Quality Assurance Project Plan: *Deepwater Horizon* Laboratory Toxicity Testing

Prepared for:

National Oceanic and Atmospheric Administration
and
State of Louisiana Trustees

Prepared by:

Michael W. Carney
Heather P. Forth
Michelle O. Krasnec
Ryan Takeshita
James V. Holmes
Jeffrey M. Morris
Abt Associates
1881 Ninth Street
Boulder, CO 80302
303-381-8000

Contact:

Jeffrey Morris

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Acronyms and Abbreviations

BTEX	benzene, toluene, ethylbenzene, and xylenes
CEWAF	chemically-enhanced water accommodated fraction
COC	chain-of-custody
CV	curricula vitae
DI	de-ionized
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DOSS	dioctyl sulfosuccinate
DOT	Department of Transportation
DWH	*Deepwater Horizon*
EDD	electronic data deliverable
EPA	U.S. Environmental Protection Agency
ERDC	Engineering Research and Development Center
FBS	fetal bovine serum
FGCU	Florida Gulf Coast University
GLP	Good Laboratory Practice
GLPP	General Laboratory Procedures and Practices
HDPE	high-density polyethylene
HEWAF	high-energy water accommodated fraction
IACUC	Institutional Animal Care and Use Committee
IATA	International Air Transport Association
ID	identification
LEWAF	low-energy water accommodated fraction
NOAA	National Oceanic and Atmospheric Administration
NRDA	Natural Resource Damage Assessment

PAH	polycyclic aromatic hydrocarbon
PCB	polychlorinated biphenyl
PI	principal investigator
PPE	Personal Protective Equipment
QA	quality assurance
QAPP	Quality Assurance Project Plan
QC	quality control
RFU	relative fluorescence unit
RNA	ribonucleic acid
RO	reverse osmosis
RPMI	Roswell Park Memorial Institute
SOP	standard operating procedure
TCT	test conditions table
TPAH	total polycyclic aromatic hydrocarbon
TPH	total petroleum hydrocarbon
TSA	Transportation Security Administration
UV	ultraviolet
VOA	volatile organic analysis
VOC	volatile organic compound
WAF	water accommodated fraction

1. Introduction

This Quality Assurance Project Plan[1] (QAPP) was developed to provide data collection guidance for *Deepwater Horizon* (DWH) laboratory toxicity testing project activities. Stratus Consulting,[2] now Abt Associates, is conducting this work on behalf of the National Oceanic and Atmospheric Administration (NOAA) and the State of Louisiana Trustees to support the DWH oil spill Natural Resource Damage Assessment (NRDA) activities. Guidance and requirements provided herein are based on U.S. Environmental Protection Agency's (EPA's) *Guidance for QAPPs* (U.S. EPA, 2002) for the purpose of data collection and analysis, with modifications to reflect project goals. This QAPP, which outlines the procedures that will be used to ensure that data are collected and analyzed to meet project requirements, contains the following information:

- Project management procedures, objectives, and approaches
- Procedures and guidelines for generating the data, including methods for documenting test results, collecting samples for laboratory analysis, and submitting results to Stratus Consulting
- Project assessment and oversight
- Data validation and assessment of data usability.

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1.1 Previous Versions of this QAPP

The first definitive tests were conducted in August 2011 under this toxicity testing program. The first version of this QAPP was completed on July 20, 2011. We have made minor clarifications to some of the text, added new principal investigators, and added new or revised protocols in

1. The recommended citation for this document is: Carney, M.W., H.P. Forth, M.O. Krasnec, R. Takeshita, J.V. Holmes, and J.M. Morris. 2016. Quality Assurance Project Plan: *Deepwater Horizon* Laboratory Toxicity Testing. DWH NRDA Toxicity Technical Working Group. Prepared for National Oceanic and Atmospheric Administration by Abt Associates, Boulder, CO.

2. Stratus Consulting merged with Abt Associates in 2015; since much of this work was conducted by Stratus Consulting prior to the merger, both firm names will appear.

subsequent versions of this QAPP. This final version represents the seventh iteration of this document, and the information herein supersedes any information presented in previous versions. The release dates for the previous six versions are listed below.

- Version 1: July 20, 2011.
- Version 2: October 13, 2011.
- Version 3: June 19, 2012.
- Version 4: February 4, 2014.
- Version 5: February 20, 2015.
- Version 6: August 31, 2015 (Draft for Public Review), December 16, 2015 (Final Draft). This version is a part of the publically available administrative record for the *Deepwater Horizon* NRDA (Morris et al., 2015).

The only substantive change to protocols that occurred across these different QAPP versions was a change to the filtration protocol, implemented on July 3, 2013 and described first in Version 4. See footnote in Appendix G of this document for more details.

2. Project Management

This section presents the project administrative functions and approaches that will be followed.

2.1 Project Organization

Stratus Consulting is performing the work described herein on behalf of NOAA and the State of Louisiana. The laboratories that conduct toxicity testing and associated analytical sample analyses are subcontractors under contract to Stratus Consulting or, in certain cases, to one of its subcontractors. The exceptions are the Northwest Fisheries Science Center, which is a NOAA laboratory and not under contract to Stratus Consulting; and the U.S. Army Corps of Engineers Engineering Research and Development Center (ERDC), which is under contract directly with NOAA. Stratus Consulting is the primary manager for all laboratories and provides oversight for work conducted at all testing laboratories. In addition, Stratus Consulting conducts toxicity testing activities at independent or subcontractor laboratories. At least one principal investigator (PI) has been designated at each laboratory. The PI is responsible for work conducted at the laboratory and for ensuring that work is conducted as described in this QAPP. Subcontractors will document deviations from project planning objectives and will provide this documentation to Stratus Consulting as soon as possible following a change so that we can implement adaptive management if needed. This QAPP will be distributed to each PI for review when finalized. Definitive toxicity tests conducted after finalization of this QAPP will be subject to requirements set forth herein.

2.2 Project Timing

The data collection and analysis methods described in this QAPP will be followed for the duration of the DWH laboratory toxicity testing project.

2.3 Special Training/Certification

All PIs and supporting staff will have experience in conducting toxicity tests, documenting test results, and collecting analytical samples. Training and certification requirements related to testing and health and safety are detailed in each testing laboratory's standard operating procedures (SOPs) and/or health and safety plans. Laboratory-specific testing methods and operating procedures are provided in their respective laboratory protocols. Any collection permits, Institutional Animal Care and Use Committee (IACUC) approvals, and respective requirements that are related to project toxicity testing activities will be the responsibility of the laboratories; Stratus Consulting may request such documentation as appropriate.

PIs and supporting staff will participate in a presentation and discussion about the NRDA process, which Stratus Consulting will host. Participants will either attend a presentation in person or join remotely via a conference call and computer with an internet connection. Testing laboratory staff must schedule a time when they can attend the presentation. Contact Stratus Consulting for scheduling information.

PIs and all supporting staff will sign a project-specific confidentiality agreement before conducting any work on this project. All project staff will submit their signed confidentiality agreements, together with their current curricula vitae (CVs) to Stratus Consulting.

2.4 Documents and Records

The laboratory toxicity test documentation may generate the following records:

- Laboratory notebooks in which testing activities are documented
- Data entry bench sheets and electronic files in which testing data and sample inventories are documented
- Electronic data files
- Photographs documenting testing activities, observations, and events

- ALS Environmental analytical chemistry laboratory documents
- Chain-of-custody (COC) and shipping forms.

Each record type is described in detail below.

2.4.1 Laboratory notebook

Laboratory notebooks will record the details of all testing activities. The notebooks will:

- Document and describe testing methods
- Record observations made in the laboratory
- Identify, locate, and track samples
- Document any deviations from the project approach, QAPP, and changes in project personnel
- Record any project-related information that would not be appropriately documented elsewhere.

2.4.2 General notebook requirements

- Notebooks will be bound. Notebooks with water-resistant covers and pages are recommended. Every notebook page will be sequentially numbered starting with the first page of the notebook. If pages are not pre-printed with page numbers, the numbers will be handwritten at the top of each page. Loose-leaf sheets, other than dedicated sampling forms, should not be used to record notes. If notes must be taken on loose sheets, the same person who took the notes will place the loose sheets securely into a notebook, and then sign and date them. The notebook and all loose-leaf notes will be retained under strict custody. Pages will not be removed from the notebook.
- All entries will be written legibly using indelible ink pen or pencil. Writing will be dark enough to allow legible photocopies to be made and should not bleed through the paper, which would cause any notes on the backside of the page to become illegible.
- The following information should be written on the front cover of the notebook; some information may need to be entered after the project has ended:

- Notebook user name, affiliation, address, and phone number
- Notebook number (1 for the first notebook, 2 for the second, etc.)
- Name of the site, city, and state
- Project name and type of activity
- Beginning and ending dates of activities entered into the notebook
- "Property of [*testing laboratory*]"
- "DWH ATTORNEY WORK PRODUCT / ATTORNEY-CLIENT COMMUNICATIONS."

- Only information that is related to the project should be entered into the notebook. All notebook entries should remain factual and objective.

- A new page should be started for each day.

- The following information should be entered at the beginning of each day:

  - Date
  - Starting time (all time entries should be recorded in military or 24-hour time format)
  - Specific location
  - General description of anticipated activities.

- A diagonal line should be drawn across any blank space of more than one line to prevent unauthorized entries.

- An approximate scale for all diagrams should be provided. If this is not feasible, write "not to scale." Orientation relative to north should be indicated with an arrow and diagram features should be labeled.

- The number, file name, orientation, and subject of all photographs should be recorded.

- Corrections should be made by drawing a single line through the corrected entry, leaving the information legible, and initialing and dating the correction.

- The following information should be entered into the notebook when collecting samples:

  - Sample information, including description/reason for sampling, stock solution preparation method [e.g., high-energy water accommodated fraction (HEWAF), species/weight/length for tissue samples, and similar information]
  - Names of sampling personnel present
  - Sample collection time (military or 24-hour time format) and time zone
  - Sample collection method (e.g., filtered/unfiltered, grab, composite)
  - Sample identification (ID) number (see naming convention in Section 4.6.1)

- Designation of sample type (e.g., definitive sample, duplicate sample)
- Sample media (water/sediment/tissue)
- Sample observations (e.g., color, odor, consistency)
- COC page and form number in which samples will be relinquished to ALS Environmental (top right-hand corner of COC sheet) – to be filled in by sampler
- Source water preparations or origin (date, time, and storage location)
- Field collections, shipments, and husbandry performance of test organisms.

- Custody of laboratory notebooks should be maintained at all times, and notebooks should be stored in a safe, secure place at all times when not in the possession of personnel.

2.4.3 Data entry bench sheet requirements

Data entry bench sheets are provided in Appendix B of this QAPP. Laboratories will print and use hard copy bench sheets to facilitate entering data into the data files (electronic versions of the bench sheets) and perform a quality assurance/quality control (QA/QC) check of 100% of the test results electronically entered into the data files. Water-resistant paper should be used for hardcopy testing bench sheets.

The following are data entry bench sheet requirements for recording information generated during toxicity testing:

- There will be one set of bench sheets for each test.

- Prior to beginning a definitive test, the PI or appropriate test manager should confirm that all required types and number of blank bench sheets are available.

- All staff conducting the test and test information recorders should be identified on each sheet. Fill out time using military or 24-hour time format.

- All bench sheet entry fields will be filled out. “NA” should be recorded in entry fields that are not applicable. Do not leave any blanks.

- All entries will be made using indelible ink.

- All mistakes should be corrected by drawing a single line through the incorrect entry and entering the correct entry in an understandable manner. The initials of the recorder should be entered next to any corrected entries.

- At the end of each day, completed bench sheets will be organized, reviewed, and, if needed, corrected by the PI or appropriate test manager. Corrections should be made by following the aforementioned method and initialing the corrected entry.

- Photocopies should be made of each day's completed bench sheets. Copied bench sheets will be separated from originals and stored in a secure place.

Toxicity testing data bench sheets and files are described in Section 4.1 and reporting requirements in Section 4.11.2.

2.4.4 Electronic data files

Electronic data files will be generated throughout the course of this project. These files may include but are not limited to data entry files, emails, photographs, scanned documents, and work plans. Electronic data files should be named with the date when the file was created. Where possible, a ReadMe should be included that describes when the file was created and by whom. If a data file is revised, a new copy should be made and the original should be preserved. Files should be securely stored in password-protected folders. Only people that are working on this project with completed confidentiality agreements on file should have access to saved files. Files should be routinely backed up during the course of this project. If the backup files are stored on an external device, this device needs to be stored in a secure location. All data files will be retained under custody procedures until notified otherwise by Stratus Consulting.

"DWH ATTORNEY WORK PRODUCT / ATTORNEY-CLIENT COMMUNICATIONS" should be included in all electronic files. This statement should be entered into the subject line when sending project-related emails. Alternatively, the confidentially statement may be written on the top line of the email.

2.4.5 Photographic log requirements

Photographs and digital videos may be taken during testing to record activities, observations, and events. Before being used to take any photographs, cameras will be set to the local time (in military or 24-hour time format) and date. Personal cameras, including those on cell phones, smartphones, and other mobile devices, are not to be used for project-related work. Photographs and video data will be collected and stored in a legally defensible fashion using requirements described in this section. There are two major elements to satisfying these requirements: preserving original files and maintaining a complete photographic record.

Photographs and digital videos will be retained as original files on the removable memory card in the camera. Once a file is created (i.e., picture taken), it will not be deleted from the microSD card and will be kept under strict COC procedures. At no time will information stored on a digital memory card or a camera's internal memory be erased or overwritten, even if the photograph is out of focus or was taken accidentally. Additionally, digital photograph files must be stored sequentially on the microSD card and not renamed.

When saving and viewing photographs and videos on a computer, each digital media file must first be downloaded to an archive file that cannot be opened, deleted, or renamed. Prior to viewing pictures, staff will upload photograph files directly from the camera to an archive file location that is secured and routinely backed up. Photos will be maintained as unopened files until notified by Stratus Consulting. After uploading unopened files, digital media files may then be copied or downloaded to a local working folder where they can be accessed. Note that files in the working folder will not be deleted. After copying files into working folders, the media storage device will be removed from the camera and sent to Stratus Consulting under COC procedures using the project-specific COC form.

A photograph/digital video log will be created for all photographs taken for this project. Microsoft Excel should be used to create the photograph/video log. Each camera used to take photographs during each test will have a unique photograph or video log. Entries in the photograph log should be made at the time each photograph is taken. The log will contain the camera ID, memory card ID, original file name, date, time, time zone, photographer name/affiliation, location where photograph was taken, and a description of the subject. The camera ID is the serial number that is clearly stamped on the camera body or other ID that is unique to the camera and can be used to identify which camera was used. If the original file name is not apparent when taking photographs (i.e., it does not display on the camera screen), the original file name can be filled out after files have been downloaded to a working folder. All other information in the photograph log should be filled out while photographs are being taken. A ReadMe worksheet should also be provided with each photograph log file, and describe when the photograph log was created and by whom, as well as any pertinent information not already provided within the log itself. Once complete, photograph logs will be checked for completeness and accuracy. The photograph log will then be provided to Stratus Consulting.

2.4.6 ALS Environmental laboratory documents

The following types of documents will be generated by analytical laboratories for this project

- A description of analytical methods and measurements performed on the collected samples and on QA/QC samples (e.g., blanks, calibration standards)
- Supporting documentation, including copies of laboratory notebooks, sample tracking forms, raw outputs from instruments, chromatograms, run logs, and similar documentation that is sufficient to conduct full data validation, if needed
- Project narrative reports.

The analytical laboratory will provide the data to Stratus Consulting in an electronic data deliverable (EDD) format.

In addition to ALS Environmental, other laboratories may be used throughout the course of this project. These laboratories will generate their own types of documents that are specific to the analyses being conducted. When used, documentation requirements will be specified in the PI SOPs prior to testing.

2.4.7 COC and shipping forms

A blank COC form is provided in Appendix C. This form will document sample retention and storage; relinquish samples; relinquish any test materials such as data sheets, microSD cards, and similar materials; and request analyses when relinquishing samples to an analytical laboratory. A description of COC requirements and procedures is provided in Section 4.8.

3. Testing Method Documentation and Review Process

This section describes the method documentation and review process for the definitive toxicity tests. Testing method documents include work plans, SOPs, and test conditions tables (TCTs). Testing laboratories will draft these test method documents and provide them to Stratus Consulting for review and approval prior to conducting definitive tests. Stratus Consulting will use finalized test method documents to draft the *General Laboratory Procedures and Practices: Deepwater Horizon Laboratory Toxicity Testing* (Krasnec et al., 2016). General Laboratory Procedures and Practices (GLPP) will document finalized methods that laboratory personnel used during definitive testing.[3]

Descriptions of each of the test methods documents follows:

- ***Work plans:*** These plans will document general testing methods and information. These work plans document elements or testing procedures that are common to all or a subset of similarly related tests being conducted by a specific laboratory. These elements may include, but are not limited to, sources of test organisms and water, test endpoint descriptions and how they will be assessed, and analytical sampling requirements. These work plan elements should be written to a level of specificity such that someone trained in the scientific discipline being utilized to conduct the test could accurately repeat it.

3. The project GLPP acronym should not be confused with Good Laboratory Practice (GLP) regulations that specify the management QC system for clinical and nonclinical research laboratories. Although the project GLPP provides GLP-like guidance and requirements, it should not be confused with a document that strictly adheres to U.S. Food and Drug Association regulations or Organization for Economic Cooperation and Development guidelines for testing of chemicals.

Where applicable, work plans may reference methods described in this QAPP or published journal articles; for example, personnel may reference this QAPP when describing methods for labeling and shipping analytical samples, or when reporting test results. Work plans may be updated as needed to reflect changes in general testing methods. New version numbers will be assigned to updated work plans.

- ***SOPs:*** These documents will detail how toxicity tests or elements of tests will be performed. SOPs should be written as a step-by-step process in chronological order. SOPs should be written to a level of specificity such that someone trained in the scientific discipline could use them to accurately repeat procedures. If appropriate, one SOP can be used to document multiple tests. For example, one SOP could detail how to perform water quality analyses, directions for calibrating diluter systems, and the process for conducting an acute toxicity exposure for multiple species or life stages. Work plans may reference laboratory-specific SOPs and project-wide SOPs provided in appendices in this QAPP when describing testing procedures. SOPs for HEWAF, chemically-enhanced water accommodated fraction (CEWAF), and low-energy water accommodated fraction (LEWAF) preparations, the most common water accommodated fraction (WAF) preparations used during definitive testing, are provided in Appendix A.1. Additional test procedures, such as spiking sediment with oil and preparing oil slick exposures for use in toxicity testing, can be found in the most recent version of the GLPP. GLPP SOPs may be referenced in laboratory method documentation materials and used and when finalized. If applicable, standard method and test-specific QA/QC limits and test performance criteria will be specified in testing laboratory SOPs or work plans if applicable to multiple testing procedures.

- ***TCTs:*** These tables will document experimental conditions for each definitive toxicity test. TCTs contain specific test information such as the number of treatments, nominal dilution series concentrations, and environmental conditions such as salinity, temperature, and feeding schedule. As such, TCTs are the final test method documents generated prior to definitive testing. Each test will have a single TCT, even if the test is repeated using the same experimental conditions. If a test is repeated, a new TCT will be drafted that clearly documents which test is being repeated in addition to any test condition changes. TCT templates are available from Stratus Consulting.

- ***GLPP:*** Work plans and SOPs will be used to create the GLPP for each laboratory conducting toxicity tests. During the course of a test, minor deviations may be made to the SOPs and work plans. The GLPP will be finalized after the completion of a definitive test and will therefore reflect the confirmed testing actions. This document will contain the final/modified versions of protocols for each definitive test. The GLPP will be regularly updated throughout the DWH laboratory toxicity testing projects lifespan (Krasnec et al., 2016).

The documents described above are required to facilitate efficient timely reviews of test methods generated by testing laboratories. Once established, work plans and SOPs may be used for multiple tests. These method documents may not have to be resubmitted to Stratus Consulting when seeking approval for each new test. In practice, TCTs may be the final method documents that are reviewed by Stratus Consulting. Stratus Consulting will give approval to start each test after review and acceptance of TCTs. To facilitate this review process, TCTs should be sent to Stratus Consulting as standalone Microsoft Word documents, so that each test will be described and provided in a separate file. Additionally, TCTs should clearly reference work plans or SOPs where appropriate. When approved, Stratus Consulting will notify the testing laboratories via email. If an approved test is rerun using the same test conditions, Stratus Consulting will assign a new test ID. If not provided, Stratus Consulting will create a new TCT file documenting the repeated test and new test ID information.

It is the responsibility of each testing laboratory to have final approved versions of work plans, SOPs, and TCTs available for reference when conducting definitive toxicity tests. If requested, these documents should be readily available for review. We recommend that testing laboratories create a hardcopy binder that contains these documents and routinely refresh it with updated work plans, SOPs, and new TCTs. Stratus Consulting will also document and inventory draft and currently approved test method documents.

4. Data Generation and Acquisition

This section describes definitive toxicity testing results and sample handling, QC, and data management for the project. A definitive test is a multi-concentration exposure that consists of control and exposure treatments that provide dose-response information within a prescribed period of time. With respect to this QAPP, range-finding or pilot tests are not considered definitive tests. The methodology described in this QAPP only pertains to work performed during definitive testing. However, general document and file COC and retention requirements described in Section 2.4 (*Documents and Records*) and the sample retention policy described in Stratus Consulting (2011) applies to all tests, including definitive, pilot, and range-finding tests.

4.1 Toxicity Testing Data Recording

As stated in Section 2.4, toxicity testing information will be documented in notebooks, bench sheets, data entry files, photograph files and logs, COC forms, and shipping forms. These data will be provided to Stratus Consulting as hard copies and/or electronic files. Electronically scanned files of hard-copy and handwritten documents are acceptable and preferred.

When a test is complete, testing laboratory staff will enter all data from notebooks and/or hardcopy bench sheets into electronic Microsoft Excel files (Appendix B). All file entries will be checked against the original notebook and/or hardcopy entry bench sheets where data were originally recorded. All hand-written information recorded on the toxicity testing results reporting bench sheets will be clearly legible. Testing laboratories will complete the transcription of test results as soon as possible after a test is ended.

Stratus Consulting will provide each laboratory with blank templates of the toxicity testing data files prior to beginning toxicity testing. Testing laboratories will use the most recent versions of these files to record test information. Data files will be printed and used as bench sheets to record test results while conducting tests. Data entry files will be in Microsoft Excel format and have separate worksheets for documenting the following items:

- ***Experimental design:*** The "Tank ID, Dilution, or Stock Code Definitions" and "Test Conditions Table" bench sheets document general testing conditions (e.g., start and end times, species and life stage tested, feeding regime) and serve as lookup tables for tracking test IDs, tank IDs, and defining stock and dilution series codes that are used in subsequent sample inventory bench sheets. These bench sheets will be printed and filled out prior to conducting tests. Test and tank ID codes are described in Section 4.3.

- ***Preparing test solutions:*** Three bench sheets document test solution preparation: "Water Accommodated Fraction Preparation and Sampling Table," "Fluorescence Analysis of Test Solutions," and "Development of Fluorescence Analysis Standard Curve." WAF documentation includes preparation start and stop times, experimental procedures, and analytical sample collection for each test and test renewal. When used, fluorescence data entry bench sheets document the preparation and analysis of solutions used to generate a standard curve, associated standard curve equation parameters, and preparation and analysis of all samples from each WAF dilution series.

- ***Daily and periodic water quality analyses:*** The "Water Quality Monitoring" bench sheet documents water quality analyses and results during the course of each test.

- ***Daily inspections:*** The "Test Performance Monitoring Bench Sheet" documents daily inspections of each tank during the course of each test. All tanks inspected will be recorded on this bench sheet. Inspectors are required to record the number of test

organisms in each tank at the start of the test and to make note of any organisms found dead. Only organisms that can be observed as dead should be counted in the "Number observed treatment mortalities" entries. These organisms should then be removed and archived/sampled. There is a separate column for treatment and non-treatment mortalities. Examples of non-treatment mortalities include accidental spilling, loss of a tank, or losing fish that jump out of the tanks. If a non-treatment mortality occurs, briefly describe the event in the "Notes" column. Each inspection will also provide the number of organisms remaining in each tank, which will be recorded in the "Number of observed alive" entry.

- ***Sample collections:*** The "Analytical Sample Inventory Bench Sheet" documents all sample collection events that occur during the course of the definitive tests. Sampling events include, but are not limited to, WAF stock, archive, fluorescence, and archive tissue samples collected during or at the end of the test. WAF stock and archive sample entries will reference codes defined by each laboratory in the "Tank ID, Dilution, or Stock Code Definitions" bench sheet. Sample IDs are also documented along with a short description of the sample event, contents, and use. Storage information should also be recorded for each sample.

In addition to the test results reporting bench sheets described above, Appendix B contains a field entry "Data Dictionary" table that defines all entry field headings found on each bench sheet.

If the data entry bench sheets that Stratus Consulting provides to the laboratories are insufficient for documenting testing results, testing laboratories will draft revised or test-specific bench sheets. Testing laboratories will draft test-specific bench sheets during the test method work plan development and provide the draft data files to Stratus Consulting for review and approval. Testing laboratories should consult with Stratus Consulting during this process to determine if a needed bench sheet has already been created and to ensure that the format is consistent with other data files. In general, bench sheets should include columns for replicate ID (tank ID), date, time of measurement, and initials of recording staff. Revised and approved data entry files should be drafted in Microsoft Excel and used as data entry bench sheets as described above.

4.2 Sample Retention Requirements

Sample retention is required for specific samples and solutions generated during project activities as stipulated in the June 24, 2011 U.S. District Court, Eastern District of Louisiana *Pretrial Order No. 37 Relating to the United States' and Natural Resource Trustees' Testing of Samples*. Project sample retention policies will apply to samples and solutions generated during all pilot, range-finding, and definitive testing and analytical activities. The following sample and solution retention requirements are summarized from the June 2011 Pretrial Order No. 37:

- All glassware, containers, and equipment used to mix or administer oil and exposure solutions [e.g., needles, syringes, gavage tubing, gas-tight syringes, exposure chambers, and sampling equipment (including analytical sample containers)] *may be washed or discarded after use.*

- Exposure solutions, including unused oil-sediment slurries, materials removed from exposure chambers as part of regular maintenance, and oil remaining in exposure chambers at the conclusion of toxicity tests, *may be discarded after any necessary samples have been collected for chemical analysis or archiving.*

- All analytical chemistry extracts of oil, water, sediment, or tissues remaining after analysis *must be retained.*

- Requirements for retention of organisms or parts of organisms used in toxicity testing vary depending upon where they were obtained or if their tissues or blood were destroyed during analysis, as follows:

    - For organisms collected in the field for this project, any organisms or parts of organisms remaining after the testing *must be retained* regardless of whether they were used for toxicity testing

    - For organisms collected in the field for this project, any organisms or parts of organisms that died or were removed from the testing program for any reason before being used in toxicity tests *must be retained*

    - For organisms purchased or otherwise obtained from third parties and available to all parties for purchase or acquisition and not used in exposure tests or for controls *do not need to be retained*

    - Organisms that were used in exposure tests, including controls, *must be retained*

    - Organisms collected in the field or from which eggs were collected *must be retained.*

- If analysis of the organism and/or its tissues or blood results in the destruction of the tissues or blood, then any extracts or preparations (e.g., histology slides and uncut paraffin-embedded tissue) *must be retained,* regardless of effective shelf lives or hold times.

- All unused oil, dispersant, and sediment *must be retained*. Under no circumstances will oil, dispersant, or sediment be used or distributed outside of tests or analyses approved by Stratus Consulting.

- If it is unclear at any time as to whether a sample of any kind (chemical or biological) should be retained, contact Stratus Consulting before attempting disposal.

4.3 Test and Tank ID Codes

Test IDs, consisting of a three-digit number, track results for each definitive test. Unique test IDs will be assigned to each proposed test for each laboratory. Upon approval of testing methods documentation and before tests begin, Stratus Consulting will assign unique test IDs to each proposed test for each laboratory. When a test is rerun or new tests are added, they will receive a new test ID. Stratus Consulting will provide additional test IDs if needed. These test IDs will be part of the sample label, which is described in Section 4.6.

Prior to starting a test, each testing laboratory will establish tank ID, WAF stock sample, and dilution series codes in the "Tank ID, Dilution, or Stock Code Table" results reporting data bench sheet. These codes will be used in subsequent data reporting bench sheets and files for each test. Codes must be specific enough to avoid duplication and facilitate easy tracking between sampling from individual tanks and samples from WAF stocks or archive/dilution series. Note that these codes are not part of sample IDs.

Tank ID codes will track data generated for individual tanks during a test. WAF stock codes will designate WAF stock water samples from other sample types in the "Analytical Sample Inventory" bench sheet. The WAF stock water sample code will be entered into the "Tank ID Dilution or Stock Code" bench sheet when WAF stock samples are inventoried. Archive samples taken from each dilution series also need to be referenced to a specific dilution series. Therefore, unique dilution series codes will be generated, defined on the "Tank ID, Dilution, or Stock Code Table," bench sheet and used when inventorying archive series samples. Given the relationship between dilution series and tank IDs, we recommend that dilution series codes also be associated with tank ID codes. For example, when using dilution code "A" for a given dilution series; tank IDs would be A-1, A-2, and so on for each replicate. Refer to the "example" test results in the reporting data entry Excel file provided to each laboratory for more examples of coding tank IDs, WAF stock samples, and dilution series.

4.4 Sampling and Chemical Analysis

Types of sample matrices that may be collected during toxicity testing include water, sediment, and biological samples such as tissue. The types of samples collected and respective analyses conducted will depend on the particular test, but will follow the basic methodology described in this section. Non-analytical samples will also be collected by testing laboratories throughout the course of this project. These samples may include, but are not limited to, bacterial cultures, molecular biology samples, test organism whole-body tissue samples for use in histological

analyses, or archives of any media type. Non-analytical samples may be analyzed in the future. Therefore, they should be collected, handled, and stored so that they do not reasonably jeopardize potential future analyses. If guidance on collection, handling, and storage of these samples is lacking herein, then testing laboratories should document collection, handling, and storage requirements in respective work plans or SOPs.

Please refer to the *Mississippi Canyon 252 (Deepwater Horizon) Natural Resource Damage Assessment Analytical Quality Assurance Plan* (analytical QAPP; Appendix D) for details on analytical methods, reporting and detection limits, and the relevant laboratory parameters for the various analytical chemistry samples collected during definitive toxicity testing.

The majority of samples that testing laboratories collect may be water samples; these include samples from each newly prepared WAF stock solution, dilution series samples from the different treatments including control treatments, and source water samples. In most cases, water samples will be collected for one of four types of analyses: (1) polycyclic aromatic hydrocarbon (PAH) with alkylated homologues; (2) benzene, toluene, ethylbenzene, and xylenes (BTEX); (3) dioctyl sulfosuccinate (DOSS); and (4) fluorescence. Section A.4 in Appendix A provides a summary guide for water sampling, including information on when to sample and what analyses are needed for different sample types, what bottles/volumes are required, basic sampling instructions, and storage information.

Note that guidance provided in the following sections may not be applicable to all tests or testing laboratories. Testing laboratories will provide sampling and analysis information to Stratus Consulting when drafting work plans, SOPs, and TCTs. Upon review of draft testing methods documents, Stratus Consulting will specify any additional or modified sampling requirements. Sampling requirements may be modified according to the capabilities of the testing laboratory and the type of test.

