

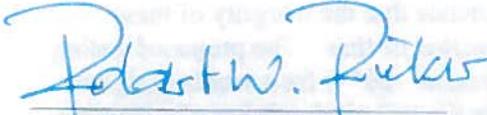
**Mississippi Canyon 252 Oil Spill
NRDA Laboratory Plan, version 2.0**

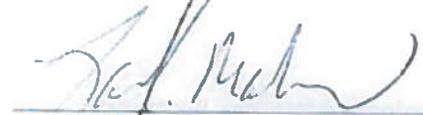
**Holding Time Study for Environmental Samples in Frozen
Archives: Laboratory Analysis Plan**

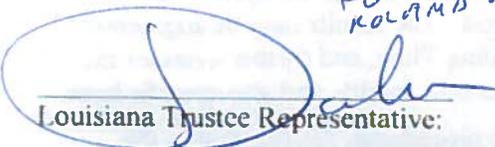
Chemistry Technical Working Group

Approval of this work plan is for the purposes of obtaining data for the Natural Resource Damage Assessment. Each Party reserves its right to produce its own independent interpretation and analysis of any data collected pursuant to this work plan.

APPROVED:

 Sept 14, 2001
Department of Commerce Trustee Representative: Date

 Aug 24, 2011
BP Exploration & Production Inc. Representative: Date

FOR HOLDING STUDY
 1/27/2012
Louisiana Trustee Representative: Date

Revision Record			
Change Date	Remove	Insert	Description of Change(s)
	Page Number(s)		

Study Plan for NRDA

Frozen Sample Chemical Stability: Laboratory Analysis Plan

Chemistry Technical Working Group

Version 2.0, August 18, 2011

1.0 Purpose:

The purpose of this analytical effort is to begin an assessment of the stability of hydrocarbons in fresh-frozen tissue samples stored for long periods of time at -20°C . A second objective is to use this information in relation to stipulations to extend the Holding Time(s) established in the Analytical Quality Assurance Plan (AQAP) for the Mississippi Canyon 252 (Deepwater Horizon) NRDA. Tissue samples collected by the Trustees and BP NRDA have been held at -20°C and some may be nearing the current 1-year Holding Time specified in Section 3.1 of the AQAP for chemical analyses. Legal retention and potential future testing requirements dictate that the integrity of these samples be maintained in archives for an extended period of time. The proposed testing of previously tested tissue samples that have been held at -20°C for a number of years will provide scientific data that can be used to assess the technical validity of a Holding Time extension.

2.0 Project Scope:

The goal of this study is to develop a sound technical basis for extending the Holding Time beyond 1 year for frozen tissue samples under the Deepwater Horizon NRDA program. The initial 11 samples to be addressed by this study will be oysters because they are one of the most common oiled tissues of interest. The results may be augmented with ongoing analyses to confirm the extended Holding Time, and further evaluate the validity of other sample and/or analyte holding times on a media- and site-specific basis.

Samples will be handled under full chain of custody procedures, as specified in the AQAP. The Mussel Watch Program¹ manager will provide documentation of the origin of these samples and approval for their use in this study. TDI-Brooks will include this documentation as well as a copy of their records showing receipt of the samples and subsequent handling from receipt to use of homogenate subsamples in this study.

Analyses of all samples will be conducted at TDI Brooks in College Station, TX. Any deviation from this analytical plan must be approved in writing by all Parties prior to implementation of the changes.

¹ Mussel Watch is a program sponsored by NOAA to monitor chemical and biological contaminant trends in U.S. coastal and Great Lakes waters. It has operated continuously at over 300 coastal sites from 1986 to present. <http://ccma.nos.noaa.gov/about/coast/nsandt/musselwatch.aspx>

3.0 Objectives:

The objectives of this study are to:

- Prioritize and analyze four replicates of the 11 oyster samples listed in Table 1 from the NOAA Status and Trends Mussel Watch Program. This testing constitutes an initial fast-track phase that might be expanded, pending review of the results, but is considered by the Trustees and RP to be a reasonable and feasible number of samples to statistically assess potential changes in PAH concentrations over time. The Mussel Watch Program samples proposed for analysis have been archived at approximately -20°C for 3 to 6 years at the TDI-Brooks laboratory. NOAA identified the candidate samples in Table 1 based on the following criteria:
 1. Eastern oysters (*Crassostrea virginica*) with an interpretable, oil-inclusive signal;
 2. Total PAH concentrations in the low parts per million range;
 3. As a secondary consideration, a diverse range of storage times and collection areas.
- Conduct all analyses as close to the original testing protocols as possible in order to minimize variability solely due to changes in methodology (Note: the Mussel Watch protocol is similar to that specified in the AQAP, including use of gas chromatography/mass spectroscopy/selective ion monitoring and reporting of recovery-corrected concentrations)
- Conduct analyses of quality control samples per the Mussel Watch protocol. Each batch of samples (< 20 samples per batch) should include (one per batch): method blank, matrix spike, and SRM 1974b. However, no sample duplicate need be included because replicate analyses are being performed on all samples. However, in addition, an MC252 Reference Oil from NIST will be analyzed on the instrument per the AQAP for the Deepwater Horizon NRDA.
- Assess long-term stability of tissue samples by using statistical tests to assess if there are significant decreases in hydrocarbon concentrations over time. The specific statistical tests and acceptance criteria will be determined through discussion with the Trustees and BP. Statistical tests will be conducted on PAH compounds reported for the original analysis of the tissue samples and various sums of those compounds.
- Acquire quantitative results for PAH compounds identified for the Mussel Watch Program in Table 2 plus percent lipids and percent moisture for each replicate analysis. Additional alkylated PAH specified in the AQAP Table 1.1a will be included to the extent feasible with the Mussel Watch protocol so that potential future testing of these tissues can include comparisons to results obtained in this study.
- The need to generate comparable data to the original Mussel Watch data is critical. TDI-Brooks conducts analyses for both the Deepwater Horizon NRDA and the NOAA Mussel Watch Program. In reporting results for this stability study, TDI-Brooks will summarize substantive differences between the

procedures performed by TDI-Brooks for the current Mussel Watch Program and the procedures followed by TDI-Brooks for these analyses under the AQAP.

Table 1. Summary of proposed tissue samples for analysis

Sample_ID	NST Site	State	FYear	Lat_DD	Lon_DD	TPAH ^a	Analyt Cont	Available Mass	Location
MW2005GBYCCV	GBYC	TX	2005			2,778	45	406.25	Galveston Bay-Yacht Club
MW2005BBGCCV	BBGC	FL	2005			2,354	40	117.71	Biscayne Bay-Gould's Canal
MW2005GBTDCV	GBTD	TX	2005			1,682	40	156.91	Galveston Bay-Todd's Dump
MW2005GBOBCV	GBOB	TX	2005			1,351	44	176.66	Galveston Bay-Offatts Bayou
MW2006MSPCCV	MSPC	MS	2006			5,664	45	644.6	Mississippi Sound-Pass Christian
MW2007IRSRCV	IRSR	FL	2007			3,032	45	317.17	Indian River-Sebastian River
MW2007SAWBCV	SAWB	FL	2007			1,509	37	263.48	St. Andrew Bay-Watson Bayou
MW2008CBJBCV	CBJB	FL	2008			1,445	44	265.1	Choctawhatchee Bay-Joe's Bayou
MW2009PCMPCV	PCMP	FL	2009			3,068	46	328.1	Panama City-Municipal Pier
MW2009CLLCCV	CLLC	LA	2009			2,578	45	459.71	Calcasieu Lake-Lake Charles
MW2009GBOBCV	GBOB	TX	2009			2,305	45	300.96	Galveston Bay-Offatts Bayou

^a TPAH values are the sum of concentrations for the 58 PAH compounds in the Mussel Watch program. Table 2 compares this list of PAH compounds with the 38 compounds analyzed in the NIST SRM 1974b tissue. Twenty-three of the same PAH compounds are analyzed in both programs.

