Environmental Samples Holding Time Studies with Alternative Preservation Strategy

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Holding Time (as defined by US EPA) = time period between sample collection and extraction

- **PAH in Water samples**
  - 7 days after collection with acid preservation and maintained at 0-6°C

- **PAH in Soil/Sediment samples**
  - 14 days after collection and maintained at 0-6°C

Opportunities:
1. Technical basis – analyte loss due to microbial degradation, volatilization, and photo oxidation
2. International sites (re-sampling with high cost)
<table>
<thead>
<tr>
<th>Matrix</th>
<th>Container</th>
<th>Preservation</th>
<th>Holding Times</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>1 Liter glass amber, PTFE lined lid</td>
<td>0 to 6°C</td>
<td>7-Days</td>
</tr>
<tr>
<td>Soil / Sediment</td>
<td>4 or 8oz glass, PTFE lined lid</td>
<td>0 to 6°C</td>
<td>14-Days</td>
</tr>
<tr>
<td>Tissue</td>
<td>Glass, PTFE lined lid or clean aluminum foil</td>
<td>Frozen -20°C</td>
<td>1 Year</td>
</tr>
</tbody>
</table>
### NOAA Deepwater Horizon Project Preservation, and Holding Times for PAHs in Water, Soil/Sediment, and Tissues

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Preservation</th>
<th>Holding Times</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Refrigeration 4°C ± 2°C Optional: Preserved with 1:1 HCL to pH &lt;2</td>
<td>7-Days if not acid preserved; 14-days if acid preserved</td>
</tr>
<tr>
<td>Soil /Sediment</td>
<td>Frozen (-20°C ± 10°C)</td>
<td>4 Years</td>
</tr>
<tr>
<td>Tissue</td>
<td>Frozen (-20°C ± 10°C)</td>
<td>4 Years</td>
</tr>
</tbody>
</table>
Problem: Condition of samples upon receipt at lab

Sampling occurred on May 17th and 20th
Samples shipped on May 28th
Samples arrived at an US lab on June 8th
Only have two blue ice package inside the cooler
No technical bases for the defined holding time requirements- it is more or less defined by the administrative requirements from 1980s

“These holding times are for sediment, water, and tissue based on guidance that is sometimes administrative rather than technical in nature”

Ref: USEPA 1995. QA/QC guidance for sampling and analysis of sediments, water and tissues for dredged material evaluations. EPA 825-B-95-001. April 1995

“Samples should be analyzed as soon as possible after collection. The times listed are the maximum times that samples may be held before the start of analysis and still be considered valid. Samples may be held for longer periods only if the permittee or monitoring laboratory has data on file to show that, for the specific types of samples under study, the analytes are stable for the longer time,”


EPA Sample Holding Time Re-Evaluation - 2005
http://www.epa.gov/esd/cmb/tasks/holding.htm
Methods
Holding Time Study Design (based on ASTM D4815)

- Low and high PAH level holding times for waters will be evaluated at pH<2 and at ambient temperature (~20°C) over a 30-day period.
- Low and high PAH level holding times for sediments will be evaluated preserved with sodium azide and at ambient temperature (~20°C) over a 3 month period.
- The number of replicates required for each time series and for each condition (low and high levels) will be determined by a Precision Study based on the RSD of 3 or 10 replicate analysis - Standard Practice for Estimation of Holding Time for Water Samples Containing Organic and Inorganic Constituents (ASTM D4515-85)
Results
BTEX in water

**Prescribed Method of Preservation:** acidification, refrigeration, or both – 7 days maximum

**Experimental Design**

Benzene, toluene, ethylbenzene, xylenes (BTEX) water holding time study design matrix.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Preservative</th>
<th>BTEX Concentration</th>
<th>Temperature</th>
<th>Measurement Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>HCl</td>
<td>Low</td>
<td>4°C</td>
<td>✓✓✓✓✓</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>Low</td>
<td>Ambient</td>
<td>✓✓✓✓✓</td>
</tr>
</tbody>
</table>

✓=Day Analyzed, Gold = Control, Green = Stable, Red = Degraded

**Results**

BTEX holding times in water were stable when preserved at ambient temperature for 21 days.

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Results
BTEX in water at Day 21 (50ng/ml)

Benzene Holding Time Study
- HCL 4°C
- HCL Ambient
- Unpreserved Ambient

Toluene Holding Time Study
- HCL 4°C
- HCL Ambient
- Unpreserved Ambient

Ethylbenzene Holding Time Study
- HCL 4°C
- HCL Ambient
- Unpreserved Ambient

Total Xylenes Holding Time Study
- HCL 4°C
- HCL Ambient
- Unpreserved Ambient
Results
PAH in Water

**Prescribed Method of Preservation:** acidification, refrigeration, or both – 7 days maximum

**Experimental Design**

**Polycyclic aromatic hydrocarbon holding time study design matrix.**

<table>
<thead>
<tr>
<th>Matrix</th>
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<th>PAH Concentration</th>
<th>Temperature</th>
<th>Measurement Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
<td>Low</td>
<td>Ambient</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>None</td>
<td>High</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NaN₃</td>
<td>Low</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NaN₃</td>
<td>High</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HCl</td>
<td>Low</td>
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**Results**

Water sample holding time results for the PAH compounds were problematic. Many exceeded 15% difference criteria in the Day 1- Day 7 low and high level PAH experiments. Due to potential Day 0 experimental artifact issues. When Day 1 substituted for Day 0, most indicated stable holding times of 10 days.
Results
Spiking anomaly present in both refrigerated and ambient (preserved) results
Results
PAHs in water (corrected based on Day 1 concentrations)
Results
PAHs in Sediment

**Prescribed Method of Preservation:** refrigeration, freezing – 14 days maximum

**Experimental Design**

Polycyclic aromatic hydrocarbon holding time study design matrix.

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<th>Temperature</th>
<th>Measurement Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sediment</td>
<td>None</td>
<td>Low</td>
<td>Ambient</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>NaN₃</td>
<td>Low</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>High</td>
<td></td>
<td></td>
<td>✓</td>
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**Results**

No statistical difference up to 60 days when preserved with sodium azide

No statistical difference up to at least 14-days with no preservative.
Low concentration PAHs in Sediment
High Concentration PAHs in Sediment
Conclusions
Technically supportable holding times at ambient temp

- BTEX holding time in water were stable up to 21 days at pH < 2 at ambient temperature
- PAHs holding time in water were stable up to 10 days at pH < 2 at ambient temperature
- PAHs holding time in sediment were stable up to 60 days with sodium azide at ambient temperature
- Even without preservation, PAHs in sediments can be stable up to 14 days at ambient temperature