Additional details on the types of samples that may be collected are described in the following sections.

4.4.1 WAF stock and dilution series samples

WAF stock or dilution series sampling may be conducted for oil exposure toxicity tests. This sampling guidance does not apply to dispersant-only tests, which are described in Section 4.4.3. Water samples may be collected from each toxicity test WAF stock solution prepared during the course of each test (Section A.1, Appendix A). Specifically, for PAH analysis, WAF stock water samples will be collected if an undiluted WAF stock will be used as the highest concentration exposure treatment. If the WAF stock is not the highest exposure concentration, then the highest exposure concentration should be sampled in lieu of sampling the WAF stock. Dilution series water samples may also be collected for analysis or extract and archive purposes only

(Section 4.4.2). Dilution series water samples may be collected and analyzed for PAHs as explained in this section. For CEWAF tests, DOSS samples may also be taken from the highest exposure concentration for each new CEWAF stock prepared. A DOSS sample may also be collected from the control water. DOSS water samples may not be needed for the other exposure concentrations in a CEWAF test. For tests using source oil, BTEX samples may be collected for the stock WAF/highest exposure concentration and control water only. See Table 1 for the stock WAF/highest exposure concentration sampling scheme. In some exposure setups such as flow-through tests, the analytical sampling may differ from what we describe above. For these tests, the analytical sampling plan will be specified in test method documents prior to conducting definitive tests.

Table 1. Analyses designations for WAF stock and dilution series solution water samples

Stock preparation method	Oil type	Analyses requested[a]
HEWAF/LEWAF	Slick A	PAH/Alk (8270C SIM/PAH)
	Slick B	PAH/Alk (8270C SIM/PAH)
	Source	PAH/Alk (8270C SIM/PAH) BTEX (8260C VOCs)
	Weathered source	PAH/Alk (8270C SIM/PAH)
CEWAF	Slick A	PAH/Alk (8270C SIM/PAH) DOSS (ALS Environmental SOP)
	Slick B	PAH/Alk (8270C SIM/PAH) DOSS (ALS Environmental SOP)
	Source	PAH/Alk (8270C SIM/PAH) BTEX (8260C VOCs) DOSS (ALS Environmental SOP)
	Weathered source	PAH/Alk (8270C SIM/PAH) DOSS (ALS Environmental SOP)

a. Testing laboratories will also check the "Archive extract?" box for each sample on the COC forms when requesting analyses.

One set of WAF stock/highest exposure concentration water samples may be collected for each new WAF stock solution prepared, according to the guidelines listed below. This will include the collection of WAF stock/highest exposure concentration water samples at each renewal for the duration of the test. WAF stocks that are used for toxicity tests can only be stored for 24 hours and need to be kept in a cool, dry, and dark location. Stock solutions stored for more than 24 hours will not be used or sampled. If the renewal occurs within 24 hours and the same stock solution from which samples were previously drawn is used to renew test media, another sample from the WAF stock/highest exposure concentration should be collected at each renewal time point and sent in for analysis. If filtered WAFs are to be tested, then sampling should be

conducted on the filtered WAF stock, unless directed otherwise. WAF stock samples should be taken by filling sample bottles directly from the stock solution aspirator bottle. Dilution series water samples should be taken by filling sample bottles as surrogate exposure vessels. Note that:

- WAF stock samples should be collected prior to diluting the stock solution to make subsequent test treatment solution
- WAF stock preparations should be sufficient to accommodate volumes needed for water samples and subsequent dilutions
- If more than one WAF preparation needs to be mixed to achieve a sufficient volume to conduct a test, all WAF solutions should be composited into one solution prior to collecting water samples for analysis and making test treatment dilutions
- The composited WAF stock solutions should be well mixed prior to taking water samples and making test treatment dilutions.

Dilution series samples may also be collected for PAH analysis from each new WAF preparation used throughout the duration of the test. These samples may be collected for extract and archive purposes. Similar to WAF stock samples, dilution series samples may be filtered or not filtered. Dilution series samples should be taken by filling sample bottles directly from the vessel in which treatment dilutions are made. As such, dilutions will be well mixed and be of sufficient volume to fill required sample bottles and dilution series test chambers. Reduced or additional dilution series sampling will be specified in test work plans, SOPs, and/or TCTs. As mentioned above, for CEWAF tests, only the highest exposure concentration and the control water are sampled for DOSS. Likewise, for source oil tests, only the highest exposure concentration and the control water are sampled for BTEX.

Table 1 provides the types of chemical analyses that may be requested for each stock preparation method and oil type. Note that in addition to analyses summarized in Table 1, the analytical laboratory will archive all WAF stock and dilution series solution sample extracts. Therefore, testing laboratories will check the "Archive extract?" box in addition to analyses requested (Table 1) for each sample on COC forms when relinquishing samples to and requesting analyses from ALS Environmental.

WAF stock and dilution series water samples will be sent to ALS Environmental for chemical analysis. Until being packaged and sent to ALS Environmental for analysis, water samples should be stored according to the requirements listed in Tables 2 and 3. Sample handling and packaging should follow methods provided in Section A.2 in Appendix A (*Analytical Sample Shipping and COC SOP*). Section A.5 in Appendix A provides a sample shipping checklist that can be used when shipping analytical samples to ALS Environmental (see Table A.4).

Table 2. Sample bottles, preservation techniques, and holding times for each type of WAF stock and dilution series water sample/analysis

Analysis requested	Sample container	Bottle volume (mL or oz); sample volume	Preservation technique	Maximum holding time before extraction or analysis
PAH/Alk (8270C SIM/PAH)	Amber glass, wide mouth (*n* = 1)	250 mL; with minimal headspace	Store at 4°C in darkness	7 days (14 days if acid preserved)
BTEX (8260C VOCs)	VOA bottle set with HCl preservative (*n* = 3)	40 mL each; with zero headspace	Store at 4°C in darkness	14 days (7 days if not acid preserved)
DOSS (ALS Environmental SOP)	Plastic centrifuge tube set (*n* = 4)	15 mL each, but only add 10 mL of sample	Freeze at -20°C ± 10°C in darkness	Not established
VOA = volatile organic analysis.				

Note that the maximum holding times listed in Tables 2 and 3 reflect the time from sample collection to the time when sample extraction occurs. ALS Environmental must receive samples such that at least one full business day (Monday–Friday) is allowed for conducting extractions within the listed holding times. Additional information on hold times for sample types not listed here can be found in the analytical QAPP (Appendix D). Ideally all samples should be shipped to the analytical laboratory the day when sampled or as soon as possible thereafter so that the sample holding times are not exceeded. Care should be taken to avoid sending samples over weekends or holidays. If unavoidable circumstances warrant sending samples to ALS Environmental over weekends or holidays, contact Stratus Consulting so that proper arrangements can be made with ALS Environmental sample intake personnel.

Table 3. Sample bottles, preservation techniques, and holding times for each type of sample/analysis

Analysis	Sample container	Bottle volume (mL or oz); sample volume	Preservation technique	Maximum holding time
Archive samples (water)	Amber glass, wide mouth	250 mL; with minimal headspace	Store at 4°C in darkness	7 days (14 days if acid preserved)
DOSS samples (water)	Plastic centrifuge tube set (*n* = 4 vials)	15 mL each, but only add 10 mL of sample	Freeze at -20°C ± 10°C in darkness	Not established
VOCs (water)	VOA bottle set with HCl preservative (*n* = 3)	40 mL each; with zero headspace	Store at 4°C in darkness	14 days (7 days if not acid preserved)

Table 3. Sample bottles, preservation techniques, and holding times for each type of sample/analysis (cont.)

Analysis	Sample container	Bottle volume (mL or oz); sample volume	Preservation technique	Maximum holding time
Fluorescence samples (water)	Borosilicate scintillation vial (n = 1 vial)	7 mL; with zero headspace	Add equal volume of 100% ethanol; store at 4°C in darkness	48 hours for freshwater; 1 week for saline
Source sample (water)	See Section 4.4.5 text		Store at 4°C in darkness	7 days
PAHs (sediment)	Glass jar	8 oz; load at least ¾ full	Store at 4°C in darkness	14 days[a]
VOCs (sediment)	Glass vial set with MeOH or $NaHSO_4$ preservative (n = 3 vials)	40 mL each; load 5 g sediment each and mix gently	Store at 4°C in darkness	14 days
Archive samples (tissue)	Wrapped in foil and bagged[b]	Whole organism tissue samples	Freeze at -20°C or colder	2 years[c]
Blood cell counts (blood smear)	Two glass microscope slides; air dry and preserve within 5 hours		Dry at room temperature in a slide box	None
Blood chemistry (plasma)	Cryovials	1.5 mL	Freeze (-20°C)	None
Blood protein levels (serum)	Cryovials	1.5 mL	Freeze (-80°C)	None
Blood endocrine markers (plasma)	Cryovials	1.5 mL	Freeze (-80°C)	None
Blood immunology (plasma, serum whole blood)	Cryovials	1.5 mL	Freeze (-20°C)	None
Microbiomics (lesions and skin secretions)	Swab tip in dry Eppy tube	2.0 mL	Freeze (-20°C)	None
Genetics (tissue)	DNA/RNA free microcentrifuge (snap-cap) tube	1.5 mL	RNALater at 4°C for 12 hrs then freeze at -20°C	None
DNA damage (tissue)	Glass scintillation vial	6 oz.	RPMI + 10% FBS + DMSO; freeze at -80°C	Two years, -80°C

Table 3. Sample bottles, preservation techniques, and holding times for each type of sample/analysis (cont.)

Analysis	Sample container	Bottle volume (mL or oz); sample volume	Preservation technique	Maximum holding time
Histology (tissue)	One HDPE screw-cap wide-mouth jar	Any	10% neutral buffered formalin, store at room temperature	None
Disease screen (tissue)	Microcentrifuge (snap-cap) tube	1.5 mL	Glycerol; freeze at -20°C	None
Dendrochronology and microchemistry (sagittae otoliths)	Manila paper scale envelope		Dry at room temperature	None
PAH metabolites (bile)	Clear glass vial with foil-lined cap; wrapped with foil	20 mL	Freeze (-80°C)	4+ years, -80°C
Chemical extracts (liquid solvent)	Depends on type of extract; contact Stratus Consulting for additional guidance[c]			

a. Maximum holding time may be extended to four years or longer if samples are stored at -20°C (Appendix D).
b. New, certified pre-cleaned borosilicate glass or polytetrafluoroethylene bottles may also be used to store archive tissue samples.
c. Archive tissue and chemical extract samples will be retained until notified otherwise regardless of holding time.

DMSO = dimethyl sulfoxide; DNA = deoxyribonucleic acid; DOSS = dioctyl sulfosuccinate; FBS = fetal bovine serum; HDPE = high-density polyethylene; RNA = ribonucleic acid; RPMI = Roswell Park Memorial Institute; VOCs = volatile organic compounds.

4.4.2 Archived water samples for PAH analysis

A single archive sample may be taken from each dilution series created from each WAF stock made at the beginning of the test and subsequent renewals for the duration of the test. Archive samples may also be collected from control treatments. Archive samples will be processed into sample extracts which will then be archived at the analytical laboratory. Archive-only samples will not be required if dilution series samples are collected for chemical analysis; extracts from these samples will be archived.

Archive samples should be taken by filling sample bottles directly from the graduated cylinder or mixing vessel in which treatment dilutions are made. Archive samples do not need to be collected for dispersant only tests.

All archive water samples will be collected in 250-mL amber glass bottles and stored at 4°C (Table 3) until sent to ALS Environmental. Archive water samples will also be collected after dilutions are made and prior to being divided into testing chambers. COC forms should indicate that these samples are to be extracted and archived but not analyzed at this time by marking the "Extract and archive only" box for each sample.

4.4.3 Dispersant-only test DOSS water samples

When conducting dispersant-only and variable dispersant tests DOSS sampling and analysis may be performed on all dilutions made from dispersant-only stocks. DOSS water samples may also be collected from control treatments used in dispersant-only tests. Samples should be taken by filling sample bottles directly from the graduated cylinder or mixing vessel in which treatment dilutions are made, just prior to filling test chambers.

For each DOSS sample, four 15-mL plastic centrifuge tubes will be filled to approximately two-thirds full (Table 3). The four sample bottles will have the same sample ID. Respective COC forms will specify that the sample is contained in four sample containers and each container with the same sample ID should be labeled "1 of 4," "2 of 4," and so on. DOSS samples will be stored at or below 4°C and shipped to ALS Environmental. If stored for more than ten days before sending to ALS Environmental, DOSS samples will be frozen (Tables 2 and 3). If not already frozen, ALS Environmental will freeze all DOSS samples when received and store frozen until analyzed.

4.4.4 Fluorescence water samples

Fluorescence analysis may be used to check accuracy of making dilution series from WAF stock solutions. The relative total PAH concentrations in WAF stock solutions and dilution series solutions may be analyzed using a fluorometer. Fluorescence analysis requires that water samples be collected from the WAF stock (or 100% WAF treatment) and from each dilution series prior to being divided into testing chambers. Fluorescence samples may also be collected from control treatments. Fluorescence samples from the WAF stock solution may be used to generate standard curves for analyzing samples. A standard curve compares the relative difference in fluorescence (e.g., total PAHs) between the WAF stock and all the dilution treatments in order to quantify the actual dilution of each treatment relative to the stock WAF.

Specifically, one fluorescence water sample may be collected from each WAF stock and each dilution series at the beginning of the test and subsequent renewals for the duration of the test. Samples should be taken by filling sample bottles directly from the aspirator bottle, graduated cylinder, or mixing vessel in which stocks and/or treatment dilutions are made. A single sample will consist of 3.5 mL of test solution added to 3.5 mL of ethanol in a 7-mL borosilicate

scintillation vial (i.e., 1:1 solution-to-ethanol ratio). Vials should have either a foil-lined or Teflon cap. After filling and capping the vial, mark the liquid level with a permanent marker, weigh the vial, add a Parafilm wrap outside of the cap, and store upright at 4°C. Rather than sending them to ALS Environmental, each testing laboratory will analyze and securely store fluorescence samples. Freshwater samples should be analyzed within 48 hours of sampling, while saline water samples should be analyzed within one week, although it is recommended that all samples be analyzed as soon as possible after collection. Fluorescence sampling and analyses should be conducted according to the *Standard Operating Procedure – Fluorescence Spectroscopy to Verify Dilutions of Water Accommodated Fraction for Toxicity Testing* (Appendix F).

4.4.5 Source water samples

Water used to make exposure solutions is referred to as source water. Source water sources vary among testing laboratories and may come from natural sources such as filtered seawater or filtered saline groundwater, or may be prepared using filtered municipal water and commercial sea salt mixes. Source water samples will be sampled periodically during the course of this project. Specifically, one unfiltered source water sample will be taken from the dilution water used for making control treatment exposure and dilution treatment solutions. The source water sample will be shipped to ALS Environmental and analyzed for PAHs, metals, polychlorindated biphenyls (PCBs), pesticides, and BTEX. Source water samples will be collected in two 1-L amber glass bottles, three VOA bottles, one 500-mL plastic bottle (1.5 L for saline waters), one 250-mL plastic bottle without acid preservative, and one 250-mL plastic bottle with acid preservative. Sample bottles will be provided to laboratories by ALS Environmental. Samples will be stored at 4°C in darkness prior to sending to ALS Environmental. Maximum hold time for all sample bottles is seven days. Source water sample IDs will be generated by each laboratory and are not described in this QAPP. COC forms will specify that all samples will be analyzed for the full contaminants scan. Stratus Consulting will be contacted prior to conducting source water sampling to set up bottle delivery and analysis requests.

4.4.6 Sediment samples

Sediment sampling may be conducted during the course of this project. Sediment samples will be collected according to laboratory-specific protocols for conducting sediment toxicity tests. Required sediment analytical sample bottles are specified in Table 3. Sample labeling and shipping methods will follow requirements outlined in Section 4.6 of this QAPP. Archive sediment samples may also be collected.

4.4.7 Tissue samples

Tissue samples will be collected during the course of this project. Tissue samples may consist of, but are not limited to, whole test organisms, blood, and excised organs (Table 3). Tissue samples not designated for specific chemical, histological, or genomic analyses will be collected as archive tissue samples. Tissue samples that are designed for specific analyses will be collected according to laboratory-specific protocols required for each analysis. Some tissue samples may be subject to special biological substance containerization and shipping requirements. More information on shipping tissue samples that are considered biological substances is provided in Section 4.9.3, *Shipping biological samples*.

Archive tissue samples

All organisms used in toxicity tests but not designated for specific analysis will be retained as archive tissue samples. Remaining tissues from samples not consumed by designated test-specific analyses will be archived. Types of tissue samples that are subject to retention under a project-wide court order are described in the *Retention of Samples and Solutions Generated during Toxicity Testing* internal confidential memorandum, dated July 14, 2011 (Stratus Consulting, 2011) and Section 4.2.

Archive tissue samples and samples subject to the organism retention order should be sampled and preserved according to established methods for conducting organic contaminant analyses on tissue samples (U.S. EPA, 2000). Specifically, tissue samples should be placed in sample containers with as little water as possible. Equipment used to process tissue samples should be made of stainless steel, anodized aluminum, borosilicate glass, polytetrafluoroethylene, ceramic, or quartz. Tissue samples should be wrapped in aluminum foil and placed into a properly labeled sample container or plastic bag. New, certified pre-cleaned borosilicate glass or polytetrafluoroethylene bottles or vials may also be used to store tissue. Archive tissue sample labeling requirements are provided in Section 4.6.1.

Organisms removed from the same exposure chamber at the same time may be included in a single archive tissue sample, unless analysis of individually identified organisms is required. It is possible that test organisms may be too small, too fragile, or decomposed to a state such that sampling is not feasible. If an archive tissue sample cannot be made, Stratus Consulting will be notified and it will be noted in a laboratory notebook. The notebook entry will provide sufficient detail to identify which test and treatment tissue samples could not be made and why the tissue sample could not be collected. If testing laboratories know that tissue sampling will not be feasible, they will contact Stratus Consulting/NOAA for approval to not collect tissue samples for that test.

Archive samples may be securely stored at -20°C at each laboratory for short-term storage or sent to ALS Environmental for long-term storage. If sent to ALS Environmental, sample shipping requirements provided in Section 4.9 will be followed.

4.4.8 Analytical chemistry sample extracts

If testing laboratories conduct chemical analyses, any remaining sample extracts will be retained (Section 4.2). This requirement pertains to pilot, range-finding, and definitive toxicity tests (i.e., all tests).

When generated, remaining chemical extracts may be securely stored under appropriate conditions at each laboratory for short-term storage or sent to ALS Environmental for long-term storage. If sent to ALS Environmental, sample shipping requirements provided in Section 4.9 will be followed.

4.5 Sample Containers, Preservation, and Holding Times

The analytical laboratory (ALS Environmental) will provide the PIs with appropriate containers for PAH, BTEX, and DOSS analysis of the WAF stock, archive water, source water, and sediment samples. Information on sample bottle type, preservation method, and holding times before extraction or analysis for each combination of matrix and analysis is provided in Tables 2 and 3 and in Appendix A, Section A.4. More details on sample containers, preservation, and holding times can be found in the *Mississippi Canyon 252 (Deepwater Horizon) Natural Resource Damage Assessment Analytical Quality Assurance Plan* (Appendix D).

4.6 Analytical Sample Labeling Procedures and Designations

All sample containers will be labeled legibly in permanent ink with the following information:

- Sample ID (details provided below)
- Time of sample collection (military or 24-hour time format)
- Sample preservative (if applicable)
- Sample collector's name.

All sample labels will be covered with clear packing tape that completely encircles the sample bottle to prevent smearing or physical damage to the label. See Section A.2 in Appendix A for more details.

Note that:

- In some cases separate tests may be run using the same WAF stock and dilution series solutions. Therefore, a single WAF stock sample and a subsequent set of archive samples could be used to characterize water chemistry for more than one test. When practiced, laboratories will generate a single sample ID for each sample type using the methodology described below and record that same sample ID for all tests in which the same WAF stock and dilution series solutions are used. Sample IDs will be documented in each test's "Sample Inventory Table" bench sheet. This methodology facilitates tracking all tests in which the same WAF stock and dilution series solutions are used, regardless of sample ID coding.

- For analysis of volatiles (BTEX), three VOA vials per sample are needed. All three vials will receive the same sample ID. This sample ID will be entered once (i.e., on only one line) on the COC forms when requesting analyses and sending samples to ALS Environmental. COC forms have an entry, "# of containers;" for volatile analysis, enter "3."

- For analysis of DOSS, four DOSS centrifuge tubes per sample are needed. All four tubes will receive the same sample ID. This sample ID will be entered once (i.e., on only one line) on the COC forms when requesting analyses and sending samples to ALS Environmental. COC forms have an entry, "# of containers;" for DOSS analysis, enter "4."

- Cryos™ Cryomarkers should be used for labeling cryogenic vials that are subject to extreme cold temperatures.

- If the sample bottle is too small to contain all the label information above, the sample ID will be the only information written on the bottle. Do not try to fit all the information on a small bottle because the information may be illegible. Record the remaining sample label information in a laboratory notebook and/or in the appropriate test results entry bench sheet.

4.6.1 Sample designations

Each sample will receive a unique alphanumeric designation to identify the sampling location, date, sample type, and sample number. This methodology is similar to NOAA field sample labeling guidance. The following format will be used:

XX-Y####-ZZ-###-###

Samples will be identified as follows:

First segment (XX): laboratory-specific two-letter designations. Two-letter designations are as follows:

- Florida Gulf Coast University (FGCU)/University of North Carolina Wilmington = FG
- Hopkins Marine Station (Stanford University) = HS
- Miami University (Ohio) = MU
- Mote Marine Laboratory =MM
- Northwest Fisheries Science Center (NOAA) = NF
- Queen's University = QU
- University of Maryland = UM
- University of Miami, the Rosenstiel School of Marine and Atmospheric Science = RS
- University of North Texas = NT
- University of Southern Mississippi, Gulf Coast Research Laboratory = GR
- Auburn University Department of Fisheries = AB
- U.S. Army Corps of Engineers/ERDC = CE
- Louisiana State University = LS
- Pacific EcoRisk = PE
- University of South Florida = SF
- Marin Biologic Laboratories = MB
- Stratus Consulting = ST
- Louisiana Universities Marine Consortium = LM
- Florida Atlantic University = FA.

Second segment (Y####): sampling date. This five-digit date code includes a letter to represent the year, with 2012 = C, 2013 = D, 2014 = E, and 2015 = F. Following the year letter code are four digits for the month and day, including zeroes. Do not use slashes or dashes between digits. For example, the date code for May 3, 2015, would be F0503.

Third segment (ZZ): two-letter sample type and matrix designations that correspond to respective sample analyses. See Table 4 for a summary of sample type/matrix two-letter designations.

Fourth segment (###): test ID. This is a unique three-digit number that identifies the toxicity test ID number that the sample is associated with. Each test will have a unique number that Stratus Consulting will assign prior to testing.

Fifth segment (###): sequential three-digit sample number for each sample. The sample number sequence will begin with 101 for each test and be sequential regardless of the sample type or matrix. Replicate samples will receive different sequential sample numbers.

Table 4. Sample type/matrix two-letter designations

Matrix	Analysis	Type code
Water – filtered	PAH	FP
Water – unfiltered	PAH	UP
Water – filtered	VOC	FV
Water – unfiltered	VOC	UV
Water – filtered	DOSS	FD
Water – unfiltered	DOSS	UD
Water – filtered	Archive	FA
Water – unfiltered	Archive	UA
Water – filtered	Fluorescence	FF
Water – unfiltered	Fluorescence	UF
Sediment	Any	SE
Sediment	Archive	SA
Tissue	Any	TS
Tissue	Archive	TA
Extracts	Archive	AX
Bacterial culture	Any	BC
Molecular biology	Any	MO

Example sample IDs: the sample ID for Mote Marine Laboratory's fourteenth sample during test number 104, a filtered water sample for PAH analysis collected on July 23, 2015, would be MM-F0723-FP-104-114. A replicate filtered sample from the same WAF would be designated as MM-F0723-FP-104-115.

4.7 Equipment Decontamination

Care should be taken to avoid any cross-contamination of testing equipment, including but not limited to test exposure chambers, laboratory glassware, water quality meter probes, and analytical samples. To the extent possible, new, certified, clean materials should be used to conduct testing and sampling activities. When new testing materials are unavailable, all equipment used during any aspect of testing will be decontaminated before and after use following one of the three protocols outlined in Section A.3 (*Decontamination SOP*) of Appendix A. Equipment or testing materials that cannot withstand any of the decontamination procedures cannot be reused.

4.8 Sample COC

All samples collected during this project will be maintained under strict COC, which is the documentation of a sample's history from the time of collection through sample analysis to final disposal or complete consumption. Sample COC forms will be used to document sample COC, request chemical analyses, transfer samples between laboratories, and transfer samples to long-term storage. A blank COC form is provided in Appendix C of this QAPP. A printable, electronic COC form will also be provided to each testing laboratory.

The individual who prepares and labels a sample is responsible for the care and custody of all samples in his/her possession. A sample is considered to be appropriately in the custody of the sampler only in the following situations:

- The sample is in the individual's possession and no one else has access to the sample
- The sample is in a sealed container that cannot be tampered with or opened without breaking a tamper-proof seal
- The sample is in a designated secure area, cold storage room, locked refrigerator, or similar storage area to which only the person with custody has access
- The sample is in a shipping cooler, envelope, or box that is tamper resistant, properly prepared for shipping, and secured using custody seal tape.

A COC transfer occurs when custody of the samples is transferred from one individual to another (e.g., from the sample collector to the sample packing/shipment individual) or when the samples are shipped to and received by the laboratory. All COC transfers that occur during the course of this project will be documented on the COC form, which will indicate the individual who is relinquishing custody of the sample and who is receiving custody. The date and time of transfer will also be recorded on the COC form. When samples are in the custody of the receiver, the person accepting the sample will sign and date the COC form.

When the samples are packed in coolers or other containers for shipment to the laboratory, the samples will be accompanied by completed original COC records (see Section A.2 in Appendix A, *Analytical Sample Shipping and COC SOP*). Each cooler will only contain the specific samples that are listed on the accompanying COC form(s). The COC record will contain the following information:

- Project name
- Sample shipper contact information
- Stratus Consulting contact information
- Any special instructions

- Sample ID (unique for each sample collected during a test)
- Date and time of sample collection
- Sample matrix (e.g., sediment)
- Analysis required for each sample (see Table 1) for definitive samples
- Name and signature of individual relinquishing custody
- Inclusive dates and times of possession for each person
- Sample shipping date and mode.

Custody seals will be used on shipping containers. These seals detect unauthorized tampering with the sample shipping container from the time the laboratories relinquish samples until they are received. Signed and dated gummed paper seals may be used for this purpose. ALS Environmental will provide the seals with the sample bottles. Seals will be attached so that the next individual to open the shipping container cannot do so without disturbing the seals, as detailed in Section A.2 of Appendix A. Custody seals will also be used when shipping any test materials and can be affixed to envelopes or boxes prior to shipment. Evidence tape may also be used as a custody seal if it is signed and dated by the person relinquishing test materials.

Only individuals authorized to receive the samples at the analytical laboratory can open coolers or other containers containing samples. The containers will first be inspected for integrity of the custody seals or other signs of tampering. The receipt of each sample in a cooler or container will be verified on the COC form. After verification, the signed COC form will be photocopied or scanned, and the copy will be mailed or emailed to the sending party. Samples will be stored in a secure area according to procedures documented for each analytical facility.

Note that these same guidelines apply for the physical transfer of written materials and file storage devices from one individual to another. For these transfers, the box on the COC labeled "Data" is to be checked and the contents being transferred (hardcopies, electronic, SD cards, etc.) are to be checked in the rows provided under the analytical sample information entry fields. Samples subject to long-term storage will be documented using the "Samples stored on-site" checkbox on respective COC forms (Appendix C). Storage location information should also be provided on the COC form.

4.9 Sample Shipping

All samples will be packed in such a manner that they are not compromised during shipment and are received in good condition by the recipient laboratory. All sample containers will be shipped in accordance with all applicable shipping regulations. If shipping frozen samples using dry ice as a refrigerant, refer to Section 4.9.2. If shipping biological samples, refer to Section 4.9.3.

All analytical samples will be packed with enough ice to maintain an ambient temperature of approximately 4°C until the laboratory receives them. Ice will be in gel packs, water frozen in

excess sample bottles, and/or ice cubes within multiple resealable plastic bags to prevent water from touching sample bottles and damaging labels. Bubble wrap or a similar packing material should be used to protect glass bottles during shipment. Glass bottles should not contact other glass bottles or hard objects in the shipping container. All shipping containers that contain analytical and archive samples being sent to ALS Environmental should include a clearly labeled temperature blank (plastic bottle containing cold water used by the analytical laboratory to verify sample temperature upon receipt). All sample coolers will be sealed using custody seals and shipped to the laboratory analyzing the samples via overnight delivery as soon as practical after collection. All sample coolers will be shipped in accordance with all applicable regulations. An original COC record, placed in a sealed plastic bag and taped to the inside of the cooler lid, will accompany each shipping container containing samples. An appropriate carbon- or photocopy of the COC record page must be retained prior to sealing the cooler. Appendix A, Section A.5, provides a sample shipping checklist that can be used as a reference guide when shipping samples to ALS Environmental.

4.9.1 Shipping archive samples

Each toxicity test may generate samples that need to be archived, including tissue samples and solvent extracts from in-house analyses, which must be retained as described in the sample retention order memorandum (Stratus Consulting, 2011). Archive samples requiring long-term cold storage can be sent to ALS Environmental. When shipping archived samples to ALS Environmental for long-term storage, samples will be shipped in separate coolers from chemical analysis samples being sent to ALS Environmental. Each individual cooler should contain no more than 100 individual archive samples. Each sample in the cooler must be properly documented on the designated DWH toxicity testing COC form (Appendix C), including sample ID and date and time of sample collection. The samples should be organized in such a way as to allow for easy ID of the samples once they arrive at ALS Environmental. For example, if 100 samples are going to be shipped, the samples will be divided by putting 10 samples in labeled zip-top plastic bags, and then indicating on the COC form which samples are in which bag. In some cases, only a handful of samples will fit in each cooler (e.g., when shipping large brood stock fish). Thus, for any cooler containing 10 or fewer samples, the sample ID for each individual sample is sufficient, and the samples do not need to be further divided using the above approach. If shipping multiple coolers, then each cooler needs a separate COC form that lists only the samples included in the corresponding cooler. In the special instructions section on the top of the COC form, include the phrase “all samples are for long-term storage only, no analysis required” to indicate that these samples are for storage only. Since ALS Environmental does not need to perform extractions or analyses on these samples, leave the “Analyses Requested” boxes unchecked. Also, the storage temperature for the samples will be indicated on the COC form (e.g., store at -20°C); samples with different storage temperature requirements must be shipped separately from each other. Note that ALS Environmental is only accepting archive samples that

require storage at 4°C or -20°C. For archive samples that have different storage requirements, please contact Stratus Consulting for further instructions.

