Groups to monitor the quantitation and internal standard ions. Optimal exact masses should be determined by monitoring 0.1 mass units near the nominal molecular weight of each PAH to determine the exact mass which gives the best signal to noise
ratio. Most ion dwell times are set at 25 ms. The higher molecular PAH's are set at 100 ms. (see method for SIM compound masses).

10.2 Initial Calibration

Prepare working stock solutions of PAHs and internal standard stock solutions of d-PAHs at the following concentrations in Acetone.

**Internal Standard:**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (ug/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene – 8</td>
<td>5</td>
</tr>
<tr>
<td>1-Methylnaphthalene-d10</td>
<td>6</td>
</tr>
<tr>
<td>Acenaphthene-d10</td>
<td>1.23</td>
</tr>
<tr>
<td>Fluorene-d10</td>
<td>1.2</td>
</tr>
<tr>
<td>Phenanthrene-d10</td>
<td>0.96</td>
</tr>
<tr>
<td>Fluoranthene-d10</td>
<td>0.93</td>
</tr>
<tr>
<td>Pyrene-d10</td>
<td>0.84</td>
</tr>
<tr>
<td>Chrysene-d12</td>
<td>0.033</td>
</tr>
</tbody>
</table>

**Target Compounds**

**PAH1 Solution**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>41.5</td>
</tr>
<tr>
<td>1-Methylnaphthalene</td>
<td>23.9</td>
</tr>
<tr>
<td>2-Methylnaphthalene</td>
<td>20.42</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>9.02</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>11</td>
</tr>
<tr>
<td>Fluorene</td>
<td>7.55</td>
</tr>
<tr>
<td>Anthracene</td>
<td>0.6</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>5.5</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>2.11</td>
</tr>
<tr>
<td>Pyrene</td>
<td>1.8</td>
</tr>
<tr>
<td>Benzo[a]anthracene</td>
<td>0.08</td>
</tr>
<tr>
<td>Chrysene</td>
<td>0.03</td>
</tr>
</tbody>
</table>

**PAH2 Solution**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>0.06</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>0.06</td>
</tr>
<tr>
<td>Benzo[e]pyrene</td>
<td>0.06</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>0.06</td>
</tr>
<tr>
<td>Perylene</td>
<td>0.06</td>
</tr>
<tr>
<td>Indeno[1,2,3-cd]pyrene</td>
<td>0.06</td>
</tr>
<tr>
<td>Benzo[g,h,i]perylene</td>
<td>0.06</td>
</tr>
<tr>
<td>Dibenzo[a,h]anthracene</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Stocks are prepared in Methylene Chloride. Spiking solutions are prepared by dilution of intermediate stocks in acetone. For calibration solutions, spiking solutions are added to reagent water.
10.3 Equipment Operation and Sample Processing

At laboratory samples and extracts are stored in the dark between 0 to 6°C.

Pore water holding time must be generated within 28 days of sediment sample collection.

Pore water must be generated and flocculated as quickly as possible, and then immediately spiked with 10ul of internal standard and surrogate solution.

Solid phase micro-extraction must be completed within 24 hrs of flocculation.

Generating Pore Water – Stir the slurry and transfer approximately 40 mL (containing a solids and liquids in proportion to the slurry provided) to a clean 40 ml vial. Cap the vial with a PTFE – Lined cap. Place the vials in the centrifuge. Spin for 30 min at 1000 g and then transfer 10ml (or what is separated) of the supernatant to a new vial.

Flocculation of Pore Water – Flocculation must be performed no more than 24 hours prior to extraction.

Prepare a Flocculation blank – 10 ml of clean reagent water in a lean vial and process the blank along with the pore water samples.

Add the working Alum solution to each pore water and QC samples. The volume of alum solution should be 1/40th of the sample volume. After the addition, swirl the vial for several rotations to incorporate the solution.

Add 3 to 5 drops of NaOH working solution to each vial. Swirl to incorporate the NaOH. Shake vials for 15 seconds. Then centrifuge for 30 minutes at 1000 g. Collect the supernatant in a clean vial.

Transfer 1.5ml into an auto sampler vial and add 10ul of internal standard.

Vials are weighed before and after adding the water sample to determine the exact sample water mass.

Extraction/Analysis of the flocculated Pore Water – Split the prepared pore water into the required replicate analysis (2) by placing 1.5ml aliquots of each into 2 ml auto sampler vials. For QC samples, add 1.5 ml of reagent water (all of the water preparation steps beginning with the centrifugation and ending with the addition of d-PAH internal standards should be conducted continuously and in the minimum amount of time possible).

Load the auto sampler following the recommended analytical sequence as stated in section 1.4. The sequence is based on a 24 hr clock. The sequence begins with the analysis of the first continuing calibration standard.