Table 2. Hydrocarbon analytes reported in Mussel Watch tissue samples (between 2005 and 2009) compared with NIST SRM 1974b established in 2003

	Mussel Watch	NIST 1974b		Mussel Watch	NIST 1974b
Decalin	X		Fluoranthene	X	X _c
C1-Decalin	X		Pyrene	X	X _c
C2-Decalin	X		C1-Fluoranthenes_Pyrenes	X	
C3-Decalin	X		C2-Fluoranthenes_Pyrenes	X	
C4-Decalin	X		C3-Fluoranthenes_Pyrenes	X	
Benzothiophene	X		Naphthobenzothiophene	X	
C1-Benzothiophene	X		C1-Naphthobenzothiophene	X	
C2-Benzothiophene	X		C2-Naphthobenzothiophene	X	
C3-Benzothiophene	X		C3-Naphthobenzothiophene	X	
Naphthalene	X	X _c	Benz[a]anthracene	X	X _c
1-Methylnaphthalene	X	X	Triphenylene		X _c
2-Methylnaphthalene	X	X	Chrysene	X	X _c
2,6-Dimethylnaphthalene	X	X	C1-Chrysenes	X	
1,6,7-Trimethylnaphthalene	X		C2-Chrysenes	X	

	Mussel Watch	NIST 1974b		Mussel Watch	NIST 1974b
2,3,5-Trimethylnaphthalene		X	C3-Chrysenes	X	
C1-Naphthalenes	X		C4-Chrysenes	X	
C2-Naphthalenes	X		Benzo[c]phenanthrene		X
C3-Naphthalenes	X		Benzo[b]fluoranthene	X	X _c
C4-Naphthalenes	X		Benzo[j]fluoranthene		X _c
Biphenyl	X	X	Benzo[k]fluoranthene	X	X _c
Dibenzofuran	X		Benzo[a]fluoranthene		X _c
Acenaphthene	X	X	Benzo[e]pyrene	X	X _c
Acenaphthylene	X	X	Benzo[a]pyrene	X	X _c
Fluorene	X	X _c	Perylene	X	X _c
C1-Fluorenes	X		Cyclopenta[cd]pyrene		X
C2-Fluorenes	X		Dibenz[a,j]anthracene		X
C3-Fluorenes	X		Dibenzo[a,h]anthracene	X	X _c
Anthracene	X	X _c	Dibenz[a,c]anthracene		X
Phenanthrene	X	X _c	Dibenz[a,j]anthracene		X
2-Methylanthracene		X	C1-Dibenzo[a,h]anthracene	X	
1-Methylphenanthrene	X	X _c	C2-Dibenzo[a,h]anthracene	X	
2-Methylphenanthrene		X _c	C3-Dibenzo[a,h]anthracene	X	
3-Methylphenanthrene		X _c	Picene		X
4,9-Methylphenanthrene		X	Benzo[b]chrysene		X
C1-Phenanthrenes_Anthracenes	X		Benzo[c]chrysene		X
C2-Phenanthrenes_Anthracenes	X		Indeno[1,2,3-c,d]pyrene	X	X _c
C3-Phenanthrenes_Anthracenes	X		Benzo[g,h,i]perylene	X	X _c
C4-Phenanthrenes_Anthracenes	X		18a-Oleanane	X	
Dibenzothiophene	X				
C1-Dibenzothiophenes	X				
C2-Dibenzothiophenes	X				
C3-Dibenzothiophenes	X				

X_c reported as certified concentrations. Certification indicates dry-weight concentrations that agree for at least two independent analytical techniques (Poster et al. 2004).

The following is a summary of the Mussel Watch procedure used for PAH analysis of oysters from 2000 –2010 by the TDI-Brooks laboratory. This procedure will be used in the current study:

The current Mussel Watch protocol (TDI-Brooks SOP 1010) is also applicable to the samples processed from 2000 to present. As summarized in Kimbrough et al. (2006), “it achieves analyte recoveries equivalent to those from Soxhlet extraction or maceration,

using less solvent and taking significantly less time. Final extracts can be used in the quantitative determination of polycyclic aromatic hydrocarbons (PAHs), aliphatic hydrocarbons, and chlorinated hydrocarbons (including planar PCBs) by chromatographic procedures. This procedure is also used to extract samples for gravimetric determination of lipids.