If samples are shipped to ALS Environmental in Kelso, Washington, the shipping address is:

ALS Environmental
1317 South 13th Avenue
Kelso, WA 98626
800-695-7222 (telephone)

4.9.2 Shipping frozen samples using dry ice

Samples may be kept frozen during shipping using dry ice. When dry ice is used as a refrigerant, special shipping regulations must be followed and special packaging materials must be used. Standard coolers are not acceptable shipping containers. Acceptable shipping containers are two-piece units made of a polystyrene (Styrofoam) inner container that is placed inside an outer corrugated cardboard box. Polystyrene cannot be used as outer packaging. The outer packaging carrying the shipment and the dry ice must be able to withstand the loading and unloading process. Note that the outer package must allow for release of CO_2 gas and should not be completely sealed with packaging tape. Dry ice should not contact or have potential for contacting sample containers. Dry ice will freeze plastic containers or bags, causing them to break apart.

Transportation Security Administration (TSA), International Air Transport Association (IATA), and Department of Transportation (DOT) requirements for the labeling the shipping container will be followed. The maximum amount of dry ice that can be shipped is 200 kg. It is recommended that shipments contain 5–10 pounds (2.3–4.5 kg) of dry ice per 24 hours of transit time. See Appendix A, Section A.6, for detailed dry ice shipping procedures.

4.9.3 Shipping biological samples

Only Biological Substance Category B, Exempt Animal Specimen tissue samples will be shipped. Examples of Category B, Exempt Animal Specimen samples that may be generated and shipped during project activities include, but are not limited to, turtle blood and its components; fresh (not frozen) tissue samples; bacteria cultures; and molecular biology samples not related to exposure and diagnosis of an infectious disease. Exempt Animal Specimen tissue samples will be shipped according to TSA, IATA, and DOT shipping package construction and labeling requirements. See Appendix A, Section A.7, for detailed Category B, Exempt Animal Specimen shipping procedures.

4.10 Sample Storage

Samples for chemical analysis will be sent to the analytical laboratory as soon as possible but may require short-term storage at the testing laboratory prior to shipment. Individual laboratories will store fluorescence samples. While stored at testing laboratories, samples will be kept under strict custody in a lockable refrigerator or freezer. Alternatively, sample custody can also be maintained if the refrigerator or freezer is in a locked room.

Archive tissue samples and remaining chemical extracts generated by testing laboratories may be sent to ALS Environmental for long-term storage as described in Section 4.9. ALS Environmental will store sample extracts, long-term archive samples, and unused portions of all samples under proper COC, temperature, and lighting conditions for at least 24 months. The analytical laboratory will contact Stratus Consulting before disposing of any samples.

4.11 Quality Control

QC is the system established for the project to assess the variability in data that arises from the sampling and analysis procedures used.

4.11.1 Laboratory QC measures

Quantitative analytical data, such as QC limits on precision, accuracy, bias, and detection limits, as well as QC samples prepared and analyzed by testing laboratories, will be specified in the laboratory work plans when applicable. For ALS Environmental QC measures refer to the *Mississippi Canyon 252 (Deepwater Horizon) Natural Resource Damage Assessment Analytical Quality Assurance Plan* (Appendix D) for details.

Non-analytical QC measures will be the responsibility of testing laboratories. Details will be provided in laboratory work plans and SOPs.

4.11.2 Terminating tests

Toxicity tests may be terminated at any time before, during, or after testing. The decision to terminate a test will be made at the discretion of the testing laboratory and/or Stratus Consulting staff. Tests may be terminated due to poor control performance, failure to maintain specified exposure, husbandry issues, COC issues, or any other circumstance that would jeopardize validity or reliability of a test. Laboratory PIs will immediately notify Stratus Consulting when it is determined that a test should be terminated. Alternatively, Stratus Consulting staff will notify PIs if Stratus Consulting staff determines that a test should be terminated.

Stratus Consulting staff will provide guidance on how to handle analytical chemistry samples taken before a test is terminated. If not already sent to ALS Environmental, Stratus Consulting may require that analytical chemistry samples be submitted for analysis. Alternatively, samples already sent to ALS Environmental prior to test termination may subsequently have the requested analyses canceled. Samples collected prior to test termination and not sent for analysis will be discarded, but only after Stratus Consulting approval. If discarded samples were documented on the *Water Accommodated Fraction Preparation and Sampling Table* or *Analytical Sample Inventory Bench Sheet*, the testing laboratory will clearly note on the bench sheets that respective samples have been discarded. As previously mentioned, the sample retention policy described in this QAPP (Section 4.2) applies to all tests, including terminated tests. Therefore, all test organisms used in a terminated test will be archived according to procedures described in Sections 4.4.7 and 4.9.1. Archive tissue samples will be documented using an *Analytical Sample Inventory Bench Sheet* and sample IDs will reference the terminated test ID.

Testing laboratories will retain all toxicity testing data entry bench sheets and any other related files or documents that were used prior to test termination, under the general retention and COC guidelines provided in Section 2.4. Stratus Consulting may request these documents at any time.

Stratus Consulting may choose, on a case-by-case basis, to salvage tests performance and/or analytical chemistry results generated during terminated tests. If this occurs, terminated test results will be subject to data entry and QA/QC (Section 4.11.3) and validation process (Section 6) described in this QAPP. If standard processes described herein are not amenable, then Stratus Consulting will develop, implement, and document an alternative data entry, QA/QC, and validation process.

4.11.3 Toxicity testing data entry

Each test laboratory will check all data entered into the Excel-based toxicity testing data entry files against the original hard-copy bench sheets before sending to Stratus Consulting. A person other than the individual who originally entered information into the data entry files should check transcription accuracy from hard-copy bench sheets. QC checks will be performed on 100% of the entries. Reviewers will correct and note transcription errors using the comment call-out boxes in affected data entry cells. When completed, the reviewer's name and date when checked will be recorded on each test's toxicity testing results reporting data entry file.

After test information is entered into the respective toxicity testing results reporting data entry files and entries are checked against original hardcopy bench sheets, a copy of the workbook will be sent to Stratus Consulting. Testing laboratories will also provide electronic scans or copies of original hard-copy bench sheets to Stratus Consulting. Copies of laboratory notebooks and other

electronic data files, photographs, and other records in which testing results are documented will also be provided to Stratus Consulting.

Please note that the toxicity testing data entry file table structure should not be edited. The files are arranged so that they are standardized among testing laboratories and can be efficiently read and analyzed. If the toxicity testing results reporting data entry files are not suitable for a given test, contact Stratus Consulting for guidance. In some cases, testing laboratories may need to use their own data entry files. Stratus Consulting will review and accept these files prior to beginning tests.

4.11.4 Data management

The laboratory will maintain original copies of all records, data, electronic files, backup files, and study documentation and will not purge the records without permission from Stratus Consulting. Laboratory analytical data will be provided to Stratus Consulting in electronic and hard-copy format. Stratus Consulting will maintain copies of laboratory notebooks, COC forms, data entry files, photographs, and photographic logs.

5. Project Assessment and Oversight

Assessment and oversight includes those actions taken to ensure that this QAPP is implemented properly (U.S. EPA, 2002).

5.1 Assessments and Response Actions

Laboratory PIs working together with Stratus Consulting will direct all testing activities. PIs are responsible for:

- Ensuring that their staff have read and understood all related protocols and this QAPP prior to conducting any definitive toxicity tests
- Directing all sampling activities, including sample collection, handling, and labeling, as well as equipment decontamination
- Reviewing test documents.

PIs will communicate directly with their staff to ensure that appropriate response actions, as well as related efforts to document them, are taken to address any problems or issues.

The analytical laboratory staff will follow their internal procedures, as well as those specified in the analytical methodologies, when performing project oversight and instituting appropriate response actions as outlined in the *Mississippi Canyon 252 (Deepwater Horizon) Natural Resource Damage Assessment Analytical Quality Assurance Plan* (Appendix D).

5.2 Reports to Stratus Consulting

PIs will communicate testing activities regularly to Stratus Consulting. The analytical laboratory will also report to Stratus Consulting, who in turn will communicate with laboratory PIs and other project staff, as appropriate.

Each testing laboratory will submit scanned test-specific toxicity testing results bench sheets as PDFs and data entry files in Microsoft Excel after each test has been completed and QC checks have been completed on all data entry. These test results should be provided to Stratus Consulting no later than one week following the conclusion of a test.

6. Data Assessments and Validation

Data assessments and validation will be conducted on the laboratory toxicity testing and analytical chemistry data according to laboratory documentation regarding the stated methods and acceptability of results. Stratus Consulting and toxicity testing laboratory staff will conduct data assessment activities. Third-party data validators will conduct the data validation.

Testing laboratories will conduct regular internal assessments of their toxicity testing program activities and data. Prior to sending test results to Stratus Consulting, the testing laboratories will assess whether data were collected as described in the testing work plans and SOPs, and whether the data meet laboratory data quality and acceptability criteria. Testing laboratories may determine that tests are invalid when data quality and acceptability criteria are not attained. Invalid tests will be identified and reported to Stratus Consulting as soon as possible so that appropriate measures can be implemented. These measures may include, but are not limited to, test termination, stopping analysis of invalid test chemistry samples, and assigning new test IDs for replacement tests.

Once a test is complete and the data have been provided to Stratus Consulting, Stratus Consulting will perform a thorough assessment of all testing materials. This review will include, but will not be limited to, assessing test performance, determining completeness of testing results, comparing electronic data entry files to hand-written data entry files, and verifying adherence to stated testing methods. Any issues identified during this assessment will be discussed with the testing laboratories on a case-by-case basis, and appropriate actions will be

taken to reconcile issues. If issues cannot be reconciled, the testing laboratories will repeat the invalid tests using new test IDs.

Analytical chemistry data may be validated by a professional, third-party data validator such as EcoChem (Seattle, WA) or Laboratory Data Consultants, Inc. (Sacramento, CA). Data validators will systematically evaluate the degree to which the analytical laboratory followed the prescribed methods, the results of internal QC sample analyses, and the implications for data usability. Analytical data validators will also determine whether samples were handled and data were generated according to the standards and criteria set forth in the standard methods and protocols being used by the analytical laboratory. In most cases these standards and criteria are stated in the *Mississippi Canyon 252 (Deepwater Horizon) Natural Resource Damage Assessment Analytical Quality Assurance Plan* (Appendix D). Data validators may assign or change chemistry data qualifiers during the data validation process. Data validators will provide validated analytical data and report any unresolved issues to Stratus Consulting. Validation methods and results will also be documented in data validation reports. When available, Stratus Consulting will use validated analytical chemistry data when analyzing and reporting testing results.

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A. Protocols and Standard Operating Procedures

A.1 Protocols for Preparing Water Accommodated Fractions

General guidelines:

- Controls should be prepared for all WAF types. Controls should be prepared using the same WAF technique, with the exception of the addition of oil. CEWAFs may require additional control preparations with dispersant only.

- For instructions on cleaning and preparing equipment, refer to the *Decontamination SOP* (see Section A.3 in this appendix).

- If filtered WAF is required, see the *Water Accommodated Fraction Filtration Standard Operating Procedure* provided in Appendix G.

- For each step during sample preparation and collection, all appropriate information must be entered into the bench sheets and data entry files provided by Stratus Consulting (see Section B.4 in Appendix B). Prior to starting an experiment, staff should acquaint themselves with the information that they are required to record.

- All unused WAF solutions left over after analytical sampling and preparing test exposure water dilution series will be disposed of according to the testing laboratory waste management procedures. All test exposure water should also be disposed of according to the testing laboratory waste management procedures when tests are concluded and any samples are taken.

- After it is prepared, WAF solution may be used to make dilution series exposure water for up to 24 hours. Similarly, dilution water may also be used for up to 24 hours after being prepared. These guidelines would only apply for tests that have a renewal within a 24-hour timeframe. WAF and dilution series will be securely stored in a cool, dry, and dark location.

SOP for HEWAF

Materials:

- Waring™ CB15 commercial food blender

- Source water (e.g., seawater or embryo culture solution)

- 1-L or larger graduated cylinder
- 1-L or larger separatory funnels and ring stand
- Heavy-duty aluminum foil, cut into 30 by 30 cm squares, rinsed with acetone/hexane or DCM
- Kimwipes
- Nitrile gloves
- Aluminum weigh boats
- Glass gastight syringes with Teflon plunger (appropriate volumes)
- Stainless steel spatulas
- Miscellaneous glassware to transfer solutions (Erlenmeyer flasks, carboys, beakers, etc.).

Procedure:

A. Preparation of blender lid (this is repeated for each WAF preparation; Figure A.1):

1. Invert blender lid on bench top
2. Center foil square over inside of lid and carefully push down into the lid; push and fold inward to avoid tearing and to keep foil centered
3. Fold excess out over the lip so it can be trimmed with scissors
4. Trim around the edge, leaving ~ 1 cm to fold over the first sealing ridge
5. Press around the edges to make the foil as flat as possible over the sealing ridge
6. Discard and replace foil for each preparation.

Figure A.1. Steps for lining the blender lid with solvent-rinsed foil.

B. Prepare oil HEWAF:

1. Obtain source water from a clean source (e.g., sand filtered, ozonated) and record temperature and salinity. Fill the pre-cleaned blender pitcher with source water; note that the blender pitcher should always be filled to capacity (blender capacity is 3.75 liters) even if the entire volume of WAF is not required. Record start time and source water volume on the "Water Accommodated Fraction Preparation and Sampling Table" bench sheet.

2. Add desired amount of oil to the blender pitcher.

    a. *Source oil and weathered source oil* should be added using a pre-cleaned gastight syringe. It is best to fill the syringe with oil and dispense it prior to taring it on a balance. This will fill the needle and any voids, allowing for a more accurate dispensing weight. Fill the syringe with the desired weight and record the initial weight. After dispensing, record the final weight and determine the actual amount added by mass difference. Note: one gram of oil is equivalent to about 1.2 mL of oil. It is best to have a syringe dedicated to source oil to avoid contamination.

b. *Slick A and B oil* should be weighed in a pre-cleaned aluminum weigh boat. Tare a weigh boat and 2–3 Kimwipes on the top loading balance. Using a stainless steel spatula, add slightly more than the desired mass of oil onto the weigh boat. With the weigh boat over the blender pitcher, slightly bend the weigh boat to create a narrower spout. Carefully transfer the oil using a spatula to scrape the oil into the pitcher. Wipe any oil remaining on the spatula using the tared Kimwipes. Reweigh the weigh boat and Kimwipes to calculate and record the actual mass transferred (Figure A.2).

Figure A.2. Taring Kimwipes and aluminum weigh boat and reweighing scraped weigh boat and Kimwipe used to clean spatula.

3. Close the blender lid.

4. Blend 30 seconds on low.

5. Transfer contents to a pre-cleaned, decontaminated separatory funnel.

6. Note time of transfer.

C. Separation:

1. Transfer separatory funnel to a ring stand, preferably in a hood

2. Allow to separate for 1 hour; be sure not to use the top layer (~ 100 mL) for downstream applications

3. Collect the bottom layer of the unfiltered HEWAF in an intermediate container (e.g., an Erlenmeyer flask); any aliquots for definitive analytical chemistry samples should be gently transferred from the intermediate container to the appropriate sample container (provided by ALS Environmental) immediately prior to the next downstream application (e.g., filtration, serial dilution, or direct-exposure testing).

SOP for LEWAF and CEWAF

Materials:

- Source water (e.g., seawater or embryo culture solution)
- 1-L or larger graduated cylinder
- Aluminum foil
- Stir plate
- Stir bars, Teflon coated
- Aspirator bottles (at least 1 liter or larger capacity)
- Tygon tubing with hose clamps
- Syringes, glass/Teflon gastight
- Container for source water, 20-L Nalgene carboy with spigot
- Top-loading bench scale (should have ≥ 300 g limit)
- Aluminum weigh boats
- Stainless steel spatula
- Kimwipes
- Nitrile gloves.

Procedure:

A. Preparing LEWAF:

1. Obtain source water from a clean source (e.g., sand filtered, ozonated) and record temperature and salinity.

2. Place clean, decontaminated aspirator bottles on stir plates.

3. Secure Tygon tubing and clamps onto bottom outlet.

4. Place stir bar in bottom of aspirator bottle, 1 in. for 1-L, 2 in. for 2-L; do not forget this step as it cannot be done after oil has been added.

5. Add desired amount of source water to each aspirator bottle.

6. Begin to stir with no vortex (180–240 rpm for 2-L with 2-in. stir bar).

7. Add desired amount of oil to aspirator bottle.

    a. *Source and weathered source oil* should be added using a pre-cleaned gastight syringe (Figure A.3). It is best to fill the syringe with oil and dispense it prior to taring it on a balance. This will fill the needle and any voids, allowing for a more accurate dispensing weight. Fill the syringe with the desired weight and record the initial weight. After dispensing, record the final weight and determine the actual amount added by mass difference. Note: one gram of oil is equivalent to about 1.2 mL of oil. It is best to have a syringe dedicated to source oil to avoid contamination.

    b. *Slick A and B oil* should be weighed in a pre-cleaned aluminum weigh boat. Tare a weigh boat and 2–3 Kimwipes on the top loading balance. Using a stainless steel spatula, add slightly more than the desired mass of oil onto the weigh boat. With the weigh boat over the aspirator bottle, slightly bend the weigh boat to create a narrower spout (Figure A.4). Gently transfer the oil using a spatula so that oil does not drop to the bottom and come into contact with the stir bar. Wipe off any oil remaining on the spatula with the tared Kimwipes. Reweigh the weigh boat and Kimwipes to calculate and record the actual mass transferred (Figure A.2).

8. Cover with aluminum foil and stir for 18–24 hours. No settling time is required for LEWAF. It is best to use it immediately to avoid VOC loss, but it may sit for up to 24 hours if necessary.

Figure A.3. Transferring source oil to aspirator bottle.

Figure A.4. Transferring slick oil into aspirator bottle.

9. Prior to use, allow 20–40 mL to drain to waste container. This will clear any water that has been sitting in the Tygon tubing.

10. Unfiltered LEWAF may be used directly for making definitive analytical chemistry samples and dilution series for exposure assays, or may be filtered and then used for making definitive analytical chemistry samples and dilution series for exposure assays; do not use the top layer (~ 100 mL).

B. Preparing CEWAF:

1. Follow steps 1–5 as for LEWAF.

2. Begin to stir with minimal vortex (less than 25% of solution height).

3. Add oil to the center of the vortex.

4. Increase the mixing speed if the vortex decreases to less than 25% of the solution height.

5. Add dispersant to center of the vortex using a gastight syringe, and again calculate the delivery mass by difference; prior to taring the syringe, prefill and dispense the syringe in order to achieve a more accurate weight, as was done with the source oil (use a syringe dedicated to Corexit to avoid contamination).

6. Adjust vortex to 25%.

7. Stir for 18–24 hours. Turn stirrer off. Let settle for 3–6 hours.

8. Prior to use, allow 20–40 mL to drain to waste container. This will clear any water that has been sitting in the Tygon tubing.

9. Unfiltered CEWAF may be used directly for making definitive analytical chemistry samples and dilution series for exposure assays, or it may be filtered and then used for making definitive analytical chemistry samples and dilution series for exposure assays; be sure not to use the top layer (~ 100 mL).

Refer to the figures for photographs of the following subjects:

- Preparing blender lid (Figure A.1)
- Reweighing scraped weigh boat and Kimwipe used to clean spatula taring Kimwipes and aluminum weigh boat (Figure A.2)

- Transferring source oil to aspirator bottle (Figure A.3)
- Transferring slick oil to aspirator bottle (Figure A.4).

A.2 Analytical Sample Shipping and COC SOP

This SOP describes how to properly ship samples for chemical analysis while maintaining established COC requirements.

As described in the project QAPP, COC forms are used to relinquish custody of samples when sent to ALS Environmental or placed in locked storage. When the samples are sent to an analytical laboratory, the COC is used to request which analyses will be conducted for each sample. After all samples have been aliquoted into appropriate bottles and labeled correctly (with clear packing tape to protect the label), each sample collected must be stored and shipped following these COC standards:

1. Wrap labeled sample bottles in bubble wrap bags or similar packing material and place into coolers with blue ice. All samples will be packed with enough ice to keep the samples cooled to approximately 4°C until received by the laboratory. Ice will be in gel packs, water frozen in excess sample bottles, and/or ice cubes in multiple resealable plastic bags to prevent water from touching sample bottles.

    a. Samples should be packed so that they are not directly touching each other or hard objects such as ice packs.

    b. A temperature blank bottle should be included in each cooler. This bottle should be in the empty cooler when it is received. The analytical laboratory will use this bottle to document the temperature of the samples when they are received. If a temperature blank bottle was not provided by ALS Environmental, make one by adding cold water to a capped 250-mL plastic bottle clearly labeled "Temperature Blank."

2. Record the ID for each sample that is placed into the cooler on the COC form(s).

    a. Note that the COC forms are used to request the chemical analyses to be performed for each sample. See the project QAPP for details on the specific analyses required for each type of sample.

3. Sign and date the COC form(s).

4. Retain one photocopy of the COC form(s) for your records in a secure location.

    a. Document shipment details and COC information in your project notebook.

5. Seal the remaining original copies of the COC form(s) in a plastic resealable bag and tape the bag to the underside of the cooler lid (or to the inside of the cooler).

6. Sign and date at least two COC seals (small stickers provided by ALS Environmental with a line for a signature) for each shipping container.

7. Place signed COC seals on opposite corners of the cooler across the seam between the cooler lid and the main body of the cooler (Figure A.5). COC seals must be arranged so that the cooler cannot be opened without disturbing the seals.

8. Place clear packing tape over the COC seals.

9. Seal the cooler by taping around the seam between the lid and body of the cooler and around the entire cooler (Figure A.6).

10. Deliver cooler(s) to a FedEx location or have FedEx pick up the cooler(s). **Do not** leave the cooler(s) at an unattended FedEx drop-off location. Samples will be shipped using overnight delivery as soon as possible following collection in order to provide ample time to perform extraction/analysis within the appropriate holding time. Avoid shipping samples over weekends. If samples are shipped to ALS Environmental in Kelso, Washington, the shipping address is:

    ALS Environmental
    1317 South 13th Avenue
    Kelso, WA 98626
    800-695-7222 (telephone)

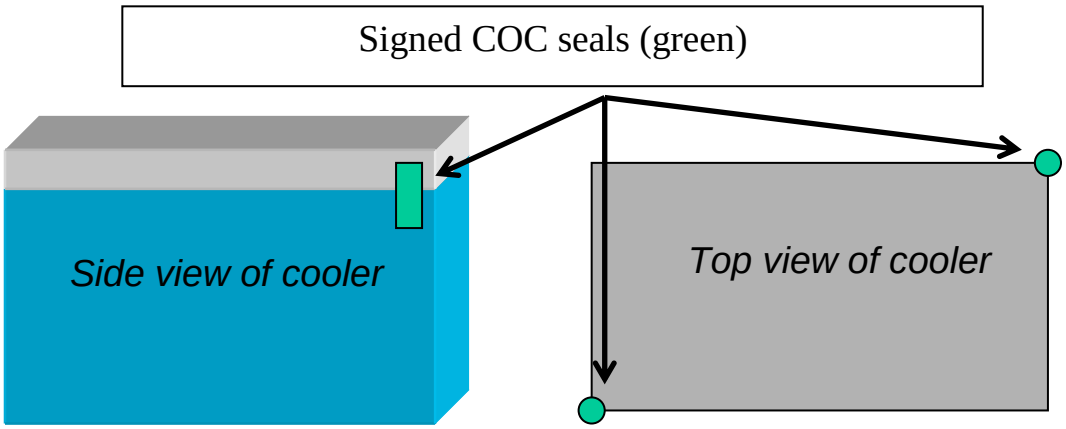

Figure A.5. Placement of signed COC seals (green) on the outside of shipping coolers.

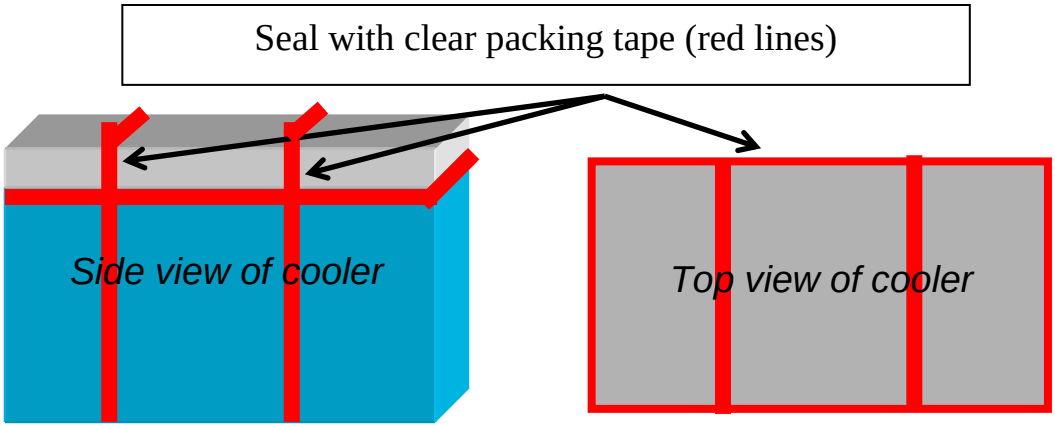

Figure A.6. Placement of clear packing tape (red lines) on shipping coolers.

A.3 Decontamination SOP

These decontamination protocols describe the procedures for preparing all non-disposable equipment prior to experimental analysis. Decontamination should be performed 24 hours in advance of experimental analysis to allow for evaporation of solvents. Three methods are presented below. The first is a solvent-based method and the second is a NaOH-based method used for glassware, stainless steel, or other materials that are inert to solvent and/or caustic chemical exposure. Either of these two methods may be used, depending on resources available to each laboratory. The third method (detergent only) should be used to decontaminate non-disposable test materials that could be destroyed by exposure to solvents and/or caustic

chemicals. Examples of such test materials may include, but are not limited to, water quality meter probes, plastic water holding tanks, and flow-through exposure tank pluming.

General considerations:

- Use gloved hands throughout all preparation steps and use commonsense laboratory safety and Personal Protective Equipment (PPE) when handling solvents.
- Hexane is helpful in removing visible residue from heavily oiled equipment. When cleaning blenders, the underside of blender prongs may need to be carefully scrubbed by hand with hexane-soaked Kimwipes, particularly after use with weathered surface oil samples; note that it is easy to puncture a glove on the blender blades.
- If glassware is too large or bulky (e.g., carboys, aquaria) to submerse into detergent or NaOH solutions, it is acceptable to rinse and scrub it three times, with a 5-minute wait between each rinse/scrub.

Acetone/Hexane/DCM method

Materials:

- Laboratory-grade soap such as Sparkleen or Liqui-Nox; Simple Green All-Purpose Cleaner
- Reagent-grade acetone and Teflon wash bottle
- Reagent-grade hexane and Teflon wash bottle
- Reagent-grade DCM and Teflon wash bottle (optional)
- Kimwipes
- Nitrile gloves (thicker reusable gloves recommended for extended contact use)
- Aluminum foil.

Procedure:

1. Wash all equipment that will come in contact with the sample (glassware, spatulas, stir bars, etc.) with laboratory-grade soap or Simple Green and hot water. When cleaning blenders, soap and hot water can be blended on low for 1 minute.

2. Rinse 3 times with reverse osmosis (RO) or organic-free de-ionized (DI) water to remove any soap residue.

3. Rinse all equipment 3 times with acetone. Allow sufficient time for full evaporation of solvent.

4. Rinse all equipment 3 times with hexane. Allow sufficient time for full evaporation of solvent.

5. If available, rinse all equipment 3 times with DCM. If DCM is not used, perform another triplicate hexane rinse. Allow sufficient time for full evaporation of solvent.

6. Cap glassware with aluminum foil and store in a closed dust-free cabinet.

7. Rinse with source water before using.

NaOH method

Materials:

- Phosphate-free laboratory detergent such as Alconox or Liqui-Nox; Simple Green All-Purpose Cleaner may also be used
- Cleaning brushes
- Reagent-grade NaOH
- Kimwipes
- Nitrile gloves
- Aluminum foil.

Procedure:

1. Dispose of any remaining waste material left in used glassware to be decontaminated in an approved manner.

2. Soak glassware in a solution of phosphate-free laboratory detergent or Simple Green using the manufacturer's suggested dilution for more than 1 hour, preferably 24 hours.

3. Thoroughly brush-wash the glassware and then rinse 6 times with tap water.

4. Soak glassware in a solution of 0.25 M NaOH (10 g NaOH/L DI water) for more than 1 hour, preferably 24 hours.

5. Rinse 6 times with RO or DI water.

6. Rinse 6 times with reagent-grade Acetone. Allow sufficient time for full evaporation of solvent.

7. Rinse 6 times with organic-free DI water.

8. Drain well and air or oven dry.

9. Cap glassware with aluminum foil and store in a closed dust-free cabinet.

10. Rinse with source water before using.

Detergent only method

Materials:

- Laboratory-grade soap such as Sparkleen or Liqui-Nox; Simple Green All-Purpose Cleaner
- Cleaning brushes
- Nitrile gloves (thicker reusable gloves recommended for extended contact use).

Procedure:

1. Wash all equipment that has and will come in contact with the exposure solutions with laboratory-grade soap or Simple Green and hot water.

2. When cleaning water quality meter probes, use a small diameter, soft bristle brush to carefully scrub inside of any orifices or seams. You may have to remove protective caps to thoroughly clean the probe. Be careful not to scratch or damage any exposed sensors.

3. Flush with ample amounts of tap water until all traces of detergent are gone.

4. Rinse 3 times with RO or DI water.

A.4 Water Sampling Matrix/Reference Guide

Table A.1. Sampling matrix for aqueous samples when conducting final/definitive DWH toxicity tests

Analyses requested	Bottles	Filling instructions	Holding time	Storage / handling	When to sample
WAF stock solution sampling: Prior to making dilution series					
PAH/Alk + archive extract	250 mL glass amber (1 per stock)	Fill to top; no headspace	7 days; 14 days if acid preserved[a]	Store at 4°C until sent to ALS Environmental	> For **all** WAF types and **all** oil types
BTEX + archive extract	40 mL glass amber VOA (3 per stock)	Fill until positive meniscus is formed, then cap vial; any bubble in vial must be < 4 mm wide	14 days; 7-days if not acid preserved[a]	Store at 4°C until sent to ALS Environmental	> **Only** WAFs made using **Source** oil type > PAH analyses will also be conducted in addition to BTEX for tests using source oil
DOSS + archive extract	15 mL plastic (4 per treatment)	Fill to ~10 mL	14 days; Indefinite when frozen at -20°C[a]	Store at 4°C until sent to ALS Environmental	> For **all CEWAF** tests > PAH analyses will also be conducted in addition to DOSS for all CEWAF tests
Dilution series – exposure solution sampling: Prior to pouring into exposure chambers; includes control treatments					
Extraction, archive only and PAHs	250 mL glass amber (1 per treatment)	Fill to top; no headspace	7 days; 14 days if acid preserved[a]	Store at 4°C until sent to ALS Environmental	> For **all** WAF types and **all** oil types
DOSS + archive extract	15 mL plastic (4 per treatment)	Fill to ~10 mL	14 days; Indefinite when frozen at -20°C[a]	Store at 4°C until sent to ALS Environmental	> For **dispersant-only** and **variable-dispersant** tests > No PAH samples are needed for **dispersant-only** tests
Fluorescence (PAH)	7 mL borosilicate[b] (1 per treatment)	3.5 mL sample/ 3.5 mL ethanol; no headspace in vial	Freshwater: 48 hours Saline: 1 week	Store at 4°C onsite prior to analysis	> For **all** WAF types and **all** oil types > If not using a 100% WAF treatment, then also need to sample the WAF stock to generate a standard curve

a. ALS Environmental must receive samples with at least 1 full business day (Monday–Friday) to conduct solvent extraction within holding time.
b. ALS Environmental will provide all aqueous sample bottles *except* for 7-mL bottles.