Replicate blanks are analyzed between replicate samples

10.4 Continuing Calibration

Prepare calibration standard spiking solutions. These are prepared by adding acetone to the stock to give the calibration solution concentrations (CS1 – CS4), as described below:

For CS1, take 5ul PAH1 and 5ul PAH2 stock to 100 ml in acetone.

For CS2, take 50ul PAH1 and 50ul PAH2 stock to 100 ml in acetone.

For CS3, take 25ul PAH1 and 25ul PAH2 stock to 10 ml in acetone.

For CS4, take 100ul PAH1 and 100ul PAH2 stock to 10 ml in acetone.
For 1 ppb std – take Initial Calibration working std and add 1.5ul into 250 ml Acetone.

Spike 4ul of PAH1/PAH2 solution to 1.5 ml of water. Spike 4ul of specific surrogate solution to appropriate CS concentration. Add 10ul internal standard solution to each concentration.

Replicate continuing calibration begins the 24 sequence as stated in section 1.4. The mean relative response factor for these duplicate daily calibration standards should agree with those from the 4-point (or 3-point) standard curve within 20% or the two-and three-ring PAH’s, and within 25% for the four-ring PAHs. No sample data will be reported if these calibration criteria are not met.

If the continuing calibration criteria is not met, identify the root cause, perform corrective action and repeat the continuing calibration. If the second consecutive continuing calibration does not meet acceptance criteria, additional corrective action must be performed.

10.5 Preventive Maintenance

The SPME GC/MS is maintained similar to all GC/MS as to source cleaning, column replacement, tuning and general upkeep. The difference with this system is the SPME injector as opposed to a liquid auto sampler. The SPME injector requires cleaning the SPME fiber before starting a sequence and after each analysis. This procedure is placing the SPME fiber in a fiber heater at 320 °C with 2 ml of inert gas flow.

11. Data Evaluation, Calculations and Reporting

The method is based on multi-concentration standards and internal standards. Chrysene-d10 (conc at 2.3 ng/ml) elutes on the tail of the previous peak, surrogate Benzo[a]anthracene-d12 (conc 250 ng/ml). Due to the differences in concentration (especially in higher calibration points and sample spiking level) Benzo[a]anthracene-d12 adds area to the Chrysene-d12. To integrate accurately, it is necessary to skim the Chrysene-d12 peak on the tail of the Benzo[a]anthracene and not square it.

The signal to noise (S/N) ratio for the GC signals present in every selected ion current profile (SICP) must be greater or equal 3:1 for target compounds in environmental samples an greater than or equal 10:1 for the labeled internal standards. If this criterion is not met, reanalyze the samples unless obvious matrix interference is present.

SIM windows for the homologs are set to at least 8 s before the first and 30 s after the last characteristic peaks to assure coverage of the elution range.

The data is quantitated using the internal standard technique.

Example: Naphthalene

Initial curve response factor = 1.060
IS Naphthalene-d8 = 620352
Naphthalene area count in sample= 1595215
IS Conc = 50 ng

$$1595215/620352 \times 50/1.060 = 2.57 \times 47.17 = (121.24 \text{ ng/1.5ml})/1.5\text{ml} = 80.8 \text{ ng/ml} = 80.8 \text{ ng/g}$$

If calculating 1-methylnaphthalene, 2-methylnaphthalene or a Homolog it is necessary to divide the above result by a SPME Factor are indicated in reference number 5.
12. **Contingencies for Handling Out-of-Control Data or Unacceptable Data**
   Data is reviewed and evaluated. Out-of-control data or unacceptable data is re-analyzed and not reported.

13. **Method Performance**

13.1 **Estimated Detection Limit Study (EDL)**
   Calculate based on ASTM-D 7363-7 section 14

13.2 **Demonstration of Capability Studies**
   Refer to Alpha SOP/08-12 for further information regarding IDC/DOC Generation.
   13.2.1 **Initial (IDC)**
   The analyst must make an initial, one-time, demonstration of the ability to generate acceptable accuracy and precision with this method, prior to the processing of any samples.

   13.2.2 ** Continuing (DOC)**
   The analyst must make a continuing, annual, demonstration of the ability to generate acceptable accuracy and precision with this method.

14. **Pollution Prevention and Waste Management**
   Refer to Alpha’s Chemical Hygiene Plan and Waste Management and Disposal SOP for further pollution prevention and waste management information.

15. **Referenced Documents**
   1. Chemical Hygiene Plan
   2. SOP/08-12 IDC/DOC Generation
   3. SOP/14-01 Waste Management and Disposal SOP
   4. ASTM-D 7363-07
   5. Solid-Phase Microextraction Measurement of Parent and Alkyl Polycyclic Aromatic Hydrocarbons in Milliliter Sediment Pore Water Samples and Determination of K_{doc} Values. S. Hawthorne, C. Grabanski, D. Miller and J. Kreitinger

16. **Attachments**
   None.