An automated extraction apparatus (Dionex ASE200 Accelerated Solvent Extractor - EPA Method SW-846 3545) is used to extract various organics from 0.5 to 15 g of wet tissue sample mixed with Hydromatrix (chemical drying agent). The extractions are performed using dichloromethane solvent inside stainless-steel extraction cells held at elevated temperature and solvent pressure. The extracted compounds dissolved in the solvent are then transferred from the heated extraction cells to glass collection vials. "Clean-up" column procedures are performed to remove biogenic organic material that may cause interference with this method, and to separate extracts into various fractions for chromatographic analysis (when required). Extracts are concentrated to a final volume of 4-mL using an evaporative solvent reduction apparatus. The concentrated extracts are processed through a "clean-up" column and HPLC, to limit matrix interference and remove lipids. After clean-up, the extract fractions are concentrated to 0.5-mL using an evaporative solvent reduction apparatus and submitted for determination of aromatic, and chlorinated hydrocarbon analytes."

The full document is on the NOAA NS&T Mussel Watch web site under:
<http://ccma.nos.noaa.gov/about/coast/nsandt/musselmethods.aspx>

Results from some government programs may be available in the near future for comparison with the Mussel Watch data to be generated under the current study. Those results will be compiled as they are identified. A PAH tissue-sample storage stability study was also conducted at North Carolina State University in the laboratory of Dr. Damian Shea using oysters exposed in the laboratory to a mixture of PAHs, including parent PAH, alkylated PAH homologues, and related petroleum heterocyclic compounds. Oyster tissue was homogenized and stored at -19 ± 1 °C, with temperature recorded daily. Subsamples of the original tissue sample were analyzed each year from 1995 (the year of receipt) until 1998. The results indicated that there is little or no change in the concentrations of PAHs in the original sample over the three year period. For some of the most volatile PAH there was some evidence of a decrease over the three years, but there is still a loss of only <20% for naphthalene, C₁-naphthalenes, and fluorene. There was no statistically significant loss of any PAHs over 2 years using a paired data t-test ($\alpha = 0.05$).

4.0 Level of Effort and Logistics:

NOAA will coordinate the release of samples from the custody of other programs for use in the stability study. NOAA will also coordinate the delivery of samples not already archived at the laboratory as necessary for future analyses should those be warranted. Oyster samples for initial analysis from the Mussel Watch Program are in storage at TDI-Brooks at -20° C. Cardno ENTRIX will supervise TDI-Brooks and consult with the Chemistry TWG as appropriate during the analyses to ensure timely delivery of results.

The lab-verified analytical results will be provided simultaneously to the Parties for distribution to the Trustees and BP. Results will be reviewed and discussed through the Chemistry TWG.

The remaining volume of all Mussel Watch samples after removal of a subsample of the homogenized tissue donated for this holding times study will still be owned by NOAA Mussel Watch Program. Extracts produced from homogenates for this holding time study will be maintained in storage at its original -20°C temperature, under custody procedures in case additional analyses are desired at some point in the future. Extracts will be held in specified storage conditions until released from TDI-Brooks by the Trustees and RP.

5.0 Milestones and Deliverables:

5.1 Sample Analysis

- TDI-Brooks will analyze Mussel Watch samples in accordance with procedures in effect for that program in 2005-2009 (Kimbrough et al. 2006; TDI-Brooks SOP 1010).
- Oyster samples already stored at TDI-Brooks have been released from the custody of the Mussel Watch Program. Following approval of this plan and authorization to proceed, analysis of the first batch of samples will begin within 14 days.
- The plan for replicate analysis of 11 samples may be modified and extended pending review of the initial round of results.
- A 28-day turn-around time is specified for each batch of samples, and successive batch extractions will proceed without interruption, unless the Parties agree otherwise.