A.5 Sample Shipping Checklist/Reference Guide

Use when sending PAH, BTEX, DOSS, and Extraction/Archive samples to ALS Environmental when conducting final/definitive DWH toxicity tests.

- ❑ Check that sample hold times are not expired (see QAPP).
  Note: ALS Environmental needs at least one business day (Monday–Friday) before hold times expire to process samples. When ALS Environmental receives samples on Saturday, they do not process them until the following Monday.
- ❑ Place clear packaging tape over each sample label to prevent loss or damage to label and check that bottle lids are secure.
  Tape should cover the entire label and circle the bottle at least one time; dry any surface water condensation prior to affixing label and tape.
- ❑ Each sample bottle should be wrapped in bubble wrap, taped again to secure the wrapping, and placed standing up in the bottom of the cooler.
  Do not over-pack coolers – can use multiple coolers; samples should not directly touch each other.
- ❑ Include one temperature blank in each individual cooler.
  Temperature blank should be clearly labeled and inventoried on COC form; temperature blank sample bottles should accompany coolers sent from ALS Environmental; if needed, make a temperature blank using a 250-mL plastic bottle filled with tap water.
- ❑ Fill remaining open cooler space with ice to keep sample temperature 4°C for 24 hours.
  Best practice is to put ice in doubled zip-top baggies placed directly on top of sample bottles until cooler is full; ice is preferred over gel-ice packs.
- ❑ Fill out a COC form (Appendix C). Make a photocopy of the completed form to keep in your records and seal the original form in a clear plastic bag taped to the inside lid of the cooler.
  Note: See QAPP for Analyses Requested.
- ❑ Place custody seals across cooler seam on opposite corners of the lid and wrap the cooler multiple times with clear packaging tape, ensuring that custody seals are taped over but still visible.
- ❑ Ship coolers using FedEx Standard Overnight Express Package Service. Hand coolers over directly to a person. Do not leave them at a pick-up location, where they will be unattended. Retain the shipping receipt. Instructions for filling out the shipping label are below.
  - 3. Recipient: ALS Environmental, 1317 South 13th Avenue, Kelso, WA 98626
    - Telephone: 800-695-7222
  - 4a. Express Package Service: check "FedEx Standard Overnight" – do not fill out Section 4b
  - 5. Packaging: check "Other"
  - 6. Special Handling: leave blank, but check "No" for *Does this shipment contain dangerous goods?*
  - 8. Residential Delivery Signature Options: check "No Signature Required"

A.6 Shipping Samples Using Dry Ice

The following procedures must be followed when shipments contain dry ice. These procedures will be followed in addition to the archive sample requirements or any other applicable sample shipping requirements described in this QAPP.

Acceptable dry ice shipping containers are two piece units made with a polystyrene (Styrofoam) inner container that is placed inside an outer corrugated cardboard box. Styrofoam cannot be used as an outer packaging. The packaging carrying the shipment and the dry ice must be able to withstand the loading and unloading process. The outer package must allow for release of CO_2 gas and should not be completely sealed with packaging tape. The maximum amount of dry ice that can be shipped is 200 kg. It is recommended that shipments contain 5–10 pounds (2.3–4.5 kg) of dry ice per 24 hours of transit time.

The following shipping container labeling and airbill guidelines are required when using dry ice as a refrigerant:

1. Obtain a "Dry Ice – Class 9" placard and write the shipper's and consignee's name and address and total weight of dry ice placed in the shipping container on the face of the placard using a permanent marker (fine-tip Sharpie).

   Note that the "Dry Ice – Class 9" placard was modified in October 2014 (Figure A.7). Use the updated placard for all dry ice shipments. If you need "Dry Ice – UN1845" placards, contact Stratus Consulting.

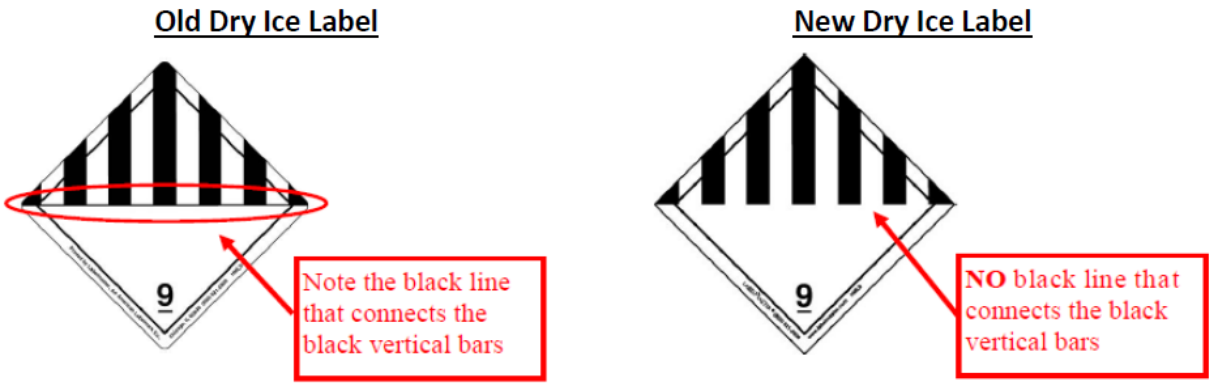

Figure A.7 "Dry Ice – Class 9" placards. Be sure to use the newer placard without the horizontal black line.

2. Affix both a "Dry Ice – Class 9" placard in the center and a "Dry Ice – UN1845" statement sticker in the upper left-hand corner of the largest side of the outer shipping container. The "Dry Ice – UN1845" statement sticker size must be 12 mm or larger. Alternatively, "Dry Ice – UN1845" can be handwritten (printed) in the upper left-hand corner if it is clearly legible and text size is 12 mm or larger.

   Note that "Dry Ice – Class 9" placards often have "Dry Ice – UN1845" printed on them. This is not sufficient to replace the 12 mm or larger "Dry Ice – UN1845" statement sticker labeling requirement described in this step.

3. Write name, address, and phone number of both the shipper and recipient on the outer shipping container on the same side as the "Dry Ice – Class 9" placard.

4. Fill out a FedEx airbill. Retain the sender's copy for your records and record the FedEx Tracking Number (located at the top of the airbill) in the laboratory notebook with respective sample information.

5. Deliver package(s) to a FedEx location or have FedEx pick up the package(s). Do not leave the cooler(s) at an unattended FedEx drop-off location. Turn coolers over directly to a person and retain shipping receipt.

Instructions for filling out the shipping label for containers containing dry ice are provided below:

- Recipient: provide recipient's name, phone number, company, and address; do not check "HOLD" Weekday or Saturday location information
- Express Package Service: check Next Business Day "FedEx Priority Overnight"
- Packaging: check "Other"
- Special Handling and Delivery Signature Options: check "No Signature Required"
- Check "Yes" for *Does this shipment contain dangerous goods?* Check "Dry Ice" and record net weight of dry ice in the shipping container

A.7 Shipping Biological Samples

This SOP describes how to properly ship biological samples while maintaining established COC requirements. Biological samples will be shipped using FedEx, and may be shipped cold or frozen using liquid or dry ice. If shipping biological materials with dry ice, both dry ice and biological shipping requirements should be followed. Biological samples that could be generated

and shipped for this project will fall into the following categories: Category B biological substances and exempt diagnostic samples:

- ***Category B biological samples*** include materials known or reasonably expected to contain a pathogen or infectious substance (e.g., bacterial, viruses, parasites, fungi, prion) that is not generally capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals when exposure occurs. This includes infectious substances transported for research, diagnosis, or investigational activities. Category B biological samples will be shipped according to UN3373 shipping requirements. Note that Category B shipments do not require completing a "Shipper's Declaration for Dangerous Goods" form.

- ***Exempt diagnostic samples*** include materials that are unlikely to cause disease in humans or animals or for which there is only a minimal likelihood that pathogens are present. Examples may include excreta, secreta, blood and its components, tissue and tissue swabs, and body parts. Exempt diagnostic samples will be shipped using similar shipping guidelines as Category B biological samples, with the exception of outer packaging labeling and airbill requirements.

Note that project staff will not ship Biological Substance Category A materials. Category A materials are infectious substances that are capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals. If you have any questions regarding biological substance category classification, contact Stratus Consulting for assistance.

Both Category B and exempt diagnostic biological samples will be shipped using the triple packaging system:

1. Confirm that the primary sample container(s) is watertight and has a positive closure cap, and that it contains no more than 1 L of liquid in aggregate; if a solid, it must not exceed the outer packaging weight. Acceptable primary sample containers include metal, plastic, or glass canisters; jars; or vials with screw-on, snap-on, or push-on lids.

   Note that selection of sample container will also consider requirements outlined in *Archive tissue samples* in Section 4.4.7 and/or work plans for test-specific analyses.

2. Wrap the primary sample container(s) lid with packaging tape for extra closure reinforcement.

3. Wrap the primary sample container(s) with cushioning material so that the samples do not contact each other or the secondary receptacle.

4. Place the prepared primary sample container(s) into a watertight secondary receptacle. Acceptable secondary receptacles include sealed plastic bags, plastic canisters, or screw-cap cans.

5. Stuff absorbent material between the primary and secondary receptacles; use enough absorbent material to absorb the entire contents of the sample(s); paper towels are acceptable absorbent material.

6. Place the sealed secondary container into a sturdy outer package. The outer packaging must be rigid and constructed of corrugated fiberboard, wood, metal, or rigid plastic (use of Styrofoam outer packaging is not permitted); must be capable of withstanding a 4-ft drop; and the package size must be at least 4"×4" on each side to accommodate placards and stickers. Note that more than one secondary container may be placed into the outer package as long as the total volume of liquid samples is less than 4 L or 4 kg of solids.

7. Stuff the outer package with absorbent or other cushioning material to prevent the secondary container from being jostled during shipping.

8. If using dry ice as a refrigerant, place it between the secondary container and outer packaging. For best results, place dry ice above and below the secondary container and place additional absorbent below the dry ice. Use cushioning material to prevent the secondary container from being jostled during shipping when the dry ice sublimates.

   Packages shipped with dry ice must comply with the dry ice shipping protocol (Section A.6).

9. Place a completed COC form sealed in a plastic bag between the secondary receptacle and the outer packaging. The COC form can be used as an itemized list of package contents (a requirement for all Category B biological shipments).

10. Once packaged, tape up the outer packaging container with packaging tape and label the largest side of the package using the following guidelines

    a. Labeling packages containing Category B biological samples

       i. Name and address of the shipper and the consignee.

       ii. Name and telephone number of the responsible person.

       iii. UN3373 placard, with each side being at least 50 mm in length; see example UN3373 placard below (Figure A.8).

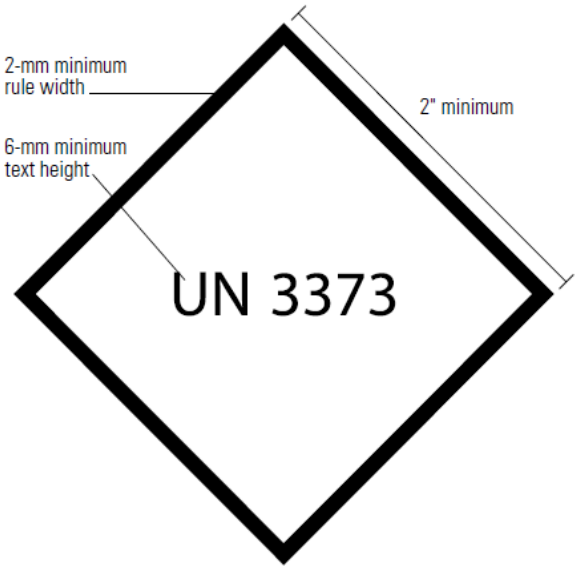

Figure A.8. Example Biological Substance, Category B "UN3373" placard.

iv. If not included on the UN3373 placard, the statement "BIOLOGICAL SUBSTANCE CATEGORY B," in capital letters at least 12 mm high, must be placed on the outer packaging adjacent to the UN3373 placard.

v. If using dry-ice as a refrigerant, affix the "Dry Ice – UN1845" placard and "Dry Ice – UN1845" statement sticker (or handwrite statement) to the outer package. Refer to A.6 SOP above for detailed package labeling procedures.

b. Labeling packages containing exempt diagnostic samples

i. Name and address of the shipper and the consignee

ii. The statement "EXEMPT ANIMAL SPECIMENS," in capital letters at least 6 mm high, must be placed on the outer packaging.

11. Use the following information when filling out biological shipment airbills.

a. In the Internal Billing Reference entry, write:

For Category B shipments – "UN 3373 Biological Substances Category B"

For exempt diagnostic shipments – "Exempt Human/Animal Specimen"

b. Recipient: provide recipient's name, phone number, company, and address; do not check "HOLD" Weekday or Saturday location information

c. Express Package Service: check Next Business Day "FedEx Priority Overnight"

d. Packaging: check "Other"

e. Special Handling and Delivery Signature Options: check "No Signature Required" and the following:

For Category B shipments – Check "Yes" for *Does this shipment contain dangerous goods?*

For exempt diagnostic shipments without dry ice – Check "No" for *Does this shipment contain dangerous goods?*

For any shipments containing dry ice – Check "Yes" for *Does this shipment contain dangerous goods?* Check "Dry Ice" and record net weight.

A.8 Shipping Histology Samples

This SOP will be used when shipping or transporting histology samples that have been fixed in solutions containing formalin. Specimens in formalin can be shipped via air cargo or land freight as "Dangerous Goods in Excepted Quantities" as long as the amount of pure formaldehyde is 30 mL or less in each individual container and less than 500 mL in the shipping container. Unless specified otherwise, a 10% buffered formalin solution will be used for fixing project-specific histology samples. Therefore, individual sample containers will not contain more than 300 mL and the shipping container will not contain more than 5 L of 10% buffered formalin. Histology samples will not be shipped in the same package as other sample types.

1. Once samples have been placed in fixative for at least 24 hrs, they can be prepared for shipment.

2. Obtain two, 1-quart Ziploc freezer bags for each sample, a few rolls of non-dyed paper towels, plain paper index cards, and a pencil.

3. Put on a new pair of nitrile gloves.

4. Check that the sample bottle contains no more than 300 mL of 10% buffered formalin and the cap is tight and does not leak. If not already done, wrap the sample container with clear packaging tape and/or Para-film™ so that the label and cap are completely covered.

5. Place each sample bottle into a Ziploc bag with enough paper towels or similar absorbent material so that they would absorb the entire contents of the sample bottle.

6. Partially seal the bag and then carefully expel as much air as possible without crushing or damaging the sample. After removing the air, completely seal the bag.

7. Write the sample ID on the outside of the sealed Ziploc bag.

8. Repeat steps 5 through 7 until all of the samples are processed.

9. Determine the total volume of 10% buffered formalin of all samples being shipped. Obtain enough shipping containers to ship all samples without going over the maximum 5 L of 10% buffered formalin per shipping container.

10. Pack prepared samples into a cooler or similar rigid shipping container that is lined with the larger plastic bag. The best way to do this is to open up and place the large plastic bag into the shipping container before filling it with samples. Fill the remaining space between the samples and the large plastic bag with more paper towels so that they are not jostled during shipping.

11. Record the ID for each sample that is placed in the cooler on the COC form(s).

12. Sign and date the COC form(s).

13. Retain one photocopy of the COC form(s) for your records in a secure location; document shipment details and COC information in your project notebook.

14. Seal the remaining copies of the COC form(s) in a plastic resealable bag and tape the bag to the inside of the shipping container.

15. Sign and date at least two COC seals (small stickers with a line for a signature) for each shipping container.

16. Place signed COC seals on opposite corners of the shipping container across the seam between the lid and the main body of the shipping container (see Figure A.1). COC seals must be arranged so that the shipping container cannot be opened without disturbing the seals.

17. Place clear packing tape over the COC seals.

18. Seal the shipping container by taping around the seam between the lid and body of the shipping container and around the entire container (see Figure A.2).

19. Label the outer shipping container with the following:

    A properly filled out "Dangerous Goods in Excepted Quantities" label with shipper signature, title, name, address, and date affixed to a vertical side of the outer container. Check "Class 9 material" and enter "UN 3334" under the "Applicable UN Number" field. These labels can be obtained from FedEx. Alternatively labels can be printed in color with overall dimensions of at least 4 in. by 4 in.

    Clearly write the shipper (From:) and consignee (To:) names, addresses, and phone numbers next to the "Dangerous Goods in Excepted Quantities" in letters at least 6-mm tall. The shipper contact information should match that on the "Dangerous Goods in Excepted Quantities" label.

20. Fill out a FedEx US Airbill using the information provided below. Retain the sender's copy for your records and record the FedEx Tracking Number (located at the top of the airbill) in the laboratory notebook with respective sample information.

21. Deliver cooler(s) to a FedEx location or have FedEx pick up the cooler(s). **Do not** leave the cooler(s) at an unattended FedEx drop-off location. Turn coolers over directly to a person and retain shipping receipt.

Histology sample shipment airbill information

Histology samples cannot be shipped using the online airbill option. Therefore, shippers will obtain and fill out a hard copy airbill as described below.

Note that only one airbill may be required when sending multiple packages to the same recipient. In this case, the FedEx agent will affix a matched barcode sticker to all other packages going to the same recipient.

1. Internal Billing Reference: DWH Task Order #X (where X is the Task Order #).

2. Recipient: provide recipient's name, phone number, company, and address; do not check "HOLD" Weekday or Saturday location information.

3. Express Package Service: check Next Business Day "FedEx Standard Overnight."

4. Packaging: check "Other."

5. Special Handling and Delivery Signature Options: check "No Signature Required" and check "Yes" for "Does this shipment contain dangerous goods?"

6. Clearly write "Dangerous Goods in Excepted Quantities" on the top of air bill above the Tracking Number.

7. Payment: check "Sender"; write $100.00 in the "Total Declared Value" entry.

B. Sample ID Look Up Tables and Toxicity Testing Results Reporting Data Entry Bench Sheets

B.1 Sample ID Look Up Tables

Sample ID laboratory codes

Laboratory name	Laboratory code
FGCU/University of North Carolina Wilmington	FG
Hopkins Marine Station (Stanford University)	HS
Miami University (Ohio)	MU
Mote Marine Laboratory	MM
Northwest Fisheries Science Center (NOAA)	NF
Queen's University	QU
University of Maryland	UM
University of Miami, the Rosenstiel School of Marine and Atmospheric Science	RS
University of North Texas	NT
University of Southern Mississippi, Gulf Coast Research Laboratory	GR
Auburn University Department of Fisheries	AB
U.S. Army Corps of Engineers/ERDC	CE
Louisiana State University	LS
Pacific EcoRisk	PE
University of South Florida	SF
Marin Biologic Laboratories	MB
Stratus Consulting	ST
Louisiana Universities Marine Consortium	LU
Florida Atlantic University	FA

Sample ID sample type codes

Matrix	Analysis	Type code
Water – filtered	PAH	FP
Water – unfiltered	PAH	UP
Water – filtered	VOC	FV
Water – unfiltered	VOC	UV
Water – filtered	DOSS	FD
Water – unfiltered	DOSS	UD
Water – filtered	Archive	FA
Water – unfiltered	Archive	UA
Water – filtered	Fluorescence	FF
Water – unfiltered	Fluorescence	UF
Sediment	All analyses	SE
Sediment	Archive	SA
Tissue	All analyses	TS
Tissue	Archive	TA
Extracts	Archive	AX
Bacterial culture	Any	BC
Molecular biology	Any	MO

B.2 Field Entry Dictionary

Data Dictionary

Worksheet name (data table title)	Column Header (data field name)	Column Contents (data field definition)
Analytical Sample Inventory Bench Sheet	Notes	Used to record additional information about each sample; Note for archive tissue samples, record the number of test organisms in sample.
Analytical Sample Inventory Bench Sheet	Number organisms per sample	Documents how many organisms are in a single archive tissue sample
Analytical Sample Inventory Bench Sheet	Recorded by	Name (or initials) of the individual that is recording test information in the respective form
Analytical Sample Inventory Bench Sheet	Sample ID	Sample ID is the concatenation of the following elements: Lab code-Date code-Sample type code-Test ID-Unique sample ID (XX-Y####-ZZ -xxx-xxx)
Analytical Sample Inventory Bench Sheet	Sampling date	Date when the given sample was collected; Use Day-Month-Year format
Analytical Sample Inventory Bench Sheet	Tank ID Dilution or Stock Code	Water samples may be taken from each tank, stocks, and/or dilution series. If samples were made using tank water then the tank ID will be recorded. If samples were made from stock and dilution water then the respective codes will be entered
Analytical Sample Inventory Bench Sheet	Sampling time	Time of day when the given sample was collected; Record in 24-hr format
Analytical Sample Inventory Bench Sheet	Storage location	Record where the given sample will be stored; If not being stored on-site, state where it has been sent
Analytical Sample Inventory Bench Sheet	Description of Sample	Short description of the sample; examples include dilution series, WAF-stock, etc.
Analytical Sample Inventory Bench Sheet	Unique ID Number	Sequential three-digit sample number for each test; First number in the sequence shall be 101. Sequential regardless of the sample type or matrix; Used to provide a unique ID number for each
Tank ID Dilution or Stock Code Definitions	Tank ID Dilution or Stock Code	Tank ID will be determined by each lab when conducting tests; Make sure that it is unique for each test; generate a stock and dilution series codes and record in this cell
Tank ID Dilution or Stock Code Definitions	Start date	Date in which test was initiated - MM/DD/YEAR format
Tank ID Dilution or Stock Code Definitions	Start time	Time of day when the given test was initiated - test organisms added to exposure solution; Record in 24-hr format
Tank ID Dilution or Stock Code Definitions	End date	Date in which test was stopped - MM/DD/YEAR format
Tank ID Dilution or Stock Code Definitions	End time	Time of day when the given test was ended; Record in 24-hr format
Tank ID Dilution or Stock Code Definitions	Nominal Treatment Concentration	Record the treatment or dilution series for the given tank
Tank ID Dilution or Stock Code Definitions	Treatment units	Record the treatment or dilution series concentration units
Tank ID Dilution or Stock Code Definitions	Replicate number	Sequential replicate number, starting from zero for controls
Tank ID Dilution or Stock Code Definitions	Notes	Used to record additional information on how test was set-up
Tank ID Dilution or Stock Code Definitions	Recorded by	Name (or initials) of the individual that is recording test information in the respective form
Test Conditions Table	Aeration (y/n)	Was aeration used to improve dissolved oxygen concentrations during the test
Test Conditions Table	Fed during test (y/n)	Documents if test organisms be fed during exposures.
Test Conditions Table	Feeding regime	If fed during the test, record the feeding schedule; Example entries could include: Daily, twice per day, or prior to renewal
Test Conditions Table	Food source	If test organisms are fed, what dietary items were they given; Example entries could include: artemia nauplii, Iso.algae, or pellets
Test Conditions Table	Life-stage/ age	Documents what life stage and age test organisms were at the beginning of the test
Test Conditions Table	Test duration	Total duration of test in hours
Test Conditions Table	Test type	Static renewal, static, or flow-through
Test Conditions Table	Oil type	Type of oil being tested - slick A, slick B, weathered source, or source
Test Conditions Table	Oil loading rate	Nominal WAF stock oil to water ratio or concentration
Test Conditions Table	Notes	Used to record additional information on how test was set-up
Test Conditions Table	Organisms per tank	Documents how many test organisms will be placed into each replicate tank
Test Conditions Table	Photoperiod (hrs light/drk)	Records how many hours of light and dark a tank will receive in a 24-hr day; Use a #/# format
Test Conditions Table	Endpoints	General description of what endpoints will be assessed
Test Conditions Table	Temperature monitoring schedule	How and when will temperature be monitored
Test Conditions Table	pH monitoring schedule	How and when will pH be monitored
Test Conditions Table	Diss. oxygen monitoring schedule	How and when will oxygen be monitored
Test Conditions Table	Conductivity monitoring schedule	How and when will conductivity be monitored
Test Conditions Table	Salinity monitoring schedule	How and when will salinity be monitored
Test Conditions Table	Alkalinity monitoring schedule	How and when will alkalinity be monitored
Test Conditions Table	Hardness monitoring schedule	How and when will hardness be monitored
Test Conditions Table	Total ammonia monitoring schedule	How and when will ammonia be monitored
Test Conditions Table	Recorded by	Name (or initials) of the individual that is recording test information in the respective form
Test Conditions Table	Test chamber cleaning	If exposure chambers are cleaned during the test, describe when and how
Test Conditions Table	Renewal frequency	Documents the schedule for renewing test solutions; Record as total number of hours between renewals
Test Conditions Table	Temperature	Optimal testing temperature
Test Conditions Table	Salinity	Optimal testing salinity
Test Conditions Table	Light source	What kind of lights are used during test; important for UV exposures
Test Conditions Table	Light intensity	General description for most tests; more details can be provided for UV testing
Test Conditions Table	Replicate number	A sequential count of the number of replicate within each treatment (must be >= 1)
Test Conditions Table	Species tested	Record the common or scientific Name (or initials) of the test organism that is being used for the given test
Test Conditions Table	Dilution/control water	Type of water used for making test solutions; identify commercial salt mixes when used
Test Conditions Table	Test chamber volume (ml)	Document the size of the exposure chamber or tank in milliliters; This is different then the volume of exposure water that is recorded in the "Test solution volume (ml)" field
Test Conditions Table	Test ID	Three digit test identification code; See Stratus Consulting Test ID assignments workbook/table
Test Conditions Table	Test solution volume (ml)	Record the approximate volume of exposure media in each replicate tank; Does not refer to the test chamber size, which is recorded in the "Test chamber volume (ml)" field
Test Conditions Table	Treatment (nominal % WAF)	Record the treatment or dilution series for the given test
Test Conditions Table	WAF prep. method	Record the method that is used for preparing the WAF stock (HEWAF or CEWAF)
Test Performance Monitoring Bench Sheet	Date	Record the date when test performance observations were made; Use Day-Month-Year format
Test Performance Monitoring Bench Sheet	Notes	Used to record additional information on test performance observations
Test Performance Monitoring Bench Sheet	Number at test start	Records the number of test organisms that were added to the tank at the beginning of the exposure/test
Test Performance Monitoring Bench Sheet	Number observed treatment mortalities	Keeps track of how many test organisms died from the listed tank for the observation date and time (not cumulative)

Test Performance Monitoring Bench Sheet	Number observed alive	Used to keep track of the number of organisms in each tank at each time point.
Test Performance Monitoring Bench Sheet	Number from nontreatment mortality	Records any mortalities that accidently occurred from the given tank for the given observation date. Examples include spilling a tank and it's contents or fish that jump out
Test Performance Monitoring Bench Sheet	Recorded by	Name (or initials) of the individual that is recording test information in the respective form
Test Performance Monitoring Bench Sheet	Tank ID	Tank ID will be determined by each lab when conducting tests; Make sure that it is unique for each test
Test Performance Monitoring Bench Sheet	Time	Record time when test performance/organisms health inspections were made for each tank; Use 24-hr format
Water Accommodated Fraction Preparation and Sampling Table	Calculated nominal concentration	Target nominal dilution series concentrations (dispersant tests) or WAF compositions (% WAF)
Water Accommodated Fraction Preparation and Sampling Table	CEWAF settle end time	Time when CEWAF was used after being stirred and left to settle; See the WAF Preparation SOP for required settle times
Water Accommodated Fraction Preparation and Sampling Table	CEWAF settle start time	Time when CEWAF was left to settle after being stirred; See the WAF Preparation SOP for required settle times
Water Accommodated Fraction Preparation and Sampling Table	CEWAF stir end date	Date when CEWAF solution mixing ended; Might be the same time as the CEWAF settle start time; See the WAF Preparation SOP for required mixing times
Water Accommodated Fraction Preparation and Sampling Table	CEWAF stir end time	Time when CEWAF solution mixing ended; Might be the same time as the CEWAF settle start time; See the WAF Preparation SOP for required mixing times
Water Accommodated Fraction Preparation and Sampling Table	CEWAF stir start time	Time when CEWAF solution began to be mixed/stirred; See the WAF Preparation SOP for required mixing times
Water Accommodated Fraction Preparation and Sampling Table	Control (y/n)	Note that control treatment test solutions should be handled similarly as WAF treatment solutions, as shown in this example.
Water Accommodated Fraction Preparation and Sampling Table	Est. total volume needed for test/renewal and samples (L)	Record the estimated total volume of WAF stock needed to fill analytical sample bottles and make treatment dilution series; Includes making each treatment-dilution series, definitive test samples
Water Accommodated Fraction Preparation and Sampling Table	Filtered before use (y/n)	Records whether or not the WAF was filtered prior to being used to make treatment dilution series
Water Accommodated Fraction Preparation and Sampling Table	Filtered DOSS sample ID	If a filtered stock sample was taken for DOSS analysis, then record it's sample ID
Water Accommodated Fraction Preparation and Sampling Table	Filtered PAH sample ID	If a filtered stock sample was taken for PAH analysis, then record it's sample ID
Water Accommodated Fraction Preparation and Sampling Table	Filtered VOC sample ID	If a filtered stock sample was taken for VOC analysis, then record it's sample ID
Water Accommodated Fraction Preparation and Sampling Table	Mass of dispersant added (mg)	Records the weight of dispersant added to seawater when making CEWAF and Corexit only stock solutions
Water Accommodated Fraction Preparation and Sampling Table	Mass of oil added (mg)	Records the weight of oil added to seawater when making the WAF stock solution
Water Accommodated Fraction Preparation and Sampling Table	Notes	Used to record additional information on how the WAF was prepared; If WAFs were composited, this information would be recorded in this field
Water Accommodated Fraction Preparation and Sampling Table	Oil type	Documents the type of oil that was used to prepare the given WAF
Water Accommodated Fraction Preparation and Sampling Table	Prep end date	Documents the day when a WAF stock preparation ended and is ready for use
Water Accommodated Fraction Preparation and Sampling Table	Prep end time	Documents the time when a WAF stock preparation ended and is ready for use; Record in 24-hr format
Water Accommodated Fraction Preparation and Sampling Table	Prep start date	Documents the day when a WAF stock preparation started
Water Accommodated Fraction Preparation and Sampling Table	Prep start time	Documents the time when a WAF stock preparation started; Record in 24-hr format
Water Accommodated Fraction Preparation and Sampling Table	Recorded by	Name (or initials) of the individual that is recording test information in the respective form
Water Accommodated Fraction Preparation and Sampling Table	Time when drain sep. funnel	Record time when the WAF solution being prepared was transferred from the separatory funnel to being used to make samples and treatment dilutions; Note that at least 1-hr settling time is
Water Accommodated Fraction Preparation and Sampling Table	Time when HEWAF X-fer to sep. funnel	Record time when the WAF solution being prepared was transferred from the aspirator bottle to the separatory funnel; Use 24-hr format
Water Accommodated Fraction Preparation and Sampling Table	Unfiltered DOSS sample ID	If a filtered stock sample was taken for DOSS analysis, then record it's sample ID
Water Accommodated Fraction Preparation and Sampling Table	Unfiltered PAH sample ID	If a filtered stock sample was taken for PAH analysis, then record it's sample ID
Water Accommodated Fraction Preparation and Sampling Table	Unfiltered VOC sample ID	If a filtered stock sample was taken for VOC analysis, then record it's sample ID
Water Accommodated Fraction Preparation and Sampling Table	WAF prep. method	Record the method that is used for preparing the WAF stock (HEWAF or CEWAF)
Water Accommodated Fraction Preparation and Sampling Table	Water/diluent volume (L)	Record the volume of seawater added to the blender or aspirator bottle when making the given WAF stock
Water Quality Monitoring	Cond (µS/cm)	Record specific conductance measurement
Water Quality Monitoring	D.O. (mg/L)	Record dissolved oxygen measurement
Water Quality Monitoring	Date	Record the date when WQ measurements were made; Use Day-Month-Year format
Water Quality Monitoring	Period	Indicates sampling period type category as "initial", "daily", "per renewal" or "final".
Water Quality Monitoring	Notes	Used to record additional information on how WQ was measured
Water Quality Monitoring	pH (S.U.)	Record pH measurement
Water Quality Monitoring	Recorded by	Name (or initials) of the individual that is recording test information in the respective form
Water Quality Monitoring	Salinity (ppt)	Record the salinity measurement in parts per thousand
Water Quality Monitoring	Tank ID Dilution or Stock Code	Water quality parameters may be measured in each tank and/or dilution water after filling all of the tanks. If measurements were made using tank water then the tank ID will be recorded. If
Water Quality Monitoring	Test ID	Three digit test identification code; See Stratus Consulting Test ID assignments workbook/table
Water Quality Monitoring	Time	Record time when water quality measurements were made; Use 24-hr format
Water Quality Monitoring	Total ammonia (mg/L)	Record total ammonia measurement
Water Quality Monitoring	Water temp. (C)	Record temperature measurement

B.3 Test Conditions Bench Sheet

Test Conditions Table
CONFIDENTIAL ATTORNEY/CONSULTANT WORK PRODUCT

Data entered by _____ Data QC _____ page_____ of _____

Test Condition Parameter	Value
Test ID number	
Species tested	
Life-stage/ age	
Test duration	
Test type	
Renewal frequency	
Oil type	
WAF preparation method	
Oil Loading rate	
Units of measure for nominal treatment levels	
Nominal Treatment 1 (control)	
Nominal Treatment 2	
Nominal Treatment 3	
Nominal Treatment 4	
Nominal Treatment 5	
Nominal Treatment 6	
Nominal Treatment 7	
Nominal Treatment 8	
Nominal Treatment 9	
Nominal Treatment 10	
Test chamber volume (ml)	
Test solution volume (ml)	
Number of replicates per treatment	
Organisms per tank	
Temperature (°C)	
Salinity	
Light source	
Light intensity	
Photoperiod (hours light/drk)	
Fed during test (y/n)	
Feeding regime	
Food source	
Test chamber cleaning	
Aeration (y/n)	
Endpoints	
Temperature monitoring schedule	
pH monitoring schedule	
Dissolved oxygen monitoring schedule	
Conductivity monitoring schedule	
Salinity monitoring schedule	
Alkalinity monitoring schedule	
Hardness monitoring schedule	
Total ammonia monitoring schedule	
Notes	
Recorded by	
Test Participants	

B.4 WAF Preparation Bench Sheet

Water Accommodated Fraction Preparation and Sampling Table

CONFIDENTIAL ATTORNEY/CONSULTANT WORK PRODUCT

TEST ID _____ Data entered by _____ Data QC_____ page_1____ of ___1___

	WAF Prep 1	WAF Prep 2	WAF Prep 3	WAF Prep 4
Prep start date				
Prep start time				
Prep end date				
Prep end time				
Control (y/n)				
WAF prep. method				
Oil type				
Est. total volume needed for test/renewal and samples (L)				
Water/diluent volume (L)				
Mass of dispersant added (mg)				
Mass of oil added (mg)				
Calculated nominal concentration				
Units of nominal concentration				
CEWAF stir start time				
CEWAF stir end date				
CEWAF stir end time				
CEWAF settle start time				
CEWAF settle end time				
Time when HEWAF X-fer to sep. funnel				
Time when drain sep. funnel				
Filtered before use (y/n)				
Unfiltered PAH sample ID				
Filtered PAH sample ID				
Unfiltered VOC sample ID				
Filtered VOC sample ID				
Unfiltered DOSS sample ID				
Filtered DOSS sample ID				
Notes				
Recorded by				

B.5 Fluorescence Analysis Standard Curve Bench Sheet

Development of Fluorescence Analysis Standard Curve

CONFIDENTIAL ATTORNEY/CONSULTANT WORK PRODUCT

TEST ID _____ Data entered _____ Data QC _____ Page _____ of _____

Item	Value	Dilution (% v/v)	Stock added (uL)	Ethanol Added (mL)	Water Added (mL)	Salt Pellet Visable (y/n)	Total Peak Area	Notes
Test ID		--------	--------	--------	--------	--------	--------	
Date		--------	--------	--------	--------	--------	--------	
Data Entered By		--------	--------	--------	--------	--------	--------	
Oil Type		--------	--------	--------	--------	--------	--------	
Dispersant Type		--------	--------	--------	--------	--------	--------	
WAF Type		--------	--------	--------	--------	--------	--------	
Salinity (ppt)		--------	--------	--------	--------	--------	--------	
Oil:Water Ratio		--------	--------	--------	--------	--------	--------	
Dispersant:Oil Ratio		--------	--------	--------	--------	--------	--------	
Centrifugation Time (min)		--------	--------	--------	--------	--------	--------	
Centrifugation Speed (x g)		--------	--------	--------	--------	--------	--------	
Volume of Sample (mL)		--------	--------	--------	--------	--------	--------	
Volume of Ethanol (mL)		--------	--------	--------	--------	--------	--------	
Wavelength Optimization Standard Used (%v/v)		--------	--------	--------	--------	--------	--------	
Wavelength OptimizationExcitation (nm)		--------	--------	--------	--------	--------	--------	
Wavelength Optimization Emission (nm)		--------	--------	--------	--------	--------	--------	
Ethanol:Water Blank	--------							
Dilution 1	--------							
Dilution 2	--------							
Dilution 3	--------							
Dilution 4	--------							
Dilution 5	--------							
Dilution 6	--------							
Dilution 7	--------							
Dilution 8	--------							
Dilution 9	--------							
Dilution 10	--------							

B.6 Fluorescence Analysis Test Solution Bench Sheet

Fluorescence Analysis of Test Solutions

CONFIDENTIAL ATTORNEY/CONSULTANT WORK PRODUCT

TEST ID _____ Data entered _____ Data QC _____ Page _____ of _____

Test ID	Sample ID	Sample Date	Expected Dilution (%v/v)	Salt Pellet Visable (y/n)	Total Peak Area	Comments

B.7 Water Quality Monitoring Bench Sheet

Water Quality Monitoring

CONFIDENTIAL ATTORNEY/CONSULTANT WORK PRODUCT

TEST ID ______________ Data entered by ______ Data QC______________

page_____ of ______

Tank ID Dilution or Stock Code	Date	Time	Period	Water temp. (C)	pH (S.U.)	D.O. (mg/L)	Cond (µS/cm)	Salinity (ppt)	Total ammonia (mg/L)	Notes	Recorded by

B.8 Test Performance Monitoring Bench Sheet

Test Performance Monitoring Bench Sheet - each tank to be inspected daily

CONFIDENTIAL ATTORNEY/CONSULTANT WORK PRODUCT

TEST ID __________ Data entered by ______ Data QC____________ **page**_____ **of** ______

Tank ID	Date	Time	Number at test start	Number observed treatment mortalities	Number from non-treatment mortality	Number observed alive	Notes	Recorded by

B.9 Tank Dilution or Stock Codes Bench Sheet

Tank ID Dilution or Stock Code Definitions - *For keeping track of Tank IDs and experimental conditions* . **TEST ID** ________ Data entered ____________ Data QC ___________ page___1_ of ___1__

CONFIDENTIAL ATTORNEY/CONSULTANT WORK PRODUCT

Tank ID Dilution or Stock Code	Start date	Start time	End date	End time	Nominal Treatment Concentration	Treatment Units	Replicate number	Notes	Recorded by

B.10 Sample Inventory Bench Sheet

Analytical Sample Inventory Bench Sheet - Document all samples taken during test

CONFIDENTIAL ATTORNEY/CONSULTANT WORK PRODUCT

TEST ID _______________ Data entered by ______ Data QC_____________ page_____ of ______

Sampling date	Sampling time	Tank ID Dilution or Stock Code	Number organisms per sample	Unique ID Number	Sample ID	Description of Sample	Storage location	Notes	Recorded by

C. Chain-of-Custody Form

NRDA Chain of Custody Form: DWH Stratus Consulting

COC Form _____ of _____

Check a box: Samples to ALS ☐ Samples to other lab _____ ☐ Samples analyzed on-site ☐ Samples stored on-site ☐ Data

Sampler/Shipper Information	Stratus Contact Information
Contact/Phone/Email:	Contact/Phone/Email:
Affiliation:	

Incident Name: Mississippi Canyon MC252 Deepwater Horizon ☐ These data require validation

Special Instructions:

ALS Contact Information	
Contact	Greg Salata
Address	1317 S. 13th Ave Kelso WA 98626
Phone	360-501-3376
ALS SR#:	

Sample ID	Sample collection Date	Sample collection Time	Matrix	Analyses requested: PAH/Alk (8270C SIM/PAH)	Total Extractable Hydrocarbons							# of containers	Comments
	mm/ddyyyy	*(24-hr local)*		*Enter x's in boxes below.*									

Relinquished by					Received by				
Date	Time	Signature	Printed Name/Org.		Date	Time		Signature	Printed Name/Org.

D. Mississippi Canyon 252 (*Deepwater Horizon*) Natural Resource Damage Assessment Analytical Quality Assurance Plan

ANALYTICAL QUALITY ASSURANCE PLAN

MISSISSIPPI CANYON 252 (DEEPWATER HORIZON) NATURAL RESOURCE DAMAGE ASSESSMENT

Version 3.0

Prepared for:

U.S. Department of Commerce
National Oceanic and Atmospheric Administration

December 7, 2011

TABLE OF CONTENTS

VERSION 3.0 CHANGES FROM VERSION 2.2:

Version 2.2 Page No.	Change
Cover	Update version # & date
Acronyms	Insert QL (Quantitation Limit) and update format to keep all acronyms and abbreviations on one page.
5	Added a brief description of the analytical methods for metals as the last bulleted item and added a column for metals in the table at the end of the section.
9 (Table 1.1d)	Changed 'Target Detection Limit' to 'Target Method Detection Limit Range'
9 (Table 1.1d)	Changed analyte name from 1-methyl-3-isopropylbenzene to 1-methyl-2-ethylbenzene (center column, bottom cell)
10 (Table 1.1e)	Based on new analytical data, two target analyte names have been revised to indicate that the data refer to a co-elution, and not single target analytes. See the attached revised Table 1.1e
10	Based on new analytical data, one target analyte name was revised to indicate that the peak is an unknown sterane rather than an identified compound. See the attached revised Table 1.1e
10	The quantitation ion was corrected for six analytes (from 217 to 218). See the attached revised Table 1.1e.
None (Table 1.1h)	Added Table 1.1h Metals Target Analyte List
13	Deleted Mark Curry as Project Coordinator and added Dennis Beckmann and Tony Penn as Project Coordinators.
14	Changed company name from ENTRIX to Cardno ENTRIX and changed email address for Cheryl Randle to cheryl.randle@cardno.com from crandle@entrix.com.
14	Changed the contact person for Alpha Analytical from Liz Porta to Susan O'Neil and updated Susan's contact information.
14	Added Battelle Duxbury and NOAA NW Fisheries Science Center with contact information as laboratories contracted for analytical work in support of the NRDA.
15	Reorganized the holding time table. Increased holding time to 2 yrs for sediment and tissue if frozen. Added metals holding time. Separated Oil and Oily Debris as individual line items for each matrix type and adjusted holding times.
21	Section 6.2 - Add as the fourth sentence (after "40CFR part 136."): The quantitation limit (QL) will be defined as the concentration that is equivalent to five times the MDL result, or equivalent to the lowest concentration standard analyzed as part of the initial calibration.
21	Section 6.2 – Second paragraph. Delete grain size from the first sentence. Add "Reporting limit for grain size will be 0.1% or lower."
21	Section 6.2 - Add as the second paragraph: Note that for the Query Manager electronic data deliverable (EDD), there are two fields: detection limit and reporting limit. The detection limit field is equivalent to the MDL, except for those cases where no MDL value exists (for example, oils). If no MDL value exists, the detection limit field is populated with the quantitation limit (QL). The reporting limit field is always populated with the QL value.
21	Section 6.2 - Add the following paragraph to the end of this section: At the discretion of the analytical laboratory, detected analytes at concentrations less than the MDL may be reported, provided that the compound meets the established identification criteria and the peak height is greater than or equal to three times the background noise level. These results will be "J" flagged by the laboratory. During validation, these results will be qualified as "F" (found) to indicate that the value is less than the MDL (see Table 7.2).
21, 29 & 30	Updated references to the table numbers in Sections 6.3 and 6.3.1 through 6.3.8.
22 (Table 6.1a)	For Matrix SRM 1941b for sediment and SRM 1974b for tissue change MQO from 20% to 30% of the NIST uncertainty range, except for fluorene in SRM 1941b extend the low end to 40%.
24 (Table 6.1c)	Added indication that analysis of MC252 Reference Oil is optional.
27 (Table 6.1f)	Added two additional methods for TOC: Standard Methods 5310C and ASTM D4129-82M and one additional method for Grain Size: PSEP 1986 Particle Size plus footnote describing reference document name.
27 (Table 6.1f)	Grain Size footnote – Added reference to use of pipettes for PSEP Particle Size method in the second sentence.

Version 2.2 Page No.	Change
	Added as the last sentence: Additionally, grain size must be reported as "True" for sediment treated with hydrogen peroxide prior to analysis or "Apparent" for sediment not treated with hydrogen peroxide.
27 (Table 6.1f)	Added as the second sentence in footnote 15: Standard Method 5310C requires that injections be repeated until consecutive measurements within 10% are obtained for a water matrix, however, duplicate analyses < 20% RPD are acceptable based on a sediment matrix.
28 (Table 6.1g)	Corrected Continuing Calibration (CCAL) minimum frequency to read: Every 12 hours or every 12 field samples and added that analysis of the MC252 Reference Oil is optional
None (Table 6.1h)	Added Table 6.1h Measurement Quality Objectives for Metals by ICP-AES and & ICP-MS and Mercury by CVAA/CVAFS.
29	For Section 6.3.2, the first sentence is revised to read: Continuing calibration verification (CCV) standards will be run at the beginning (opening) and end (closing) of each analytical sequence, and at the frequencies indicated in Tables 6.1a - 6.1d, and 6.1f - 6.1h.
29	For Section 6.3.3, second paragraph, change second and third sentence to read: The laboratory's value must be within 30% of either the upper or lower end of NIST's 95% uncertainty range for SRM 1941b and SRM 1974b. For oil, water, filters, and inert sorbent materials analyses, SRM 1582 is not extracted, but only diluted and analyzed on the instrument thus the laboratory's value must be within 20% of the NIST uncertainty range.
30	Include the following as Section 6.3.8: 6.3.8 Surrogates All field and QC samples will be spiked with surrogates prior to extraction, as required by the analytical methods. Control criteria for the surrogate recovery are listed in Tables 6.1a - 6.1d, and 6.1g. For the PAH and saturated hydrocarbon analyses, the target analyte concentrations will be corrected for surrogate recovery as specified in the laboratory SOPs.
33 (Table 7.2)	The definition of a "J" qualifier is replaced with the following: Reported concentration is an estimate with potentially more bias or less precision than an unqualified result, as determined by the associated quality control results.

Acronyms and Abbreviations

%D	Percent difference
%R	Percent recovery
ASTM	American Society for Testing and Materials
BS/BSD	Blank spike/blank spike duplicate
CCV	Continuing calibration verification
CRM	Certified reference material
DISP	Dispersant
DOSS	Dioctylsulfosuccinate salt
DOT	U.S. Department of Transportation
DQO	Data quality objectives
EDD	Electronic data deliverable
EIP	Extracted ion Profile
EPA	U.S. Environmental Protection Agency
GC/MS-SIM	Gas chromatography with low resolution mass spectrometry using selected ion monitoring
GC-FID	Gas chromatography with flame ionization detection
LC	Liquid chromatography
MC 252	Mississippi Canyon 252 (Deepwater Horizon)
MDL	Method detection limit
MQO	Measurement quality objectives
MS/MSD	Matrix spike/matrix spike duplicate
NIST	National Institute of Standards and Technology
NOAA	National Oceanic and Atmospheric Administration
NRDA	Natural resource damage assessment
OPA	Oil Pollution Act
OSHA	Occupational Safety and Health Administration
PAH	Polycyclic aromatic hydrocarbons
PIANO	Paraffins, isoparafins, aromatics, napthenes, olefins
QA	Quality assurance
QAP	Quality assurance plan
QC	Quality control
QL	Quantitation Limit
RM	Reference material
RPD	Relative percent difference
RSD	Relative standard deviation
SHC	Saturated hydrocarbons
SOP	Standard Operating Procedures
TEH	Total extractable hydrocarbons
TEM	Total extractable matter
TEO	Total extractable organics
TOC	Total organic carbon
USEPA	U.S. Environmental Protection Agency
VOC	Volatile organic compounds

INTRODUCTION

On April 20, 2010, a fatal explosion struck the Deepwater Horizon offshore oil platform approximately 50 miles off the Louisiana coast in the Gulf of Mexico, ultimately leading to the destruction of the platform and the connecting riser pipe to the seafloor a mile below the water surface, and the ongoing release of thousands of barrels of crude oil from the seafloor per day. The incident has been declared a Spill of National Significance by the U.S. Secretary of Homeland Security and a major spill response effort is in progress. The spill threatens a broad expanse of the U.S. Gulf Coast in addition to the natural resources in the path of the oil slick which has spread across thousands of square miles at sea. Federal and state natural resource trustees have begun collecting ephemeral data to support a natural resource damage assessment (NRDA). Currently, NOAA is the lead administrative trustee. Although a formal agreement has not yet been reached, BP America has indicated an interest in cooperating with the natural resource trustees in the damage assessment.

This Analytical Quality Assurance (QA) Plan describes the minimum requirements for the chemical analysis of the environmental samples that are collected in support of this NRDA. This plan does not address the actual field collection or generation of these samples. The scope of the laboratory work is twofold: (1) generate concentrations for key chemicals used in injury determinations for crude oil releases, and (2) produce more extensive chemical data to use in fingerprinting for source identification. The applicable chemicals, need and frequency of environmental sample analyses, quality control requirements, and data usage vary for these two purposes, although implementation of this plan enables both to be achieved. In recognition of these differences, sampling plans may reference the Analytical QA Plan and cite to specific tables of chemical analyses that are appropriate to the needs of the particular sampling effort.

The requirements specified in this plan are designed to: (1) monitor the performance of the measurement systems to maintain statistical control over the reported concentrations of target analytes and provide rapid feedback so that corrective measures can be taken before data quality is compromised and; (2) verify that reported data are sufficiently complete, comparable, representative, unbiased and precise so as to be suitable for their intended use.

The analytes of concern addressed in this QA Plan are polycyclic aromatic hydrocarbons (PAHs) including alkyl homologues, saturated hydrocarbons (SHC), total extractable hydrocarbons (TEH)[1], and volatile organic compounds (VOCs) and petroleum biomarkers. Additional analytes of concern are potentially toxic polar and non-polar components found within or formed from the dispersant agents utilized during the response to the incident, although the appropriate target analytes and methods are not yet established. A variety of matrices may be analyzed including water, filters, sediment/soil, tissues, vegetation, absorbent materials (e.g. Teflon nets, etc.), oils and oil debris. In addition to the primary analytes of concern, ancillary tests may include: percent moisture, total

[1] TEH is the total aromatic and aliphatic content as determined by GC-FID. If the sample extract is not "cleaned up" to remove biogenic material prior to the GC-FID analysis, then the result from the GC-FID analysis is termed Total Extractable Matter (TEM).

organic carbon (TOC) and grain size for sediment samples, and total extractable organics (TEO) for tissues. Additional tests not currently addressed in the QAP but may be of interest are: SARA (%Saturate, %Aromatic, %Resin, % Asphaltene) content in oil[2]; carbon, hydrogen, and nitrogen (CHN)[3] for sediments and particulate material in water. Performance criteria will be added to the QAP for additional tests when requested under the NRDA program.

The work plans and associated QA plans under which these samples were generated or collected are independent documents and not included or considered herein. This Analytical QA Plan describes the minimum requirements to be taken to provide for the chemical analyses (and associated physical normalizing parameters) of the previously generated or collected samples in a technically sound and legally defensible manner.

This Analytical QA Plan is consistent with the intent of NRDA regulations under OPA (33 U.S.C. §§ 2701 *et seq.)* and satisfies the requirements listed in the relevant EPA guidance for QA plans (USEPA 2002 and USEPA 2001) as far as the documents relate to analytical testing services. This QA plan will be revised as appropriate, as changes are made to the NRDA and the QA program.

[2] SARA according to method published by Zumberge et al (2005) or equivalent. [Zumberge, J., J.A. Russell, and S.A. Reid . 2005. Charging of Elk Hills reservoirs as determined by oil geochemistry AAPG Bull. v. 89, pp. 1347-1371]

[3] CHN by micro elemental analyzer using the Dumas method of complete and instantaneous oxidation (flash dynamic combustion) at >1,000 °C following exposure of the sample to HCl fumes to remove inorganic carbon.

1.0 PROJECT DESCRIPTION

A number of laboratories will be analyzing samples associated with this NRDA. The intent of this plan is to present the minimum requirements for the performance criteria for the laboratories providing data in support of this investigation. The analytes of specific interest and brief descriptions of the analytical methods are as follows:

- PAHs including alkyl homologues by gas chromatography with low resolution mass spectrometry using selected ion monitoring (GC/MS-SIM). The analytical procedure is based on EPA Method 8270D with the GC and MS operating conditions optimized for separation and sensitivity of the target analytes. Alkyl PAH homologues are quantified using a response factor assigned from the parent PAH compound. Analytes, associated response factors and target detection limits are listed in **Table 1.1a.** The following references discuss the method options in further detail:

  Federal Register 40CFR300, Subchapter J, Part 300, Appendix C, 4-6-3 to 4-6-5 pp. 234-237.

  Murphy, Brian L. and Robert D. Morrison (Editors). 2007. *Introduction to Environmental Forensics*, 2nd Edition. Chapter 9, p. 389 – 402;

  Page, D.S., P.D. Boehm, G.S. Douglas, and A.E. Bence. 1995. Identification of hydrocarbon sources in the benthic sediments of Prince William Sound and the Gulf of Alaska following the *Exxon Valdez* oil spill. *In*: *Exxon Valdez Oil Spill: Fate and Effects in Alaskan Waters, ASTM STP 1219*, P.G. Wells, J.N. Bulter, and J.S. Hughes, Eds, American Society for Testing and Materials, Philadelphia. pp 44-83.

  Kimbrough, K.L., G.G. Lauenstein and W.E. Johnson (Editors). 2006. *Organic Contaminant Analytical methods of the National Status and Trends Program: Update 2000-2006*. NOAA Technical Memorandum NOS NCCOS 30. p. 25- 37.

  Sauer, T.C. and P.D. Boehm. 1995. *Hydrocarbon Chemistry Analytical Methods for Oil Spill Assessments*. MSRC Technical Report Series 95-032, Marine Spill Response Corporation, Washington, D.C. 114 p.

  USEPA. 2008. *Test Methods for Evaluating Solid Waste, Physical/Chemical Method* (SW846).

  Wang, Z. and S.A. Stout. 2007. Chemical fingerprinting of spilled or discharged petroleum – methods and factors affecting petroleum fingerprints in the environment. In: *Oil Spill Environmental Forensics: Fingerprinting and Source Identification*. Z. Wang and S.A. Stout, Eds, Elsevier Publishing Co., Boston, MA, pp. 1-53.

- Saturated hydrocarbons by gas chromatography with flame ionization detection (GC/FID) based on EPA Method 8015. Analytes and target detection limits are listed in **Table 1.1b**.

- Total Extractable Hydrocarbons (TEH[4]) representing the total aromatic and aliphatic hydrocarbon content of sample extracts after silica gel clean-up and analysis by GC/FID (**Table 1.1b**). The result is reported based on integration of the FID signal over the entire hydrocarbon range from *n*-C9 to n-C44 and calibrated against the average alkane hydrocarbon response factor.

  If the sample extract does not receive any clean-up then the result will be reported as Total Extractable Matter (TEM) because the extract may contain non-hydrocarbon compounds. Either TEH or TEM may reported by the laboratory depending on the handling of the extract.

- Standard volatile organic compounds (VOC) by GC/MS based on EPA Method 8260B but for aromatics hydrocarbons only. Analytes and target detection limits are listed in **Table 1.1c.**

- Extended list of VOCs for a specialized fingerprinting analysis of paraffins, isoparaffins, aromatics, napthenes, and olefins (PIANO) by GC/MS. Analytes and target detection limits are provided in **Table 1.1d** for this source identification list.

- Petroleum biomarkers by GC/MS-SIM. Two methods for the analysis of petroleum biomarkers are contained herein, viz., quantitative and qualitative. The difference between these two analyses is that quantitative analysis produces absolute concentrations of target analytes whereas qualitative analysis produced pattern, or fingerprints, only. The proposed target analyte list for quantitative biomarkers is provided in **Table 1.1e.** This list may be expanded if warranted. This method is discussed in further detail in:

  Murphy, Brian L. and Robert D. Morrison (Editors). 2007. *Introduction to Environmental Forensics*, 2nd Edition. Chapter 9, p. 389 – 402;

  Wang, Z.. Stout, S.A., and Fingas, M. (2006) Forensic fingerprinting of biomarkers for oil spill characterization and source identification (Review). *Environ. Forensics* **7(2)**: 105-146.

- Qualitative biomarker patterns may also be acquired using GC/MS-SIM with monitoring of selected ions (m/z) as provided in **Table 1.1f**. Since no concentration data are generated by qualitative analysis the results are reported as hardcopy PDF files of each ion over the appropriate retention time(s) and scale and included in the hardcopy data package produced by the laboratory.

- Corexit indicator compounds can be identified and (semi-) quantified by conventional GC/MS-SIM. The indicator compounds presently identified include: 2-butoxyethanol, three closely-eluting glycol ether isomers (reported together as a single analyte), and

[4] Note that the term TEH is being used for the total hydrocarbon analysis. The term "Total Petroleum Hydrocarbon" (TPH) may be used to refer to TEH, in some instances. For this QAP, the term TEH is used to avoid confusion with state-regulated gasoline or diesel determinations, rather TEH is used to refer to the sum of hydrocarbons from C_9 to C_{44}.

bis-(2-ethylhexyl)fumarate (the latter of which is a thermal degradation product of DOSS formed in the GC injection port). These indicator compounds can be identified in samples prepared for alkylated PAH analysis using conventional solvent extraction and preparation. These indicator compounds can be analyzed for concurrently with the alkylated PAHs during the same GC/MS acquisition by adding appropriate ions to the file. Suggested ions for monitoring are listed in **Table 1.1.g**. Indicator compound identifications are confirmed by analyzing a Corexit standard (i.e., a mixture of Corexit 9500 and 9527) under the same conditions as used for samples by comparing ion patterns and GC retention times. Semi-quantitative results for these indicator compounds can be based on a normalized response factor of 1 (without surrogate correction), and then the concentrations reported flagged by the laboratory as semi-quantitative.

- Corexit 9500/9527 dispersant (DISP) by liquid chromatography (LC)/MS for quantitative assessment, particularly dioctylsulfosuccinate sodium salt (DOSS). Proposed measurement performance criteria are presented in **Table 6.1g**. Because the method is under development the laboratory may develop appropriate performance criteria based on past method performance.

- GC/MS may have use for qualitative assessments of solvent package components (e.g. glycol ethers) or primary degradation products of DOSS (alkyl diesters), pending further method development. Standard methods are not available for either technique but provisional analytical criteria and detection limits are under development.

- Total metals in sediments and tissues by inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma-mass spectrometry (ICP-MS) and total mercury in sediments and tissues by cold vapor atomic absorption (CVAA) or cold vapor atomic fluorescence spectrometry (CVAFS). The analytical procedures are based on EPA SW-846 Methods 6010C, 6020A, 7470A, 7471A, 7471B, 7474, and 7742. The target analyte list and target MRLs for each matrix are included in **Table 1.1h**. In order to meet the target MRLs, if may be necessary to use an increased sample size to account for the high moisture content in marine sediments.

Analyses will include a number of different sample matrices. Matrices that will be analyzed will be determined in sampling plans and may not include all analyses for each matrix. The following table provides a summary of which analyses may be applicable to each matrix (analyses may be added or deleted as warranted over time).