5.2 Reporting

The following items will be provided simultaneously as deliverables to the Trustees and BP NRDA. Delivery will be in accordance to the Data Sharing agreement in Section 5.3, including by e-mails from TDI-Brooks to the recipients listed below for immediate access to laboratory records by all Parties, or if size-limited, by CD-ROM:

- On behalf of the Trustees: William B. Driskell [REDACTED]
- On behalf of the Louisiana Trustees: Amanda Vincent [REDACTED]
- On behalf of BP NRDA: Dreas Nielsen [REDACTED]
- Excel spreadsheet in standard TDI-Brooks format with histogram plots and quantitative results for samples, quality control sample, and SRMs.

- Electronic data deliverable (EDD) in NOAA QueryManager format.
- Level IV data package for quantitative analysis of PAH, percent lipids, and percent moisture (PDF format).
- Photographs of all samples and sample jars
- All laboratory notes regarding handling and homogenization of replicates
- Any additional chemical data besides PAH results that may exist for the historical Mussel Watch analyses, if available.
- The holding time study data released by the laboratory will be immediately available for technical evaluation by the Trustees and BP while they are undergoing any validation as cooperative data by 3rd party validators for the Trustees and BP NRDA (EcoChem and Cardno ENTRIX). There shall be no restriction on use of the holding time study data by the Parties as released by the laboratory, recognizing that the final study data for public release may be subject to qualification as appropriate.

Chain-of-custody forms will be filled out for all sample custody transfers and will accompany the samples. Copies of the new COC forms and the original COC form from Mussel Watch will be included in the final data packages, along with the latter authorizing release of tissue homogenate subsamples from the Mussel Watch program.

All documentation associated with analyzing the samples will be preserved in accordance with the legal documentation preservation order.

All materials associated with the collection or analysis of samples must and will be handled, retained, or disposed of in accordance with the requirements set forth in applicable Court Orders governing tangible items that have been or may be issued in MDL No. 2179 IN RE: Oil Spill by the Oil Rig "DEEPWATER HORIZON" (E.D. LA 2010)

5.3 Data Sharing

TDI-Brooks shall simultaneously deliver laboratory-verified data, including all necessary metadata, generated as part of this work plan as a Laboratory Analytical Data Package (LADP) to EcoChem, Inc. on behalf of the Trustees, the Louisiana Oil Spill Coordinator's Office (LOSCO) on behalf of the State of Louisiana and to BP (or Cardno ENTRIX on behalf of BP).

Upon completion of all analyses, the final TDI-Brooks data report and electronic files along with the results of original analyses performed on the same samples will be validated and made available to all Parties.

6.0 Key Personnel:

- Study Director – Dennis Beckmann (BP).
- Analytical Leaders – Robert Barrick (Cardno ENTRIX on behalf of BP), Greg Baker (NOAA).

- QA Managers – Ann Bailey (EcoChem on behalf of the Trustees), Cheryl Randle (Cardno ENTRIX on behalf of BP)
- Lead Data Analysts – Linda Cook (Exponent on behalf of BP), William Driskell on behalf of the Trustees

7.0 Safety Plans:

Health and safety protocols in accordance with good laboratory practice will be followed at TDI-Brooks, who is responsible for developing and implementing requirements.

8.0 References:

- Kimbrough, K.L., G.G. Lauenstein, and W.E. Johnson (eds.) 2006. Organic Contaminant Analytical Methods of the National Status and Trends Program: Update 2000-2006. NOAA Technical Memorandum NOS NCCOS 30 Silver Spring, MD.
- NOAA. 2011. Analytical Quality Assurance Plan, version 2.2. Mississippi Canyon 252 (Deepwater Horizon) Natural Resource Damage Assessment. Prepared by EcoChem. January 20, 2011.
- Poster, D.L., M.M. Schantz, J.R. Kucklick, M.J. Lopez de Alda, B.J. Porter, R. Pugh, S. A. Wise. 2004. Three new mussel tissue standard reference materials (SRMs) for the determination of organic contaminants. *Anal Bioanal Chem.* 378(5):1213-31.

References to the studies cited in this work plan are for background and context only. Approval of this work plan does not constitute endorsement of, or agreement with, the methods, analysis, or conclusions of any study cited herein.

9.0 Costs:

All laboratory costs will be direct-billed to BP. BP will not be responsible for any additional cost other than those associated with the ordinary review of the work as described here without BP approval.