Matrix	PAH	SHC/TEH	BIOMARK	DISP	VOC	Metals
Water	X	X	X	X	X	
Filters	X	X	X			
Sediment/Soil	X	X	X	X	X	X
Tissue	X		X	X		X
Vegetation	X	X	X	X		
Inert Sorbent Materials	X	X	X	X	X	
Oil/Oily Debris	X	X	X	X	X	

TABLE 1.1a
Extended PAH (Parent and Alkyl Homologs) and Related Compounds

	Compound	RF Source[5]
D0	cis/trans-Decalin	
D1	C1-Decalins	D0 or tD0[6]
D2	C2-Decalins	D0 or tD0
D3	C3-Decalins	D0 or tD0
D4	C4-Decalins	D0 or tD0
BT0	Benzothiophene	
BT1	C1-Benzo(b)thiophenes	BT0
BT2	C2-Benzo(b)thiophenes	BT0
BT3	C3-Benzo(b)thiophenes	BT0
BT4	C4-Benzo(b)thiophenes	BT0
N0	Naphthalene	
N1	C1-Naphthalenes	N0
N2	C2-Naphthalenes	N0
N3	C3-Naphthalenes	N0
N4	C4-Naphthalenes	N0
B	Biphenyl	
DF	Dibenzofuran	
AY	Acenaphthylene	
AE	Acenaphthene	
F0	Fluorene	
F1	C1-Fluorenes	F0
F2	C2-Fluorenes	F0
F3	C3-Fluorenes	F0
A0	Anthracene	
P0	Phenanthrene	
PA1	C1-Phenanthrenes/Anthracenes	P0
PA2	C2-Phenanthrenes/Anthracenes	P0
PA3	C3-Phenanthrenes/Anthracenes	P0

	Compound	RF Source
PA4	C4-Phenanthrenes/Anthracenes	P0
RET	Retene	RET or P0
DBT0	Dibenzothiophene	
DBT1	C1-Dibenzothiophenes	DBT0
DBT2	C2-Dibenzothiophenes	DBT0
DBT3	C3-Dibenzothiophenes	DBT0
DBT4	C4-Dibenzothiophenes	DBT0
BF	Benzo(b)fluorene	BF or FL0
FL0	Fluoranthene	
PY0	Pyrene	
FP1	C1-Fluoranthenes/Pyrenes	FL0 or PY0
FP2	C2-Fluoranthenes/Pyrenes	FL0 or PY0
FP3	C3-Fluoranthenes/Pyrenes	FL0 or PY0
FP4	C4-Fluoranthenes/Pyrenes	FL0 or PY0
NBT0	Naphthobenzothiophenes	
NBT1	C1-Naphthobenzothiophenes	NBT0
NBT2	C2-Naphthobenzothiophenes	NBT0
NBT3	C3-Naphthobenzothiophenes	NBT0
NBT4	C4-Naphthobenzothiophenes	NBT0
BA0	Benz[a]anthracene	
C0	Chrysene/Triphenylene	
BC1	C1-Chrysenes	C0
BC2	C2-Chrysenes	C0
BC3	C3-Chrysenes	C0
BC4	C4-Chrysenes	C0
BBF	Benzo[b]fluoranthene	
BJKF	Benzo[j,k]fluoranthene	BKF[8]
BAF	Benzo[a]fluoranthene	BKF or BAF

	Compound	RF Source
BEP	Benzo[e]pyrene	
BAP	Benzo[a]pyrene	
PER	Perylene	
IND	Indeno[1,2,3-cd]pyrene	
DA	Dibenz[a,h]anthracene	
GHI	Benzo[g,h,i]perylene	
4MDT	4-Methyldibenzothiophene	DBT0
2MDT	2/3-Methyldibenzothiophene	DBT0
1MDT	1-Methyldibenzothiophene	DBT0
3MP	3-Methylphenanthrene	P0
2MP	2/4-Methylphenanthrene	P0
2MA	2-Methylanthracene	P0
9MP	9-Methylphenanthrene	P0
1MP	1-Methylphenanthrene	P0
	2-Methylnaphthalene	
	1-Methylnaphthalene	
	2,6-Dimethylnaphthalene	
	1,6,7-Trimethylnaphthalene	
	Other	
	Carbazole	
	C30-Hopane[7]	

Target Method Detection Limit Range

Sediment/Soil = 0.1 – 0.5 ng/g dry weight
Tissue = 0.2 – 1.0 ng/g wet weight
Water = 1 – 5 ng/L

Target Reporting Limit

Oil = 2.0 mg/kg

[5] Response factor (RF) to be used for quantitation. If blank, compound is included in the calibration mix.

[6] tD0 = transD0 (used if cis/trans in separate standards)

[7] Quantitative concentrations of C29-hopane and 18α-oleanane may be provided if laboratories are calibrated to do so; the C30-hopane is a minimum requirement.

[8] BKF = Benzo(k)fluoranthene. Benzo(j)fluoranthene and Benzo(k)fluoranthene coelute and will be reported as Benzo(j,k)fluoranthene (BJKF).

TABLE 1.1b
Saturated Hydrocarbons (Alkanes/Isoprenoids Compounds) and Total Extractable Hydrocarbons

Abbr.	Analyte
nC9	n-Nonane
nC10	n-Decane
nC11	n-Undecane
nC12	n-Dodecane
nC13	n-Tridecane
1380	2,6,10 Trimethyldodecane
nC14	n-Tetradecane
1470	2,6,10 Trimethyltridecane
nC15	n-Pentadecane
nC16	n-Hexadecane
nPr	Norpristane
nC17	n-Heptadecane
Pr	Pristane
nC18	n-Octadecane
Ph	Phytane
nC19	n-Nonadecane
nC20	n-Eicosane
nC21	n-Heneicosane
nC22	n-Docosane

Abbr.	Analyte
nC23	n-Tricosane
nC24	n-Tetracosane
nC25	n-Pentacosane
nC26	n-Hexacosane
nC27	n-Heptacosane
nC28	n-Octacosane
nC29	n-Nonacosane
nC30	n-Triacontane
nC31	n-Hentriacontane
nC32	n-Dotriacontane
nC33	n-Tritriacontane
nC34	n-Tetratriacontane
nC35	n-Pentatriacontane
nC36	n-Hexatriacontane
nC37	n-Heptatriacontane
nC38	n-Octatriacontane
nC39	n-Nonatriacontane
nC40	n-Tetracontane

TEH	$\Sigma(C_9\text{-}C_{44})$ Integration of the FID signal over the entire hydrocarbon range from n-C9 to n-C44 after silica gel cleanup.
TEM	$\Sigma(C_9\text{-}C_{44})$ Integration of the FID signal over the entire hydrocarbon range from n-C9 to n-C44 no silica gel cleanup.

	Target Method Detection Limit
Sediment (Alkanes) =	0.01 µg/g dry weight
Sediment (TEH) =	1 µg/g dry weight
Water (Alkanes) =	0.8 µg/L
	Target Reporting Limit
Oil (Alkanes) =	200 mg/kg
Oil (TEH) =	200 mg/kg
Water (TEH/TEM) =	200 µg/L

TEH = Total Extractable Hydrocarbons with silica gel "clean-up"
TEM = Total Extractable Matter with no extract "clean-up"

TABLE 1.1c
Standard Volatile Organic Compounds

Analyte
1,2,4-Trimethylbenzene
1,3,5-Trimethylbenzene
4-Isopropyltoluene
Benzene
Ethylbenzene
Isopropylbenzene
m,p-Xylenes
Naphthalene[9]
n-Butylbenzene
n-Propylbenzene
o-Xylene
sec-Butylbenzene
Styrene
tert-Butylbenzene
Toluene

Target Method Detection Limit Range

Sediment/Soil = 0.1 – 1 ng/g

Water = 0.05 – 0.5 µg/L

Target Reporting Limit

Oil = 2 mg/kg

[9] Naphthalene is also included on the **Table 1.1a** target analyte list of PAH compounds. The PAH analysis is the preferred method, rather than this volatile method. Thus, if a sample location is analyzed for both PAH and VOC the result from the PAH analysis will be noted in the database as the preferred result.

TABLE 1.1d
C5-C13 Volatile Compounds for PIANO Forensic Assessment

Abbrev.	Analyte
IP	Isopentane
1P	1-Pentene
2M1B	2-Methyl-1-butene
C5	Pentane
T2P	2-Pentene (trans)
C2P	2-Pentene (cis)
TBA	Tertiary butanol
CYP	Cyclopentane
23DMB	2,3-Dimethylbutane
2MP	2-Methylpentane
MTBE	MTBE
3MP	3-Methylpentane
1HEX	1-Hexene
C6	Hexane
DIPE	Diisopropyl Ether (DIPE)
ETBE	Ethyl Tertiary Butyl Ether (ETBE)
22DMP	2,2-Dimethylpentane
MCYP	Methylcyclopentane
24DMP	2,4-Dimethylpentane
12DCA	1,2-Dichloroethane
CH	Cyclohexane
2MH	2-Methylhexane
B	Benzene
23DMP	2,3-Dimethylpentane
THIO	Thiophene
3MH	3-Methylhexane
TAME	TAME
1H	1-Heptene/1,2-DMCP (trans)
ISO	Isooctane
C7	Heptane

Abbrev.	Analyte
MCYH	Methylcyclohexane
25DMH	2,5-Dimethylhexane
24DMH	2,4-Dimethylhexane
223TMP	2,2,3-Trimethylpentane
234TMP	2,3,4-Trimethylpentane
233TMP	2,3,3-Trimethylpentane
23DMH	2,3-Dimethylhexane
3EH	3-Ethylhexane
2MHEP	2-Methylheptane
3MHEP	3-Methylheptane
T	Toluene
2MTHIO	2-Methylthiophene
3MTHIO	3-Methylthiophene
1O	1-Octene
C8	Octane
12DBE	1,2-Dibromoethane
EB	Ethylbenzene
2ETHIO	2-Ethylthiophene
MPX	p/m-Xylene
1N	1-Nonene
C9	Nonane[10]
STY	Styrene
OX	o-Xylene
IPB	Isopropylbenzene
PROPB	n-Propylbenzene
1M3EB	1-Methyl-3-ethylbenzene
1M4EB	1-Methyl-4-ethylbenzene
135TMB	1,3,5-Trimethylbenzene
1D	1-Decene
1M2EB	1-Methyl-2-ethylylbenzene

Abbrev.	Analyte
C10	Decane[10]
124TMB	1,2,4-Trimethylbenzene
SECBUT	sec-Butylbenzene
1M3IPB	1-Methyl-3-isopropylbenzene
1M4IPB	1-Methyl-4-isopropylbenzene
1M2IPB	1-Methyl-2-isopropylbenzene
IN	Indan
1M3PB	1-Methyl-3-propylbenzene
1M4PB	1-Methyl-4-propylbenzene
BUTB	n-Butylbenzene
12DM4EB	1,2-Dimethyl-4-ethylbenzene
12DEB	1,2-Diethylbenzene
1M2PB	1-Methyl-2-propylbenzene
14DM2EB	1,4-Dimethyl-2-ethylbenzene
C11	Undecane[10]
13DM4EB	1,3-Dimethyl-4-ethylbenzene
13DM5EB	1,3-Dimethyl-5-ethylbenzene
13DM2EB	1,3-Dimethyl-2-ethylbenzene
12DM3EB	1,2-Dimethyl-3-ethylbenzene
1245TMP	1,2,4,5-Tetramethylbenzene
PENTB	Pentylbenzene
C12	Dodecane[10]
N0	Naphthalene[11]
BT0	Benzothiophene[11]
MMT	MMT
C13	Tridecane[10]
2MN	2-Methylnaphthalene[11]
1MN	1-Methylnaphthalene[11]

Target Method Detection Limit Range

Sediment/Soil = 0.1 – 10 ng/g

Water = 0.2 - 2.0 µg/L

Target Reporting Limit

Oil = 2 mg/kg

[10] These compounds are also included on the **Table 1.1b** target analyte list of saturate hydrocarbons. Because of the extraction technique, the GC-FID method for hydrocarbons is the preferred method, rather than this volatile method. Thus, if a sample location is analyzed for both saturate hydrocarbons by GC-FID and VOC the result from the GC-FID analysis will be noted in the database as the preferred result.

[11] These compounds are also included on the **Table 1.1a** target analyte list of PAH compounds. Because of the extraction technique, the PAH analysis is the preferred method, rather than this volatile method. Thus, if a sample location is analyzed for both PAH and VOC the result from the PAH analysis will be noted in the database as the preferred result.

TABLE 1.1e
Petroleum Biomarkers for Quantitative Analysis

Compound *	Quant Ion m/z
C23 Tricyclic Terpane (T4)	191
C24 Tricyclic Terpane (T5)	191
C25 Tricyclic Terpane (T6)	191
C24 Tetracyclic Terpane (T6a)	191
C26 Tricyclic Terpane-22S (T6b)	191
C26 Tricyclic Terpane-22R (T6c)	191
C28 Tricyclic Terpane-22S (T7)	191
C28 Tricyclic Terpane-22R (T8)	191
C29 Tricyclic Terpane-22S (T9)	191
C29 Tricyclic Terpane-22R (T10)	191
18a-22,29,30-Trisnorneohopane-Ts (T11)	191
C30 Tricyclic Terpane-22S (T11a)	191
C30 Tricyclic Terpane-22R (T11b)	191
17a(H)-22,29,30-Trisnorhopane-Tm (T12)	191
17a/b,21b/a 28,30-Bisnorhopane (T14a)	191
17a(H),21b(H)-25-Norhopane (T14b)	191
30-Norhopane (T15)	191
18a(H)-30-Norneohopane-C29Ts (T16)	191
17a(H)-Diahopane (X)	191
30-Normoretane (T17)	191
18a(H)&18b(H)-Oleananes (T18)	191
Hopane (T19)	191
Moretane (T20)	191
30-Homohopane-22S (T21)	191
30-Homohopane-22R (T22)	191
T22a-Gammacerane/C32-diahopane	191
30,31-Bishomohopane-22S (T26)	191
30,31-Bishomohopane-22R (T27)	191
30,31-Trishomohopane-22S (T30)	191

Compound	Quant ion m/z
30,31-Trishomohopane-22R (T31)	191
Tetrakishomohopane-22S (T32)	191
Tetrakishomohopane-22R (T33)e	191
Pentakishomohopane-22S (T34)	191
Pentakishomohopane-22R (T35)	191
13b(H),17a(H)-20S-Diacholestane (S4)	217
13b(H),17a(H)-20R-Diacholestane (S5)	217
13b,17a-20S-Methyldiacholestane (S8)	217
14a(H),17a(H)-20S-Cholestane/ 13b(H).17a(H)-20S-Ethyldiacholestane (S12)	217
14a(H),17a(H)-20R-Cholestane 13b(H),17a(H)-20R-Ethyldiacholestane (S17)	217
Unknown sterane(S18)	217
13a,17b-20S-Ethyldiacholestane (S19)	217
14a,17a-20S-Methylcholestane (S20)	217
14a,17a-20R-Methylcholestane (S24)	217
14a(H),17a(H)-20S-Ethylcholestane (S25)	217
14a(H),17a(H)-20R-Ethylcholestane (S28)	217
14b(H),17b(H)-20R-Cholestane (S14)	218
14b(H),17b(H)-20S-Cholestane (S15)	218
14b,17b-20R-Methylcholestane (S22)	218
14b,17b-20S-Methylcholestane (S23)	218
14b(H),17b(H)-20R-Ethylcholestane (S26)	218
14b(H),17b(H)-20S-Ethylcholestane (S27)	218
C26,20R- +C27,20S- triaromatic steroid	231
C28,20S-triaromatic steroid	231
C27,20R-triaromatic steroid	231
C28,20R-triaromatic steroid	231

* Peak identification provided in parentheses.

Target Reporting Limit

Sediments/Soil = 2 ug/Kg dry weight
Waters = 10 ng/L

Target Reporting Limit

Oil = 2 mg/Kg

TABLE 1.1f
Suggested Hydrocarbon Groups and Petroleum Biomarkers for Qualitative Analysis

n-Alkycyclohexanes (m/z 83)
n-Alkanes (m/z 85)
Diamondoids (m/z 135, 187)
Sesquiterpanes (m/z 109, 123)
Isoprenoids (m/z 183)
Triterpanes (m/z 191)
Regular Steranes (m/z 217)
Rearranged β,β-steranes (m/z 218)
Methyl steranes (m/z 232, 245)
Methyl and triaromatic steroids (m/z 231)
Monoaromatic steroids (m/z 253)
Diasteranes (m/z 259)

TABLE 1.1g
Corexit Indicator Compounds for Qualitative Analysis in Water Only
(monitoring mass/charge ion)

2-Butoxyethanol (m/z 87, 75)
Glycol ether Isomers (m/z 59, 103)
Bis-(2-ethylhexyl) fumarate (m/z 112, 211)

TABLE 1.1h
Metals Target Analyte List

Analyte	Method	Target Reporting Limits (RL)	
		Sediment (mg/Kg) dry weight	Tissues (mg/Kg) wet weight
Aluminum	ICP/ICP-MS	10	5
Antimony	ICP/ICP-MS	0.05	NA
Arsenic	ICP-MS	0.5	0.5
Barium	ICP/ICP-MS	0.1	0.05
Beryllium	ICP/ICP-MS	0.05	NA
Cadmium	ICP/ICP-MS	0.05	0.02
Calcium	ICP/ICP-MS	100	NA
Chromium	ICP/ICP-MS	0.2	0.2
Cobalt	ICP/ICP-MS	0.05	0.05
Copper	ICP/ICP-MS	0.1	0.1
Iron	ICP/ICP-MS	10	2
Lead	ICP/ICP-MS	0.05	0.02
Magnesium	ICP/ICP-MS	50	NA
Manganese	ICP/ICP-MS	0.2	0.2
Mercury	CVAA/CVAFS	0.01	0.01
Nickel	ICP/ICP-MS	0.5	0.5
Potassium	ICP/ICP-MS	100	NA
Selenium	ICP/ICP-MS	0.1	0.1
Silver	ICP/ICP-MS	0.05	0.05
Sodium	ICP/ICP-MS	100	NA
Strontium	ICP/ICP-MS	2.00	NA
Thallium	ICP/ICP-MS	0.1	NA
Vanadium	ICP/ICP-MS	0.5	0.5
Zinc	ICP/ICP-MS	1	0.5

Method detection limits (MDL) should be at least 3 times lower than the target reporting limits.

2.0 PROJECT ORGANIZATION AND RESPONSIBILITIES

2.1 Assessment Manager

Greg Baker
Office of Response and Restoration
NOAA
345 Middlefield Road, MS-999
Menlo Park, CA 94025
(650)329-5048 FAX (650)329-5198
greg.baker@noaa.gov

The Assessment Manager is the designated natural resource trustee representative who is responsible for the review and acceptance of specific work plans and associated QA plans.

2.2 Project Coordinators

The Project Coordinators are responsible for administration of the contracts with the laboratory(ies). The Project Coordinators will oversee the proper scheduling and transmittal of the data from the time of sampling to data reporting.

Project Coordinator for Battelle:

Dennis Beckmann
Data and QA Manager
Gulf Coast Restoration
BP America, Inc.
501 Westlake Park Boulevard
Houston, TX 77079
dennis.beckmann@noaa.gov

Project Coordinator for Alpha Analytical, Columbia Analytical Services, and NOAA NW Fisheries Science Center:

Tony Penn
Deputy Division Chief
NOAA Assessment and Restoration Division
1305 East West Highway
Building SSMC4
Silver Spring, MD 20910
tony.penn@noaa.gov

2.3 Quality Assurance

Ann Bailey is the QA Coordinator reporting directly to the Assessment Manager. Ms. Bailey is responsible for the implementation of this Analytical QA Plan. She will receive assistance in the coordination and performance of laboratory technical audits and independent data validation from the QA Contractor (EcoChem). The QA Coordinator has the authority and responsibility to cease or temporarily halt activities not in keeping with this QA Plan. The QA Coordinator will work closely

with laboratory representatives and the project team to assure that project and data quality objectives are met. The QA Coordinator may be reached at:

Ann Bailey
EcoChem, Inc.
710 Second Avenue, Suite 660
Seattle, WA 98104
(206)233-9332 x106 FAX (206)233-0114
abailey@ecochem.net

Cheryl Randle is a QA Reviewer conducting data validation on behalf of BP America. Ms. Randle is responsible for working closely with the Assessment Manager's QA Coordinator to assure the validity of the final data in accordance with this Analytical QA Plan. The QA Reviewer will conduct spot validation of up to 25 percent of the reported data, unless substantial problems are discovered in which case up to 100 percent validation may be performed. The QA Reviewer may be reached at:

Cheryl Randle
Cardno ENTRIX, Inc.
1000 Hart Road, Suite 130
Barrington, IL 60010
(847)277-2865 FAX (847)381-6679
cheryl.randle@cardno.com

2.4 Analytical Laboratories

The laboratories planned to be contracted at this time for analytical work in support of the NRDA are TDI-Brooks B&B Laboratories (B&B), Newfields/Alpha Analytical (Alpha), and Columbia Analytical Services (CAS). The laboratory project managers are responsible for assuring that all analyses performed meet project and measurement quality objectives. The Laboratory Project Managers are:

Juan Ramirez
TDI-Brooks B&B Laboratories
1902 Pinon
College Station, TX 77845-5816
(979)693-3446 FAX: (979)693-6389
juanramirez@TDI-BI.com

Jonathan Thorn
Battelle
397 Washington Street
Duxbury, MA 02332
781-952-5271
thornj@battelle.org

Susan O'Neil
Alpha Analytical
320 Forbes Boulevard
Mansfield, MA 02048
508-844-4117
soneil@alphalab.com

Gina Ylitalo
NOAA NW Fisheries Science Center
2725 Montlake Boulevard
Seattle, WA 98112
206-860-3325
gina.ylitalo@noaa.gov

Greg Salata, PhD.
Columbia Analytical Services (CAS)
1317 S. 13th Ave.
Kelso, WA 98626
(360)577-7222
gsalata@caslab.com

As additional analytical laboratories are brought under contract, this QAP will be updated to include their names and project managers.

3.0 SAMPLE HANDLING AND CHAIN OF CUSTODY PROCEDURES

Chain of custody procedures will be used for all samples throughout the analytical process and for all data and data documentation, whether in hard copy or electronic format. Sampling procedures, including sample collection and documentation, are part of the work plans of the individual projects and as such, are not considered here.

3.1 Sample Preservation and Holding Times

Sample preservation and field treatment of samples for analyses should be described in relevant field work plans. Based on EPA guidance, "advisory" sample holding times prior to analysis and holding times for the extracts are presented in **Table 3-1**. These holding times may be extended or preservation guidance amended, as options are assessed.

3.2 Chain of Custody

Chain of custody records will be completed in ink.

A sample is considered in “custody” if:

- it is in the custodian’s actual possession or view, or
- it is retained in a secured place (under lock) with restricted access, or
- it is placed in a container and secured with an official seal(s) such that the sample cannot be reached without breaking the seal(s).

Samples are kept in the custody of designated sampling and/or field personnel until shipment.

3.3 Sample Shipping

Any transfer or movement of samples will use chain of custody procedures. The original signed and dated chain of custody record accompanies the sample(s); a copy is retained by the sample shipper. All shipments will comply with DOT regulations (*49CFR, Parts 172 and 173*).

TABLE 3-1
Sample Holding Times

Matrix/Analysis	Storage for Samples	Holding Time to Extraction	Holding Time to Analysis
VOC Analyses			
Water	Refrigeration 4ºC ±2º with no headspace; Optional: Preserved with HCl in the field in VOA vial.	Not applicable	7 days if not acid preserved; 14 days if acid preserved
Sediment	Refrigeration 4ºC ±2º For preservation requirements, see SW-846 Method 5035A.	Not applicable	14 days
Oil	Above freezing to 30ºC	Not applicable	No holding time
Oily Debris	Refrigeration <6ºC	Not applicable	No holding time
PAH, SHC/TEH, Biomarker Analyses			
Water	Refrigeration 4ºC ±2º; Optional: Preserved with 1:1 HCl to pH<2	7 days if not acid preserved; 14 days if acid preserved	40 days from extraction[12]; except biomarkers no holding time
Filters	Frozen (-20ºC ±10ºC)	2 Years	40 days from extraction[12]; except biomarkers no holding time
Sediment/Soil (also total solids, grain size and TOC)	Frozen (-20ºC ±10ºC), except Grain Size should not be frozen – store at 4ºC ±2º	2 Years, except not applicable for Grain Size, Total Solids, and TOC	40 days from extraction[12]; except biomarkers grain size, total solids and TOC no holding time.
Tissue (Total Extractable Organics aka Lipids)	Frozen (-20ºC ±10ºC)	2 Years	40 days from extraction[12]; except biomarkers and TEO no holding time.
Vegetation	Frozen (-20ºC ±10ºC)	2 Years	40 days from extraction[12]; except biomarkers no holding time
Inert Sorbent Material	Frozen (-20ºC ±10ºC)	2 Years	40 days from extraction[12]; except biomarkers no holding time
Oil	Above freezing to 30ºC	No holding time	40 days from extraction[12]; except biomarkers no holding time
Oily Debris	Refrigeration <6ºC	No holding time	40 days from extraction[12]; except biomarkers no holding time
Dispersants (DOSS) Analyses			
Water	Frozen (-20º ±10ºC), 15mL plastic centrifuge tubes	Not established	Not established
Sediment and Tissue	Frozen (-20º ±10ºC), glass jars	Not established	Not established
Metals Analyses			
Water	Preserve with HNO_3 to pH <2	Not applicable	6 months except Mercury: 28 days
Sediment and Tissue	Frozen (-20°C ±10°C)	Not applicable	2 years except Mercury: 1 year[13]

[12] 40 days is an advisory extraction holding time. Extracts should be held at -20C in the dark, and may be analyzed past 40 days and results not qualified if surrogates are within criteria.

[13] Holding time for metals, except mercury, is based on *Puget Sound Dredged Disposal Analysis Data Quality Guidance Manual* (PTI July 1988). Holding time for mercury is based on *Appendix to Method 1631 Total Mercury in Tissue, Sludge, Sediment, and Soil by Acid Digestion and BrCl Oxidation* (EPA-821-R-01-013, January 2001)

3.4 Sample Receipt

Immediately upon receipt of samples, the recipient will review the shipment for consistency with the accompanying chain of custody record and sample condition, before signing and dating the chain of custody record. Sample condition(s) will be noted on the laboratory's sample receipt form and maintained with the chain of custody records. If there are any discrepancies between the chain of custody record and the sample shipment, the recipient will contact the sample shipper immediately in an attempt to reconcile these differences. Reconciliation of sample receipt differences will be maintained with the chain of custody records and discussed in the laboratory narrative which accompanies the data report.

3.5 Intra-Laboratory Sample Transfer

The laboratory sample custodian or designee will maintain a laboratory sample-tracking record, similar to the chain of custody record that will follow each sample through all stages of laboratory processing. The sample-tracking record will show the name or initials of responsible individuals, date of sample extraction or preparation, and sample analysis.

3.6 Inter-Laboratory Sample Transfer

Transfer of samples from one analytical laboratory to another, e.g. for grain size or TOC analysis, will follow chain of custody, sample shipping and receipt procedures described above. Transfer of samples between laboratories will be noted in the laboratory case narrative which accompanies the data report.

3.7 Sample Archival

All unanalyzed samples and unutilized sample aliquots or extracts will be held by the laboratory in a manner to preserve sample integrity at a secure location with chain of custody procedures for one (1) year after the QA Contractor has validated the data package for that particular set of samples. All archived materials will be accessible for review upon request. At the end of the archival period, the laboratory shall contact the QA Coordinator to obtain directions for handling remaining samples. The samples will not be disposed of by the laboratory unless provided with written approval from the Assessment Manager.

3.8 Data and Data Documentation

The laboratories will provide the QA Contractor with hardcopy data tables, QC documentation and instrument printouts suitable for QA assessment/data validation. Required laboratory deliverables are listed in **Table 7.1**. Data packages will include all related instrument print-outs ("raw data") and bench sheets. A copy of the data and data documentation developed by the laboratory for a given data package will be kept by the laboratory in a secure location using chain of custody procedures for five (5) years after the QA Contractor has validated that data package. All archived data and documentation will be accessible for review upon request. These materials will become the responsibility of the Assessment Manager upon termination of the archival period.

The original data will be transferred from the laboratory to the QA Contractor by means such that a signature is required at the time of document delivery. The QA Contractor will document receipt of packages and maintain a record of the method and date of data submittal with the complete data package. The QA Contractor will maintain the copy of the data packages and related validation documentation in a secure location for a period of one (1) year from the date of validation. These materials will become the responsibility of the Assessment Manager upon termination of the archival period.

4.0 LABORATORY OPERATIONS

All laboratories providing analytical support for the MC252 Damage Assessment must have the appropriate facilities to store and prepare samples, and appropriate instrumentation and staff to provide data of the required quality within the time period dictated. Laboratories are expected to conduct operations using good laboratory practices, including:

- Training and appropriate certification of personnel.
- A program of scheduled maintenance of analytical balances, laboratory equipment and instrumentation.
- Routine checking of analytical balances using a set of standard reference weights (ASTM class, NIST Class S-1, or equivalents).
- Recording all analytical data in secure electronic system with date and associated analyst identification, and/or logbooks with each entry signed and dated by the analyst.
- Monitoring and documenting the temperatures of cold storage areas and freezer units.

Laboratory operations may be evaluated by the QA Coordinator through technical systems audits, performance evaluation studies, and performance in a NIST-managed intercomparison program. Personnel in any laboratory performing analyses for this damage assessment should be well versed in good laboratory practices, including standard safety procedures. It is the responsibility of the laboratory manager and /or supervisor to ensure that safety training is mandatory for all laboratory personnel. The laboratory is responsible for maintaining a current safety manual in compliance with the Occupational Safety and Health Administration (OSHA) or equivalent state or local regulations. Proper procedures for safe storage, handling and disposal of chemicals should be followed at all times; each chemical should be treated as a potential health hazard and good laboratory practices should be implemented accordingly.

4.1 Quality Assurance Documentation

All laboratories must have the latest revision of the MC 252 NRDA Analytical QA Plan. In addition, the following documents and information must be current and available to all laboratory personnel participating in the processing of MC 252 samples:

- Laboratory Quality Assurance Management Plan
- Laboratory Standard Operating Procedures (SOPs) – Detailed instructions for performing routine laboratory procedures.

- Control charts or data tables – These must be developed and maintained throughout the project for appropriate analyses and measurements, including:
  - Alkyl PAH pattern book for MC252 reference oil.

4.2 Laboratory Systems Audits

Prior to or during sample analysis, QA systems audits will be performed. The laboratory audits will be conducted by the QA Coordinator or designee. The checklists used for the laboratory audits are based on requirements outlined in "Good Laboratory Practice Standards" (*40 CFR Part 792*) and audit procedures of the EPA National Enforcement Investigations Center, "NEIC Procedures Manual for the Contract Evidence Audit and Litigation Support for EPA Enforcement Case Development" (*EPA 330/9-89-002*). The Laboratory Project Managers will be informed of the findings and recommendations of the audit before the auditors leave the facility. A written report discussing the audits will be submitted to the Assessment Manager.

Additional laboratory audits may be performed at any time throughout the duration of the NRDA.

4.3 Participation in Intercomparison Exercises

Each analytical laboratory performing analysis will be required to participate in potential intercomparison exercises that may be organized by NS&T and/ or NIST during the duration of the laboratory's participation in this NRDA analytical program. A variety of samples including sample extracts and representative matrices (e.g., sediment or tissue samples) may be utilized in these exercises. Laboratories are required to analyze only those matrices or analytes that they are providing in like manner for the NRDA analytical program. When participating in the intercomparison exercise, the laboratory should analyze the sample(s) in the same manner as routinely performed for this NRDA and as specified in this Analytical QA Plan. Laboratories which fail to achieve acceptable performance will be required to provide an explanation to the QA Coordinator and/or undertake appropriate corrective actions.

5.0 ASSESSMENT OF DATA QUALITY

The purpose of this Analytical QA Plan is to develop and document analytical data of known, acceptable, and defensible quality. The quality of the data is presented as a set of statements that describe in precise quantitative terms the level of uncertainty that can be associated with the data without compromising their intended use. These statements are referred to as Data Quality Objectives (DQOs) and are usually expressed in terms of precision, bias, sensitivity, completeness, and comparability.

5.1 Precision

Precision is the degree of mutual agreement among individual measurements of the same property under prescribed similar conditions, such as replicate measurements of the same sample. Precision is concerned with the "closeness" of the results. Where suitable reference materials (RMs) are available,

precision will be expressed as the relative standard deviation (RSD) for the repeated measurements. This use of RMs allows for the long-term measurement of precision but does not include homogenization as a source of analytical variability.

In addition to the tracking precision of replicate RM analyses, precision will be expressed as the relative percent difference (RPD) between a pair of replicate data from environmental samples prepared and analyzed in duplicate.

5.2 Bias

Bias is the degree of agreement of a measurement with an accepted reference value and may be expressed as the difference between the two measured values or as a percentage of the reference value.

The primary evaluation of bias will be through the use of RMs. RMs with certified values (from NIST or a similar source) will be used if they are available. The laboratory will maintain control charts to track the RM performance. Spiked matrix samples will also be analyzed to assess bias for those analytes that are not available in suitable reference materials.

5.3 Comparability

Comparability expresses the confidence with which one data set can be evaluated in relationship to another data set. Comparability of the chemical analytical data is established through the use of:

- Program-defined general analytical methodology (e.g., low resolution MS), detection limits, bias and precision requirements and reporting formats;
- NIST-traceable calibration materials;
- Reference material with each sample batch;
- Analysis of a common “reference oil”.

5.4 Completeness

Completeness is a measure of the proportion of data specified in the sampling plan which is determined to be valid. Completeness will be assessed by comparing the number of valid sample results to the total number of potential results planned to be generated. The DQO for completeness is 95%, i.e. no more than 5% of the analytical data missing or qualified as unreliable (rejected).

6.0 QUALITY CONTROL PROCEDURES

No particular analytical methods are specified for this project, but the QA/QC requirements will provide a common foundation for each laboratory’s protocols. This “common foundation” includes: (1) the specification of the analytes to be identified and quantified and the minimum sensitivity of the analytical methods and (2) the use of NIST reference materials, and (3) the use of a common MC252 Reference Oil.

Prior to the analysis of samples, each laboratory must provide written protocols for the analytical methods to be used; calculate detection limits for each analyte in each matrix of interest and establish an initial calibration curve in the appropriate concentration range for each analyte. The laboratory must demonstrate its continued proficiency by participation in refereed intercomparison exercises (as available) and repeated analyses of reference materials, calibration checks, and laboratory method blanks. Laboratories will be expected to take corrective actions promptly if measurement quality objectives described in this plan are not met.

A laboratory may be audited at any time to determine and document that they have the capability to analyze the samples and can perform the analyses in compliance with the QA plan. Independent data validation will be undertaken promptly after analyses of each sample batch to verify that measurement quality objectives are met. The data validator will discuss any unacceptable findings with the laboratory as soon as possible, and assist the laboratory in developing a satisfactory solution to the problem.

6.1 Standard Operating Procedures for Analytical Methods

Prior to the analysis of field samples, each laboratory is required to submit to the QA Coordinator for review and approval, written Standard Operating Procedures (SOPs) detailing the procedures used in sample receipt and handling, sample preparation and analysis, data reduction and reporting. Once approved, the SOPs for each analytical method and from each analytical laboratory will be archived with this plan as part of the QA documentation.

6.2 Determination of Method Detection Limit, Quantitation Range, and Reporting Limits

The analytical laboratory will establish and report a method detection limit (MDL) for each analyte of interest in each matrix, with the exception of oil for which MDLs cannot be accurately determined. The target detection ranges or limits are specified in **Tables 1.1a – 1.1e and 1.1h**. The actual MDLs will be established by following the method in *40CFR part 136*. The quantitation limit (QL) will be defined as the concentration that is equivalent to five times the MDL result, or equivalent to the lowest concentration standard analyzed as part of the initial calibration. Results that are less than 5X the MDL or less than the lowest calibration standard will not be required to meet the measurement quality objectives (MQOs) for precision and bias, because these results may be outside the "quantitation range". Thus, these results may be flagged by the laboratory with a J, to indicate the results are possibly an estimate and have not been required to meet the MQOs. If the analyte is not detected in a sample, the result will be reported as non-detected at the MDL and flagged with a "U".

Note that for the Query Manager electronic data deliverable (EDD), there are two fields: detection limit and reporting limit. The detection limit field is equivalent to the MDL, except for those cases where no MDL value exists (for example, oils). If no MDL value exists, the detection limit field is populated with the quantitation limit (QL). The reporting limit field is always populated with the QL value.

Reporting limits for the supporting analyses (percent moisture, percent total extractable organics [TEO], and total organic carbon) will be 0.01%. Reporting limit for grain size will be 0.1% or lower. The reporting limit will be demonstrated by the laboratory to be greater than 5X the detection limit.

Target detection limits, as shown at the bottom of **Tables 1.1a through 1.1e** and in **Table 1.1h**, may not be met due to required dilutions, interferences, and/or limited sample size. If a laboratory MDL does not meet the target detection limit, the reason for the elevated detection limits should be discussed in the laboratory case narrative.

At the discretion of the analytical laboratory, detected analytes at concentrations less than the MDL may be reported, provided that the compound meets the established identification criteria and the peak height is greater than or equal to three times the background noise level. These results will be "J" flagged by the laboratory. During validation, these results will be qualified as "F" (found) to indicate that the value is less than the MDL (see Table 7.2).

6.3 Quality Control Criteria

MQOs and required minimum frequency of analysis for each QC element or sample type are summarized in **Tables 6.1a – 6.1h**. The analytical laboratory will determine when MQOs have not been met, and perform appropriate corrective actions before continuing the analyses or reporting of the data. If the "Corrective Action" in the Method Performance Criteria table states "Resolve before proceeding", the laboratory must perform an adjustment to the analytical process and subsequently demonstrate the criteria will be met before proceeding with analysis for project samples. In addition, if results associated with a non-compliant QC element have been obtained, the laboratory must repeat those analyses until acceptable QC results are obtained. If the laboratory determines the non-compliance does not affect the quality of the data, the laboratory will discuss the non-compliance and the rationale, used to conclude the data are not affected, in the case narrative which accompanies the data report. If the laboratory determines the non-compliance is due to interferences or circumstances outside the laboratory's control, the laboratory will discuss the reason for the non-compliance in the case narrative and the results reported.

At this time, no criteria for evaluating the target analyte concentrations in the MC252 Reference Oil have been established. Chromatographic resolution criteria for specific compound (peaks) are specified in **Tables 6.1a through 6.1e** and **Table 6.1g** below. When additional criteria are developed they will be added to this Analytical QAP.

TABLE 6.1a
Method Performance Criteria for Extended PAH (Parent and Alkyl Homologs) and Related Compounds

Element or Sample Type	Minimum Frequency	Measurement Quality Objective/ Acceptance Criteria	Corrective Action
Tuning	Prior to every sequence	Tune as specified in laboratory SOP	Resolve before proceeding.
Initial Calibration (All parent PAH and selected alkyl homologue PAH)	Prior to every sequence, or as needed based on continuing calibration/verification check.	5-point calibration curve over two orders of magnitude %RSD ≤ 20	Resolve before proceeding.
Continuing Calibration (CCAL)	Every 12 hours or every 12 field samples	%D ≤ 25 for 90% of analytes %D ≤ 35 for 10% of analytes	Perform instrument maintenance. Re-analyze affected samples.
Initial Calibration Verification (Second Source or can be met if CCAL is second source)	Per initial calibration	%R target analytes 80-120%	Resolve before proceeding.
Matrix SRM 1941b for sediment; SRM 1974b for tissue	One per batch/every 20 field samples	Within ±30% of NIST 95% uncertainty range for analytes within the quantitation range. 2 analytes may be greater than 30% outside, however average %D must be <35%[14]	Resolve before proceeding.
Oil SRM 1582 (Oil and Water only)	One per batch of oil/every 20 field samples	Within ±20% of NIST 95% uncertainty range for analytes within the quantitation range. 2 analytes may be greater than 20% outside, however average %D must be <35%	Resolve before proceeding.
MC 252 Reference Oil	One per batch/every 20 field samples	Peak resolution >80% of 9-methylphenanthrene from 1-methylphenanthrene (m/z 192). Plus additional criteria to be developed.	Resolve before proceeding.
Matrix Spike/Matrix Spike Duplicate (Sediments, Soils, Tissues only)	One per batch/every 20 field samples	%R 50% - 125% for target analytes detected at >5X the spiked amount; RPD ≤30%, except biphenyl (40%-140%) and decalin (25%-125%)	Evaluate impact to data, discuss with manager, and determine if corrective action is needed.
Blank Spike/Blank Spike Duplicate (Aqueous Samples)	One per batch/every 20 field samples	%R 50% - 125% for target analytes, RPD ≤30%, except biphenyl (40%-140%) and decalin (25%-125%)	Resolve before proceeding.
Procedural Blank	One per batch/every 20 field samples	No more than 2 analytes to exceed 5x target MDL unless analyte not detected in associated samples(s) or analyte concentration >10x blank value	Resolve before proceeding. QA coordinator may be contacted to resolve issues surrounding 'minor exceedance'.
Sample Duplicate (not required for water matrix)	One per batch/every 20 field samples	RPD ≤ 30% if analyte concentration is greater than QL	Evaluate impact to data, discuss with manager, and determine if corrective action is needed.
Mass Discrimination	Initial calibration and CCVs (mid-level)	Ratio for the concentration of Benzo[g,h,i]perylene to phenanthrene ≥0.70	Resolve before proceeding.
Internal Standard (IS)	Every sample	50% - 200% of the area of the IS in the associated calibration standard	Resolve before proceeding.
Surrogates	Every sample	%R 40-120% except d12-perylene which is 10-120%	Re-extract affected samples. Evaluate impact to data, discuss with manager, if corrective action is needed.

[14] Except for fluorene in SRM 1941b, extend the low end to 40%.

TABLE 6.1b
Method Performance Criteria for Alkanes/Isoprenoids Compounds and Total Extractable Hydrocarbons

Element or Sample Type	Minimum Frequency	Measurement Quality Objective/ Acceptance Criteria	Corrective Action
Initial Calibration (Standard solution - all target analytes, except phytane, and C_{31}, C_{33}, C_{35}, and C_{39} n-alkanes)	Prior to every sequence, or as needed based on continuing calibration/verification check.	5-point calibration curve %RSD ≤ 20	Resolve before proceeding.
Continuing Calibration (CCAL)	Every 12 hours or every 12 field samples	%D ≤ 15 for 90% of analytes %D ≤ 20 for 10% of analytes	Perform Instrument Maintenance. Re-analyze affected samples.
Initial Calibration Verification (Second Source or can be met if CCAL is second source)	Per initial calibration	%R target analytes 80-120%	Resolve before proceeding.
SRMs - no SRMs for SHC or TPH are available at this time			
MC 252 Reference Oil	One per batch/every 20 field samples	Peak resolution >80% of n-C17 from pristane; Additional criteria to be developed.	Resolve before proceeding.
Matrix Spike/Matrix Spike Duplicate (Sediments, Soils, Tissues only)	One per batch/every 20 field samples	%R 50% - 125% for target analytes detected at >5X the spiked amount; RPD ≤30%.	Evaluate impact to data, discuss with manager, and determine if corrective action is needed.
Blank Spike/Blank Spike Duplicate (Aqueous Samples)	One per batch/every 20 field samples	%R 50% - 125% for target analytes, RPD ≤30%.	Resolve before proceeding.
Procedural Blank	One per batch/every 20 field samples	No more than 2 analytes to exceed 5x target MDL unless analyte not detected in associated samples(s) or analyte concentration >10x blank value	Resolve before proceeding. QA coordinator may be contacted to resolve issues surrounding 'minor exceedances'.
Duplicate Sample Analysis (not required for water matrix)	One per batch/every 20 field samples	RPD ≤ 30% if analyte concentration is greater than QL	Evaluate impact to data, discuss with manager, determine if corrective action is needed.
Mass Discrimination	Initial calibration and CCVs (mid-level)	Ratio for the raw areas of n-C36 / n-C20 ≥0.70	Resolve before proceeding.
Surrogates	Every sample	%R 40-125%	Re-extract affected samples. Evaluate impact to data, discuss with manager, and determine if corrective action is needed.

TABLE 6.1c
Method Performance Criteria for VOCs

Element or Sample Type	Minimum Frequency	Measurement Quality Objective/ Acceptance Criteria	Corrective Action
Tuning	Prior to every sequence	Per SW846 8260B	Resolve before proceeding
Initial Calibration (ICAL)	Prior to every sequence, or as needed based on continuing calibration/verification check.	Minimum of 5 concentration levels %RSD ≤ 25% for 90% of analytes %RSD ≤ 35% for all analytes >C6	Resolve before proceeding.
Continuing Calibration (CCAL)	Every 12 hours or every 12 field samples	%D ≤ 25% for 90% of analytes %D ≤ 35% for all analytes >C6 Except t-butanol <50%	Perform Instrument Maintenance. Re-analyze affected samples.
Initial Calibration Verification (Second Source or can be met if CCAL is second source)	Per initial calibration	%R target analytes 80-120%. Except 2 analytes can be at 60 - 140%	Resolve before proceeding.
SRMs – No SRMs are available at this time			
MC 252 Reference Oil (optional)	One per batch/every 20 field samples	To Be Determined	Resolve before proceeding.
Matrix Spike/Matrix Spike Duplicate (Sediments, Soils)	One per batch/every 20 field samples	%R 50% - 130% for target analytes detected at >5X the spiked amount; RPD ≤30%.	Evaluate impact to data, discuss with manager, and determine if corrective action is needed.
Blank Spike/Blank Spike Duplicate (Aqueous Samples)	One per batch/every 20 field samples	%R 50% - 130% for target analytes, RPD ≤30%.	Resolve before proceeding.
Procedural Blank	One per batch/every 20 field samples	No more than 2 analytes to exceed 5x target MDL unless analyte not detected in associated samples(s) or analyte concentration >10x blank value	Resolve before proceeding. QA coordinator may be contacted to resolve issues surrounding 'minor exceedances'.
Sample Duplicate	One per batch/every 20 field samples	RPD ≤ 30% if analyte concentration is greater than QL	Evaluate impact to data, discuss with manager, and determine if corrective action is needed.
Internal Standard (IS)	Every sample	50% - 200% of the area of the IS in the associated calibration standard	Resolve before proceeding.
Surrogates	Every sample	%R 70-130%	Re-extract or re-analyze affected samples. Evaluate impact to data, discuss with manager, and determine if corrective action is needed.

TABLE 6.1d
Method Performance Criteria for Quantitative Biomarkers

Element or Sample Type	Minimum Frequency	Measurement Quality Objective/ Acceptance Criteria	Corrective Action
Tuning	Prior to every sequence	Tune as specified in laboratory SOP	Resolve before proceeding.
Initial Calibration	Prior to every sequence, or as needed based on continuing calibration/verification check.	5-point calibration curve over two orders of magnitude %RSD ≤ 20	Resolve before proceeding.
Continuing Calibration (CCAL)	Every 12 hours or every 12 field samples	%D ≤ 25 for 90% of analytes %D ≤ 35 for 10% of analytes	Perform instrument maintenance. Re-analyze affected samples.
Oil SRM 1582 (Oil and Water only)	One per batch of oil/every 20 field samples	Biomarker concentrations are not certified; Peak resolution (*m/z* 191) of: (a) oleanane (T18) from hopane (T19); (b) C26 Tricyclic Terpane stereoisomers 22R (T6b) from 22S (T6c) and from C24 Tetracyclic Terpane (T6a)	Resolve before proceeding.
MC 252 Reference Oil	One per batch/every 20 field samples	Peak resolution (*m/z* 191): 30-Norhopane (T15) from 30-Norneohopane (T16) from Diahopane (X). Add'l. criteria To Be Determined.	Resolve before proceeding.
Method Blank	One per batch/every 20 field samples	No more than 2 analytes to exceed 5x target MDL unless analyte not detected in associated samples(s) or analyte concentration >10x blank value	Resolve before proceeding. QA coordinator may be contacted to resolve issues surrounding 'minor exceedance'.
Sample Duplicate	One per batch/every 20 field samples	RPD ≤ 30% if analyte concentration is greater than QL	Evaluate impact to data, discuss with manager, and determine if corrective action is needed.
Internal Standard (IS)	Every sample	50% - 200% of the area of the IS in the associated calibration standard	Resolve before proceeding.
Surrogate	Every sample	%R 50-130%	Evaluate impact to data, discuss with manager, if corrective action is needed.

TABLE 6.1e
Method Performance Criteria for Qualitative Biomarkers

Element or Sample Type	Minimum Frequency	Measurement Quality Objective/ Acceptance Criteria	Corrective Action
Oil SRM 1582 (Oil and Water only)	One per batch of oil/every 20 field samples	Peak resolution (m/z 191) of: (a) oleanane (T18) from hopane (T19); (b) C26 Tricyclic Terpane stereoisomers 22R (T6b) from 22S (T6c) and from C24 Tetracyclic Terpane (T6a)	Resolve before proceeding.
MC 252 Reference Oil	One per batch/every 20 field samples	Peak resolution (*m/z* 191): 30-Norhopane (T15) from 30-Norneohopane (T16) from Diahopane (X). Add'l. criteria To Be Determined.	Resolve before proceeding.
Method Blank	One per batch/every 20 field samples	No interference with biomarker patterns	Resolve before proceeding. QA coordinator may be contacted to resolve issues surrounding 'minor exceedance'.
Sample Duplicate	One per batch/every 20 field samples	Qualitative comparison meets laboratory SOP	Evaluate impact to data, discuss with manager, and determine if corrective action is needed.

TABLE 6.1f
Method Performance Criteria for General/Conventional Chemistry

Conventional Sediment Parameters: Total Organic Carbon (TOC), Grain Size, Total Solids
Tissues: Total Extractable Organics (TEO)

QC Element or Sample Type	Minimum Frequency	Acceptance Criteria	Relevant Parameter(s) Reference Methods*
Initial Calibration	Prior to analysis (method and instrument specific procedures & number of standards)	For multipoint calibration, Correlation coefficient (r) >0.995	TOC
Continuing Calibration	Must start and end analytical sequence and every 10 samples	%R 90%-110%	TOC
Method Blanks	One per batch/every 20 field samples	Not to exceed QL	TOC, TEO
Blank Spike Samples	One per batch/every 20 field samples	%R 75% - 125%	TOC
Matrix Spike Samples	One per batch/every 20 field samples	%R 75% - 125% If MS/MSD analyzed, RPD ≤ 25%	TOC
Replicate Analyses[15]	Each sample must be analyzed at least in duplicate. The average of the replicates shall be reported.	RPD or %RSD < 20% for concentrations > QL	TOC
Sample Duplicates[16]	One per batch/every 20 field samples	RPD ≤ 25% for analyte concentrations greater than QL	TOC, Grain Size, TS, TEO
Reference Materials TOC NIST 1941B TEO NIST 1974B	One per batch/every 20 field samples	Values must be within ±20% of NIST uncertainty range	TOC, TEO

*** Reference Methods**

TOC	Plumb 1981 or SW 846 Method 9060A or Standard Methods 5310C or ASTM D4129-82M, or equivalent
Grain Size	ASTM D422 or PSEP 1986 Particle Size. If using sieve analysis only, report as percent gravel, coarse sand, medium sand, fine sand, very fine sand, and silt/clay. If using sieve with hydrometer or sieve with pipette, report as percent gravel, coarse sand, medium sand, fine sand, very fine sand, silt, and clay. Additionally, grain size must be reported as "True" for sediment treated with hydrogen peroxide prior to analysis or "Apparent" for sediment not treated with hydrogen peroxide.
TS (percent)	EPA 160.3

Method 9000 series - analytical methods from SW-846 (U.S. EPA 1986) and updates
The SW-846 and updates are available from the web site at: http://www.epa.gov/epaoswer/hazwaste/test/sw846.htm
Plumb (1981) - U.S. EPA/U.S. Army Corps of Engineers Technical Report EPA/CE-81-1:
http://yosemite.epa.gov/r10/CLEANUP.NSF/ph/T4%20Technical%20Documents/$FILE/Plumb.pdf
PSEP. 1986. "Recommended Protocols for Measuring Conventional Sediment Variables in Puget Sound." Prepared for the Puget Sound Estuary Program.

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[15] Method SW9060 requires quadruplicate analyses, however duplicate or triplicate analyses are acceptable. Standard Method 5310C requires that injections be repeated until consecutive measurements within 10% are obtained for a water matrix, however, duplicate analyses < 20% RPD are acceptable based on a sediment matrix.

[16] Method SW9060 requires a duplicate spike. A matrix spike and sample duplicate are acceptable in lieu of matrix spike/matrix spike duplicates. For grain size, RPD criteria only applied if fraction is greater than 5%.

TABLE 6.1g
Method Performance Criteria for Analysis of Dioctylsulfosuccinate sodium salt (DOSS)

Element or Sample Type	Minimum Frequency	Measurement Quality Objective/ Acceptance Criteria	Corrective Action
Initial Calibration	Prior to every sequence, or as needed based on continuing calibration/verification check.	5-point calibration curve over two orders of magnitude %RSD ≤ 20	Resolve before proceeding.
Continuing Calibration (CCAL)	Every 12 hours	%D ≤ 30	Perform instrument maintenance. Re-analyze affected samples.
Initial Calibration Verification (Second Source or can be met if CCAL is second source)	Per initial calibration	%R target analytes 70-130%	Resolve before proceeding.
MC 252 Reference Oil (optional)	One per batch/every 20 field samples	Criteria to be developed	Resolve before proceeding.
Matrix Spike/Matrix Spike Duplicate (Sediments, Soils, Tissues only)	One per batch/every 20 field samples	%R 50% - 125% if sample concentration detected at >5X the spiked amount; RPD ≤30%	Evaluate impact to data, discuss with manager, and determine if corrective action is needed.
Blank Spike/Blank Spike Duplicate (Aqueous Samples)	One per batch/every 20 field samples	%R 50% - 125; RPD ≤30%	Resolve before proceeding.
Method Blank	One per batch/every 20 field samples	Not to exceed 5x target MDL unless analyte not detected in associated samples(s) or analyte concentration >10x blank value	Resolve before proceeding.
Sample Duplicate (not required for water matrix)	One per batch/every 20 field samples	RPD ≤ 30% if analyte concentration is greater than QL	Evaluate impact to data, discuss with manager, and determine if corrective action is needed.
Internal Standard (IS)	Every sample	50% - 200% of the area of the IS in the associated calibration standard	Resolve before proceeding.
Surrogates	Every sample	%R 40-120%	Re-extract affected samples. Evaluate impact to data, discuss with manager, if corrective action is needed.

TABLE 6.1h
Measurement Quality Objectives for Metals by ICP-AES & ICP-MS and Mercury by CVAA/CVAFS

Element or Sample Type	Minimum Frequency	Measurement Quality Objective/ Acceptance Criteria	Corrective Action
ICP-MS Tune	Daily at the beginning of each 24 hour shift. Must start each analytical sequence.	Tuning solution must contain elements spanning all the mass regions of interest (see EPA methods 200.8 & 6020). Analyze 5 times with RSD ≤ 5% Resolution <0.9amu at 10% peak height Mass calibration < 0.1 amu difference from target mass	Resolve before proceeding
Initial Calibration	Daily prior to sample analysis.	Minimum of a 2 point curve for ICP-AES/ICP-MS (1 blank + 1 standard containing all target analytes) Min 5 point curve for CVAA/CVAFS r>0.995 for multi-point curves	Resolve before proceeding
Independent (Initial) Calibration Verification (ICV)	Analyzed immediately after calibration and prior to samples	Different source than calibration standards Concentration near mid-point of calibration curve Must contain all target analytes to be reported %R[1] = 90% - 110%	Resolve before proceeding
Initial Calibration Blank (ICB)	Must be analyzed after each ICV	ICB < RL for all target analytes	Resolve before proceeding
Reporting Limit Standard (CRI)	Daily prior to sample analysis if initial calibration did not contain a low-level standard at the RL for each target analyte. If initial calibration includes the RL as the low-level standard in the initial calibration curve, then RL Std is not required.	Prepare using same source as calibration standards all target analytes at a concentration = RL %R[1] = 70% - 130%	Resolve before proceeding unless all target analytes in associated samples are > 10x RL
Interelement Interference Check Standards (ICSA & ICSAB)	Daily prior to sample analysis	See EPA methods for ICSA & ICSAB concentrations of interferents and other analytes; for ICP-AES checks on background points and instrument interelement interference corrections; for ICP-MS checks on isobaric interference corrections. ICSA & ICSAB: %R[1] = 80% - 120%	Resolve before proceeding
Continuing Calibration Verification (CCV)	Must be analyzed before samples, after every 10 samples, and at end of each analytical sequence	CCV concentration should be near mid-point of calibration curve and contain all target analytes %R = 90% - 110%	Perform instrument maintenance. Re-analyze affected samples.
Continuing Calibration Blank (CCB)	Must be analyzed after each continuing calibration verification (CCV)	CCB < RL for all target analytes Unless: analyte not detected in associated sample(s) or sample analyte concentrations are >10x the blank value	Resolve before proceeding

TABLE 6.1h
Measurement Quality Objectives for Metals by ICP-AES & ICP-MS and Mercury by CVAA/CVAFS

Element or Sample Type	Minimum Frequency	Measurement Quality Objective/ Acceptance Criteria	Corrective Action
Method Blank	Every batch (max. 20 field samples).	No analytes to exceed the reporting limit unless analyte not detected in associated sample(s) or detected in samples at >10x the blank value	Resolve before proceeding
Laboratory Control Sample or Reference Material Possible sediment RMs: NRCC MESS-3 or PACS-2 NIST 1646A*, 1944*, 2702 ERA 540 Possible tissue RMs: NRCC DOLT-4, DORM-3, or TORT-2; NIST 2976 or 1947	Every batch (max. 20 field samples).	Reference Material or laboratory control sample must be matrix-matched to the field samples and prepared/analyzed with the sample batch. Aqueous: %R = 80% - 120% Sediment & Tissue: Values must be within ±30% of the vendor 95% confidence limits for true values >RL	Resolve before proceeding
Matrix Spike (MS)	Every batch (max. 20 field samples).	Must be performed on a NOAA sample from same preparation/analysis batch. Must contain all target analytes to be reported. Sediment/Tissue: %R[2] = 70% - 130% (For native conc. < 4X spike added)	If a MS %R is <30%, a post digestion spike should be analyzed and fall within 75%-125%. See EPA Method 6010C and 6020A for details on spike levels and evaluation. Report QC exceedance in data package narrative.
Sample Duplicate (or matrix spike duplicate) [3]	Every batch (max. 20 field samples).	Sediment/Tissue: RPD[3] ≤ 30% if value > RL	Report this QC exceedance in data package narrative.
Internal Standards (ICP-MS only)	Every sample (QC & field samples)	See EPA methods 200.8 and 6020 for recommended IS elements.Relative intensity of IS %R = 70% - 130% compared to IS of standard in calibration curve (or mid-point standard of calibration for multi-level curve).	Check for instrument drift. If IS in assoc. CCB is acceptable, then dilute sample 5X and re-analyze until IS in control for affected analyte(s). If instrument drift is indicated, recalibrate and re-analyze.
General Reporting	Every sample	* Non-detected values should be reported to the sample-specific MDL or RL to achieve the target sensitivity levels listed in Table 1.1h for each target analyte (using all preparation/dilution factors). * Reporting of detected results less than the RL must be qualified "J" as estimated values. * Results > the linear range must be diluted to within the LR; the diluted result will be reported for the affected analyte.	Include explanation of all non-compliances observed in sample receipt, holding times, preparation, or analysis in the laboratory narrative of the data report.

* These SRMs do not have a certified value for Mercury

6.3.1 Initial Calibration

Acceptable calibration (initial and continuing) must be established and documented before sample analyses may begin. NIST-traceable calibration materials must be used where available in establishing calibration. Initial calibrations will be established according to the criteria in **Tables 6.1a – 6.1d and 6.1f - 6.1h**. A specific requirement for this project is to use methodology (and tune instrumentation) for low detection limits, therefore, samples with analytes above the calibration range will be diluted and reanalyzed. If samples require a dilution, results from the initial analytical run that were within the calibration range should be reported. Results from the diluted analyses should be reported for only those analytes which exceeded the calibration.

6.3.2 Continuing Calibration Verification

Continuing calibration verification (CCV) standards will be run at the beginning (opening) and end (closing) of each analytical sequence, and at the frequencies indicated in **Tables 6.1a – 6.1d and 6.1f - 6.1h**. If CCV results do not meet the specified criteria, then the instrument must be re-calibrated and all samples analyzed since the last acceptable CCV must be re-analyzed.

6.3.3 Reference Materials

Reference materials of a matrix appropriate to the samples being analyzed, will be analyzed every 20 samples throughout the analytical program, if available. The data resulting from the analysis of these samples will be reported in the same manner as that from the field samples. These data will be the prime materials used to determine and document the accuracy and precision of the associated field sample data. The reference materials to be used are listed in the criteria tables.

Accuracy is computed by comparing the laboratory's value for each analyte against either end of the range of values reported by the certifying agency. The laboratory's value must be within 30% of either the upper or lower end of NIST's 95% uncertainty range for SRM 1941b and SRM 1974b except the low end for fluorine for 1941b is extended to 40%. For oil, water, filters, and inert sorbent materials analyses, SRM1582 is not extracted, but only diluted and analyzed on the instrument, thus the laboratory's value must be within 20% of the NIST uncertainty range. The MC252 Reference Oil will be run with each batch of samples (e.g., GU2988-A0521-O9805 or equivalent as approved by the QA Coordinator). Chromatographic resolution criteria of selected peak pairs in the Reference Oil are indicated in **Tables 6.1a-6.1e, and 6.1g**. After initial data sets are acquired, additional criteria for the Reference Oil will be determined.

6.3.4 Method Blanks

Method (procedural) blanks are laboratory derived samples which have been subjected to the same preparation or extraction procedures and analytical protocols as project samples. A method blank will be analyzed with every 20 field samples analyzed. Acceptance criteria are provided in **Tables 6.1a – 6.1g**. Failure to meet acceptance criteria requires definitive corrective action to identify and eliminate the source(s) of contamination before the subsequent reanalysis and re-extraction of the blank and affected samples. Sample results will not be blank corrected.

6.3.5 Sample Duplicates

A duplicate sample aliquot from a representative matrix will be prepared and analyzed with every 20 field samples, except for water samples, filters, and inert sorbent materials for SHC/TEH and PAH. Water samples, filters and inert sorbent materials for SHC/TEH and PAH will not be analyzed in duplicate because of the difficulty in subsampling representative aliquots. If duplicate VOA vials are collected, then volatile organic analyses may be performed in duplicate. Acceptance criteria are provided in **Tables 6.1a – 6.1h**.

6.3.6 Matrix Spike/Matrix Spike Duplicates or Blank Spike/Blank Spike Duplicate

Matrix spike/matrix spike duplicates (MS/MSDs) will be analyzed every 20 samples, except for water samples, filters and inert sorbent materials. MS/MSDs will not be analyzed with the water sample batches because of the difficulty in subsampling representative aliquots from a sample container. Instead, blank spike/blank spike duplicates (BS/BSDs) will be analyzed with each batch of water samples. Samples will be spiked prior to extraction. Spike solution concentrations for the MS must be appropriate to the matrix and anticipated range of contaminants in the sample; that is 2 to 10 times analyte concentration. However, because it is not possible to know the concentration of contaminants prior to analysis, professional judgment may be exercised in choosing concentrations that are reasonable under the circumstances. Acceptance criteria are provided in **Tables 6.a – 6.1c, 6.1g, and 6.1h.** Acceptance criteria for conventionals matrix spike and blank spike samples are provided in **Table 6.1f**.

6.3.7 Internal Standards

All samples will be spiked with internal standards prior to analysis, when required by the analytical method. Control criteria for internal standard recovery are listed in **Tables 6.1a, 6.1c, 6.1d, and 6.1g**.

6.3.8 Surrogates

All field and QC samples will be spiked with surrogates prior to extraction, as required by the analytical methods. Control criteria for the surrogate recovery are listed in **Tables 6.1a – 6.1d, and 6.1g**. For the PAH and saturated hydrocarbon analyses, the target analyte concentrations will be corrected for surrogate recovery as specified in the laboratory SOPs.

7.0 DATA REDUCTION, VALIDATION AND REPORTING

7.1 Data Reduction

Data reduction is the process whereby raw data (analytical measurements) are converted or reduced into meaningful results (analyte concentrations). This process may be either manual or electronic. Primary data reduction requires accounting for specific sample preparations, sample volume (or weight) analyzed, and any concentrations or dilutions required.

Primary data reduction is the responsibility of the analyst conducting the analytical measurement and is subject to further review by laboratory staff, the Laboratory Project Manager and finally, independent reviewers. All data reduction procedures will be described in the laboratory SOPs. Any deviations from the laboratory SOPs will be discussed in the laboratory case narratives.

- Concentrations will be reported as if three figures were significant.
- Data generated from the analysis of blank samples will not be utilized for correction of analyte data.
- Surrogate compounds, matrix spikes, and spike blanks will be evaluated as %R.
- Reference materials will be reported in units indicated on the certificate of analysis.
- Continuing calibration factors will be presented as %D
- Duplicate sample results will be expressed as RPD.

7.2 Data Review and Validation

Data review is an internal review process where data are reviewed and evaluated by personnel within the laboratory. Data validation is an independent review process conducted by personnel not associated with data collection and generation activities.

Data review is initiated at the bench level by the analyst, who is responsible for ensuring that the analytical data are correct and complete, the appropriate SOPs have been followed, and the QC results are within the acceptable limits. The Laboratory Project Manager has final review authority. It is the Laboratory Project Manager's responsibility to ensure that all analyses performed by that laboratory are correct, complete, and meet project data quality objectives.

External and independent data validation will be performed for all samples by the QA Contractor using a full data package containing sufficient information to allow the independent validation of the sample identity and integrity, the laboratory measurement system, and resulting quantitative and qualitative data. The required information with associated instrument print-outs are listed in **Table 7.1**.

TABLE 7.1 Laboratory Data Deliverables Per Sample Batch

Chain-of-Custody/ Sample Receipt Checklist	
Sample Data:	Result summaries including surrogate recoveries, percent total solids, dilutions, etc
Standards Data:	Target MDL data based on the method in *40 CFR, 136* Calibration summaries: Initial calibration data, standard curve equation, correlation coefficient or %RSD, continuing calibration %D.
Quality Control Data (Method Blanks, CRMs, Duplicates, Matrix Spikes, Spike Blanks):	Results summaries including surrogate recoveries, plus %R and RPD, as applicable.
Case Narrative:	Special handling or analysis conditions. Any circumstance that requires special explanation such as an exception to QA/QC conditions or control criteria, dilutions, reanalysis, etc. Corrective actions/procedure alterations
Chromatograms and Extracted Ion Profiles	Appropriately scaled (1) GC/FID chromatograms for samples and associated QC analyzed for extractable hydrocarbons; (2) GC/MS EIPs for samples and associated QC analyzed for qualitative biomarkers
Electronic Data Deliverable:	As specified in laboratory contract.

Three levels of data validation will be performed (see USEPA, *Guidance for Labeling Externally Validated Laboratory Analytical Data for Superfund Use.* EPA-540-R-08-005. January 2009 for definitions): full (stage 4), summary (stage 2B), or cursory (stage 2A) validation. Full validation will consist of a review of the entire data package for compliance with documentation and quality control criteria for all the following items, plus recalculations of instrument calibration curves, sample and QC results. Summary validation will consist of a review of all the following items, but without recalculations. Cursory validation will consist of a review of only the starred (*) items:

- Package completeness*
- Holding times from extraction to analysis*
- Instrument calibration, initial and continuing
- Blank results*
- Instrument performance
- Spike recoveries*
- Standard reference material results*
- Laboratory duplicate results*
- Reported detection limits*
- Compound quantitation
- Compound identification
- Verification of electronic data deliverable (EDD) against hardcopy (10% verification)*

As the project proceeds and the quality of the data is verified and documented, the level of validation will decrease at the discretion of the QA Coordinator. At a minimum, cursory validation will be performed on all data packages, i.e., only the starred items will be reviewed.

Qualifiers (**Table 7.2**) may be assigned to individual data points by the QA Contractor. These validation qualifiers will not replace qualifiers or footnotes provided by the laboratory, but will be added to the data summary tables to inform the data user whether or not the data met all project quality objectives. Both sets of qualifiers will be maintained in the database.

TABLE 7.2 Data Validation Qualifier Codes

U	Analyte concentration is not significantly greater than the associated blank result. The result is judged to be the detection limit.
R	Unreliable result. Data should not be used.
N	The analysis indicates the present of an analyte for which there is presumptive evidence to make a "tentative identification".
NJ	The analysis indicates the presence of an analyte that has been "tentatively identified" and the associated numerical value represents its approximate concentration.
J	Reported concentration is an estimate with potentially more bias or less precision than an unqualified result, as determined by the associated quality control results.
UJ	Not detected. Detection limit is an estimate with potentially more bias or less precision than an unqualified detection limit as judged by the associated quality control results
DNR	Do not report; A more appropriate result is reported from another analysis or dilution.
F	Found. Analyte detected at less than the MDL, however, peak height is greater than 3 times the noise level and ID criteria are met.

All discrepancies and requests for additional corrected data will be discussed with the laboratory prior to issuing the formal data validation report. Review procedures and findings during data validation will be documented on worksheets. A validation report will be prepared for each data group/data package summarizing QC results, qualifiers, and possible data limitations. Only validated data with appropriate qualifiers will be released for general use. Data are not considered final until QA Coordinator has performed assessment and accepted the data.

In addition, the validated data will be reviewed by the QA Reviewer on behalf of BP America. The following process shall be used should the independent validation of the laboratory data results in a material difference in how qualifiers have been assigned or in the actual value itself:

- The QA Coordinator and QA Reviewer will meet to determine the source of the difference, and resolve. No changes to validated results will be made if the differences are considered immaterial to both the QA Coordinator and QA Reviewer.

- If the validated data have already been released by the QA Coordinator, then the data will be updated in accordance with the resolution and reposted.

- Should there be no agreement on how to resolve the difference, the QA Coordinator and QA Reviewer shall request further assistance from the Assessment Managers and BP America, respectively.

- The basis for all material changes to validated results will be documented along with the resubmitted validated data.

8.0 CORRECTIVE ACTION AND PROCEDURE ALTERATION

The analytical laboratories are required to adhere to the SOPs submitted by them to the QA Coordinator for this project. When the data from the analyses of any quality control sample exceeds the project specified control limits or indicates that the analytical method is drifting out of control, it is the

immediate responsibility of the analyst to identify and correct the situation before continuing with sample analysis.

A narrative describing the problem noted, the steps taken to identify and correct the problem and the treatment of the relevant sample batches must be prepared and submitted with the relevant data package. If the action indicates a revision to the accepted SOP is warranted, the laboratory will revise the SOP and resubmit the SOP to the QA Coordinator within 30 working days after the problem was noted. Until the revised SOP is approved, any data sets reported with the revised method will have the any changes to the method noted in the laboratory's case narrative.

9.0 QUALITY ASSURANCE REPORTS TO MANAGEMENT

Quality Assurance/Quality Control (QA/QC) reports will be submitted periodically to the Assessment Manager(s) by the QA Coordinator. These reports may be either formal or informal in response to the Assessment Manager's request. Upon termination of the analytical work for this damage assessment, a formal QA report will be submitted. This report will include:

- General compliance with QA objectives
- Summary of technical and performance evaluation audits
- Summary of data validation reports
- Summary of laboratory control charts

10.0 REFERENCES

Bence, A.E., K.A. Kvenvolden, and M.C. Kennicutt, II. 2006. Organic geochemistry applied to environmental assessments of Prince William Sound, Alaska, after the Exxon Valdez oil spill--a review. *Org. Geochem.* 24(1):7-42.

Pu, F., R.P. Philp, L. Zhenxi and Y. Guangguo. 1990. Geochemical characteristics of aromatic hydrocarbons of crude oils and source rocks from different sedimentary environments. *Org. Geochem.* 16(1-3):427-443.

USEPA, 2002. *Guidance for Quality Assurance Project Plans*, (EPA QA/G-5) EPA/240/R-02/009, December 2002. http://www.epa.gov/quality/qs-docs/r5-final.pdf

USEPA, 2001. *EPA Requirements for Quality Assurance Project Plans*, (EPA QA/R-5) EPA/240/B-01/003, March, 2001. http://www.epa.gov/quality/qs-docs/g5-final.pdf

E. Retention of Samples and Solutions Generated during Toxicity Testing Memorandum

This appendix was not intended for public release and has been redacted. Note that information contained in this confidential memorandum is provided throughout Section 4 of the project QAPP.

F. Standard Operating Procedure – Fluorescence Spectroscopy to Verify Dilutions of Water Accommodated Fraction for Toxicity Testing

Preamble

When excited by ultraviolet (UV) light, substances containing conjugated double bonds will emit light (or heat) at longer wavelengths, causing fluorescence. The fluorescent properties of a substance are measurable and increase proportionally with concentration. PAHs are found in complex mixtures such as oil, and fluoresce when exposed to UV light. Although fluorescence cannot provide an exact measurement of total PAH (TPAH) or total petroleum hydrocarbons (TPHs) in water, fluorescence varies in direct proportion to the concentrations of aromatic compounds that fluoresce at specific excitation wavelengths. Therefore, assuming that these constituents are present in the same proportions in as the stock sample, fluorescence can be used to estimate relative concentrations across a dilution series of that stock.

Fluorescence has many benefits, including ease of use, low cost, minimal sample preparation, quick sample analysis, and the ability to analyze large numbers of samples on a daily basis. One primary benefit of this method is the ability to rapidly verify the relative PAH concentrations in the different dilution splits of a toxicity test. This allows near real-time data to ensure that the experiment has been set up properly. As described in detail in this SOP, this involves:

- Optimizing the excitation and emission wavelengths of the fluorescent spectrometer for the oil and WAF preparation type being tested
- Measuring the fluorescence of the test solutions prepared by diluting the stock solutions
- Verifying the accuracy of test dilutions by comparing to a standard curve (Figure F.1).

1. Wavelength optimization

a. Optimize excitation wavelengths *for each oil and WAF preparation type* or each time a composition change is suspected.
b. Use a high-concentration standard solution to optimize wavelength (e.g., 1 ppm).
c. Put the sample in the spectrometer.
d. Warm up the lamp according to manufacturer's instructions.

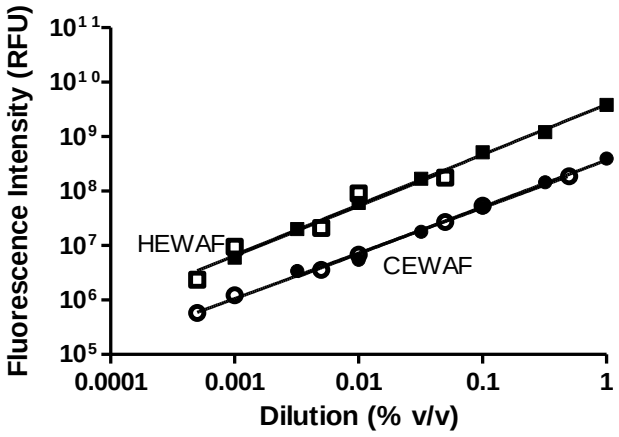

Figure F.1. Relationship between standard curves prepared from stock solutions of HEWAF and CEWAF of crude oil and dilution curves for toxicity test solutions prepared from the same stock solutions and sampled at Time 0, immediately after preparation. Open symbols represent standard curve data and closed symbols represent toxicity test dilutions. The data for toxicity test solutions fall on top of standard curves because test solutions were sampled immediately after preparation.

e. Open an emission scan. Enter an excitation wavelength of approximately 300 nm and an emission range of 310–460 nm. Ensure that the emission wavelength range always begin at least 5 nm higher than the excitation wavelength to avoid recapturing excitation light. The initial emission range should span 150 nm to ensure the entire peak is captured. This can be reduced to speed up analysis if the end of the range is determined to not be useful for measuring oil in water.
f. Optimize excitation wavelengths *for each oil and WAF preparation type* or each time a composition change is suspected.
g. Use a high-concentration standard solution to optimize wavelength (e.g., 1 ppm).
h. Put the sample in the spectrometer.
i. Warm up the lamp according to manufacturer's instructions.
j. Open an emission scan. Enter an excitation wavelength of approximately 300 nm and an emission range of 310–460 nm. Ensure that the emission wavelength range always begin at least 5 nm higher than the excitation wavelength to avoid recapturing excitation light. The initial emission range should span 150 nm to ensure the entire peak is captured. This can be reduced to speed up analysis if the end of the range is determined to not be useful for measuring oil in water.

k. Set the step size to 2 nm to further increase analysis speed. Ensure that the step size does not exceed 2 nm; exceeding 2 nm will decrease the integrity of the analysis.

l. Start the emission scan. You will hear some clicking sounds as the monochromators are set to the wavelengths you selected.

m. After the scan is complete, record the wavelength at which the peak maximum occurs.

n. Open an excitation scan. Set the emission wavelength to the recorded peak wavelength from the previous step. The excitation range should span 150 nm. Set the highest wavelength in this range a minimum of 5 nm lower than the emission wavelength as mentioned above. Set the step size to 2 nm.

o. Start the excitation scan.

p. After the scan is complete, record the wavelength at which the peak maximum occurs. If this peak excitation is different from the excitation wavelength used in the first emission scan, use this new peak excitation wavelength for a new emission scan, and begin again at step e. The resulting curve should lie higher than the initial emission scan as the optimal wavelength range is approached.

q. Repeat this process of using peak wavelengths to run excitation and emission scans in tandem until the peak wavelengths do not differ from previous scans. The maximum peaks from both the optimal excitation and emission scans should be about the same height and the curves will look roughly symmetrical (Figure F.2).

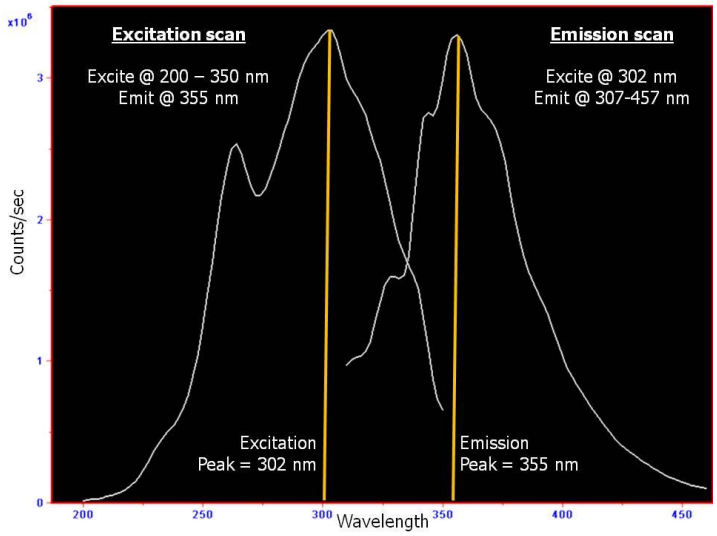

Figure F.2. Optimal excitation and emission scan curves for a 0.032% v/v CEWAF using Bunker C fuel oil.

2. Sampling and storage

Take a single 3.5-mL sample from each WAF stock and dilution series and add to 3.5 mL of ethanol in a 7-mL borosilicate scintillation vial with a foil- or Telfon-lined cap. Wrap Teflon tape or parafilm around the cap to minimize ethanol evaporation during storage. Analyze freshwater samples within 48 hours of sampling; analyze saline water samples within 1 week of sampling. Store samples in the dark at 4°C when not being handled for analysis. All samples, controls, exposure solutions, and standards should be prepared using the same water source.

3. WAF standard curve

A standard curve indicates the range of WAF concentrations over which the relationship between fluorescence and hydrocarbon concentrations are linear. It also demonstrates variance due to pipetting, and the reproducibility of test solutions from day-to-day and from experiment-to- experiment. Standard curves are characterized by their slopes, intercepts, and statistics related to "goodness of fit." With time, each laboratory will accumulate a database of standard curves that demonstrates the extent to which they vary, and the dilutions of each type of WAF needed to fall within a linear range. These data provide the confidence needed to reduce the frequency and number of dilutions on standard curves to streamline routine checks on preparation of test solutions.

a. Standard curves can be prepared for WAF, HEWAF, CEWAF, or any other solution that requires direct comparison. The WAF used for the standard curve should be the same WAF used for test dilutions.
b. Prepare the WAF and dilute in water to obtain a range of exposure concentrations. Add equal parts ethanol to preserve the sample.
c. Open an emission scan and enter the optimized excitation and emission wavelengths. Change the step size to 2 nm.
d. Before analyzing standards, measure the fluorescence of an ethanol control, water control, and a 50:50 ethanol:water solution control. Prepare the controls using the same water and ethanol source as the standards and samples. The ethanol and water controls are a check against contamination, The 50:50 ethanol:water control provides a baseline with a total area less than 1,000,000 relative fluorescence units (RFUs). Prepare new controls and re-run if contamination is suspected.
e. Prepare standards in 20-mL glass scintillation vials using test solution water. Add water to the vials first, then ethanol, and finally the sample.
f. Vortex each solution before sampling for dilution and after preparation. The dilutions (below) can be altered to reflect the test dilutions, time constraints, and the linear portion of the curve.

i. 100% v/v stock solution
ii. 50% v/v
    1. 5 mL of 100% v/v stock +5 mL EtOH
iii. 10% v/v
    1. 1 mL of 100% v/v stock + 4 mL water + 5 mL EtOH
iv. 3.2% v/v
    1. 320 µL of 100% v/v stock + 4.68 mL water + 5 mL EtOH
v. 1.0% v/v
    1. 100 µL of 100% v/v stock + 4.9 mL water + 5 mL EtOH
vi. 0.32% v/v
    1. 32 µL of 100% v/v stock + 4.968 mL water + 5 mL EtOH
vii. 0.1% v/v
    1. 10 µL of 100% v/v stock + 4.99 mL water + 5 mL EtOH
viii. 0.032% v/v
    1. 320 µL of 1% v/v stock + 4.84 mL water + 4.84 mL EtOH
ix. 0.01% v/v
    1. 100 µL of 1% v/v stock + 4.95 mL water + 4.95 mL EtOH

g. Run your standards on the fluorometer before the corresponding samples.
h. Re-run your standards each time the lamp is changed, major adjustments are made to the fluorescence spectrometer (slits adjusted, etc.), or daily if analysis will take several days.

4. WAF sample analysis

Sample preparation

a. Vortex samples for 5 seconds.
b. Sonicate samples for 3 minutes. Ensure that the sonicator heater option is not on. Note: When preparing samples of saline water for analysis, the addition of ethanol may cause the salt to precipitate, and the resulting turbidity can affect fluorescence readings. Thus, additional steps are needed to remove salt before analysis.
c. To remove precipitated salt from samples prior to fluorescence analysis, transfer the sample to microcentrifuge tubes. If using 1.5-mL microcentrifuge tubes, use two tubes for each sample in order to provide 3 mL for the fluorometer cuvette. Perform this step with a limited number of samples each time (e.g., four samples) to ensure the samples are not sitting in the microcentrifuge tubes for an extended period of time. Spin samples at 10,000 rpm for 10 minutes. A salt pellet will be visible at the bottom of the tube.
d. Remove the supernatant from the microcentrifuge tube while avoiding the pellet.

e. Transfer the sample to a cleaned quartz cuvette using a glass Pasteur pipette. The sample is ready to be analyzed.
f. Prepare and analyze all controls in the same manner as the samples.

Sample analysis

a. Before analyzing the samples, measure the fluorescence of an ethanol control, a water control, and a 50:50 ethanol:water control, as described above for standards. For controls with saltwater and ethanol, prepare the same way as saltwater samples.
b. Prepare new controls and re-run if contamination is suspected. Run a new 50:50 ethanol:water control after every 20 samples. This value is the baseline corresponding to the 20 samples following the control.
c. Open an emission scan. Enter the excitation and emission wavelengths determined from the wavelength optimization process above. Set the step size to 2 nm.
d. Wipe the cuvette on all sides with a Kimwipe and place the cuvette into the fluorescence spectrometer (ensure cuvette is always placed into instrument with the same orientation).
e. Label scan with the Sample ID.
f. Start emission scan.
g. Repeat the process with the next sample.
h. Save the file periodically while running samples.
i. Between each sample, rinse the cuvette twice with distilled or de-ionized water then rinse twice with ethanol.
j. At the end of the day, rinse the cuvette twice with distilled or de-ionized water and then once with ethanol. Use a cotton-tipped applicator to wipe the inner walls of the cuvette then rinse a second time with ethanol. Cuvettes are fragile and expensive – store in a box.

5. Determining the measured concentration

a. Subtract the fluorescence background (area of 50:50 ethanol:water control) from the fluorescence of each standard, and plot the log area of standards vs. the log of dilution (%v/v). The relationship can be described statistically by a log-log linear regression. If the R^2 value for the regression is less than 0.95, rerun your standards. The linear regression formula is the ***log area = intercept + (slope * log concentration)***. If the relationship is not linear, remove data points outside the linear range of the curve. If the linear portion of the curve is represented by fewer than 5 data points, rerun the standard curve with at least five concentrations within the linear range.

b. For each sample, subtract the background (area for 50:50 ethanol:water control) from the total area of the sample. Multiply this area by 2 to take into account the 50% dilution of samples with ethanol. Note: Standards should be prepared as exact concentrations/dilutions, therefore the fluorescence total area for standards should not be multiplied by 2.

c. Log-transform the resulting area and plot as a function of the corresponding log-transformed % dilution (%v/v). Compare the results to those obtained from the standard curve to determine if the test dilutions were prepared correctly. The relationship of area vs. concentration for samples should be identical to the standard curve (Figure F.1). Quick observations will determine which dilutions were not prepared correctly and the correct dilution can be estimated from the standard curve.

d. If standard curves are repeatable over time and among experiments with the same oil, this procedure can be shortened by measuring the fluorescence of one standard dilution and the fluorescence of freshly-prepared test solutions. The fluorescence readings of test samples should be predictable from the fluorescence of the one standard solution, using the average slope and intercept calculated from previous measurements of standard curves. ***This will only work within one oil and one method for preparing WAF***.

6. Supplemental information

a. Machine maintenance
   i. Each time the fluorescence spectrometer is used, fill in a usage log with the total time the lamp was turned on. This includes time for warming up, sample analysis, breaks, lunch, and other time increments.
   ii. The Xenon lamp life is about 500–700 hours. When changing out lamps, always wear gloves and eye protection because the bulb is under pressure. Never touch the quartz envelope with bare hands. This could cause the lamp to explode.
   iii. After the lamp is changed, optimize the signal by centering the lamp within the housing. Use a sample compound (e.g., retene) that has a clear and obvious peak, and run a time-based scan to detect the peak. Adjust the knobs on the lamp housing until the maximum intensity is reached.

b. Cuvettes
   i. Use high-quality quartz cuvettes for analyses. If more than one cuvette will be used for a set of samples, ensure that the cuvettes have been matched so that they can be used interchangeably. If the set of cuvettes changes at any point during analysis of a group of samples, a new standard curve to reflect the corresponding cuvettes.

ii. Between samples, rinse cuvettes twice with distilled water (essential for samples containing saltwater,) then twice with ethanol. Periodic control samples will ensure that the cleaning procedure was adequate. If necessary, use a cotton-tipped applicator to wipe the inner walls of the cuvette.

iii. Before placing a cuvette into the spectrometer, wipe the outside of the cuvette with a Kimwipe to remove any smudges or liquid that may interfere with the fluorescence reading.

c. Repetition of analyses

i. To conduct sample repeats, use a fresh sub-sample from the 7.0 mL original sample, or sample again.

ii. Because the fluorometer subjects each sample to a beam of high intensity light, many of the aromatic compounds that fluoresce will be partially or completely degraded. A repeated analysis of the same sample may give progressively lower fluorescence measurements.

G. Water Accommodated Fraction Filtration Standard Operating Procedure

Purpose

This SOP describes the general techniques and procedures for filtration of laboratory-prepared WAFs (including LEWAF and HEWAF) and CEWAFs. The methods described in this SOP can be used to filter water samples to assess the "dissolved" phase of the WAF or CEWAF, or they can be used to filter WAF or CEWAF exposure solutions prior to using the water for toxicity tests.

Materials

1. Stainless steel (solvent-rinsed) forceps.

2. 90-mm glass filter holder/funnel with sintered glass frit base (e.g., Sterlitech Item# 352100-KG90). We prefer sintered glass to stainless steel mesh or porcelain base; materials should be rinsed with solvent before each use.

3. Two glass aspirator flasks of appropriate volume(s), rinsed with solvent. One flask will be used for waste and the other for collecting filtrate. Using a filtrate aspirator flask appropriate for the volume required (i.e., to filter 1 L use a 2-L flask) reduces the chance of any potential contamination from the stopper coming into contact with the solutions or from the solution being pulled into the vacuum.

4. Filter holder/funnel to aspirator flask clamp.

5. Rubber stopper, cleaned with soap and warm water, and then rinsed with DI water. Do not clean the rubber stopper with solvents, as it can cause leaching of organic contaminants from the stopper.

6. Thick-walled rubber tubing to connect vacuum flask to pump or hydroaspirator. Tubing should be of appropriate diameter to securely fit all fittings without using adaptors.

7. No-oil vacuum pump with a very low setting (e.g., FisherSci Cat. No. 01-257-508) or access to a faucet with a hydroaspirator connection. We recommend vacuum pressure of no more than 5 cm Hg (2 in. Hg vac) for all preparations *except* for the 100% Slick A/B HEWAFs, for which we recommend 10 cm Hg vacuum pressure.

8. Two 0.3 µm pore size glass fiber filters[1] – 90 mm diameter (e.g., Sterlitech Item #GF7590100).[2]

9. Sample bottles, glass beakers, and/or designated containers rinsed with solvent.

10. WAF solutions and clean source water.

Procedure

1. Ensure all glassware and equipment is cleaned and decontaminated (as described in Appendix A) before starting. Ensure that solvents from all glassware have fully dried, so there is no solvent residue contamination.

2. Set up the filter apparatus with two stacked 0.3 µm glass fiber filters. To reduce the potential for contamination, use a pair of solvent rinsed forceps to transfer filters from the package to the frit.

3. Collect slightly more than the desired amount of WAF into an intermediate glass vessel (e.g., beaker). If filtering a WAF immediately following its preparation, WAF should not be collected until after its requisite settling time (as described in Appendix A.1). Also, when draining a settled WAF be sure not to use the top layer from the separatory funnel or aspirator bottle. Ensure that all collected solutions are homogeneous prior to filtering.

4. Place the first filter on the glass frit base. Add a small amount of clean source water (~ 3 mls) to moisten the filter so that it adheres to the glass frit. For source water, use the same seawater, or seawater with similar salinity and chemistry, that you used to prepare the WAF. Repeat this process after layering the second filter on top of the first one. Add the glass filter funnel on top of the filter papers and clamp it in place and assemble the filter apparatus with an aspirator flask designated for waste solutions, turn on the vacuum so that you can remove the excess source water from the filters.

1. We found that droplets can break through a filter when using typical 0.7-µm filters in the filtration of our CEWAFs. This may be caused by the very small droplet sizes produced when preparing a CEWAF. Samples filtered prior to July 3, 2013 and all samples filtered at Marin Biologic Laboratories, Inc. used one 0.7-µm glass fiber filter. After that time, we changed the CEWAF filtration protocol to use two stacked 0.3-µm filters to reduce breakthrough. Depending on the source oil and the volume of filtrate required, filtration with stacked filters might need to be done multiple times.

2. A table presenting comparable glass fiber filters of different brands can be found at the following website: http://www.sterlitech.com/glass-fiber-comparison-table.html.

5. Remove the waste aspirator flask and replace with a new, decontaminated filtrate aspirator flask of the appropriate capacity to collect required volume of filtrate and reassemble the filter apparatus.

6. Pour the WAF solution to be filtered into the funnel.

7. To start the vacuum, turn it onto a low setting (5–10 cm Hg vac) or slightly turn on the faucet connected to the hydroaspirator. Use gravity filtration if needed. If possible, record the vacuum pressure on the Analytical Sample Inventory Bench Sheet.

8. As the sample is filtered, add additional volumes of WAF to the funnel as needed to get the desired volume of filtrate. Do not let the filter run dry during filtration.

9. If the filtration starts to slow significantly, you may need to replace the filter paper. Do not increase the vacuum pressure or overload the filter paper; this may lead to droplet breakthrough. A 90 mm diameter filter is better than a smaller diameter filter, as the larger surface area helps reduce filter overload.

10. To replace filter, pour any residual WAF solution from the funnel, disassemble the filter apparatus to remove used filters, and then repeat steps 4 through 10, as needed.

11. Once you have filtered the desired volume of WAF, shut off the vacuum, make sure the sample is well mixed, and pour the filtrate into your designated container or sample bottle.

H. Shipping Instructions for Sample Retention

Each toxicity test may generate samples that need to be archived, including tissue samples and solvent extracts from in-house analyses, which must be retained as described in the sample retention order memorandum (Stratus Consulting, 2011). Archive samples requiring long-term cold storage can be sent to ALS Environmental. If frozen samples are being shipped using dry ice as a refrigerant, follow procedures outlined in Appendix A.6 (*Shipping Samples Using Dry Ice*).

When shipping archived samples to ALS Environmental for long-term storage, ship samples in separate coolers from samples being sent to ALS Environmental for chemical analysis. Each individual cooler should contain no more than 100 individual archive samples. Each sample in the cooler must be properly documented on the designated DWH toxicity testing COC form (Appendix C), including sample ID, date and time of sample collection and sample matrix. The samples should be organized in such a way as to allow for easy ID of samples once they arrive at ALS Environmental. For example, if 100 samples are going to be shipped, the samples will be divided by putting 10 samples in labeled zip-top plastic bags; indicate which samples are in which bag on respective COC form in the comments entry.

In some cases, only a small number of samples will fit in each cooler (e.g., when shipping large brood stock fish). For any cooler containing 10 or fewer samples, the sample ID numbers for each individual sample are sufficient, and the samples do not need to be further divided using the above approach. If shipping multiple coolers, then each cooler needs a separate COC form that lists only the samples included in the corresponding cooler.

In the special instructions section on the top of the COC form, include the phrase “all samples are for long-term storage only, no analysis required.” Since these samples do not need any extractions or analyses done by ALS Environmental, do not check the “Analyses Requested” box on the COC form.

Indicate the storage temperature for the samples on the COC form (e.g., store at -20°C). Samples with different storage temperature requirements must be shipped separately from each other. ALS Environmental is only accepting archive samples that require storage at 4°C or -20°C. For archive samples that have different storage requirements, please contact Stratus Consulting for further instructions.

Ship coolers using FedEx Standard Overnight Express Package Service and retain the shipping receipt as described in Appendix A.2. Hand coolers over directly to a FedEx representative; do not leave them at a pick-up location where they may be unattended. Instructions for filling out the shipping label are below.

- 3. Recipient: ALS Environmental, 1317 South 13th Avenue, Kelso, WA 98626
  - Telephone: 800-695-7222
- 4a. Express Package Service: check "FedEx Standard Overnight" – do not fill out Section 4b
- 5. Packaging: check "Other"
- 6. Special Handling: leave blank, but check "No" for *Does this shipment contain dangerous goods?*
- 7. Payment: check "Sender"
- 8. Residential Delivery Signature Options: check "No Signature Required."