

Washington State Department of Ecology

Spill Prevention, Preparedness, & Response Program

Standard Operating Procedure for Collecting Oil Spill Shellfish Tissue Samples

Version 1.0

Author – Dale Davis

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Reviewer – Rebecca Post

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QA Approval - William R. Kammin, Ecology Quality Assurance Officer

Date -

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Signatures on File

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*Any reference to specific equipment, manufacturer, or supplies is for descriptive purposes only and does not constitute an endorsement of a particular product or service by the author or by the Department of Ecology.*

*Although Ecology follows the SOP in most instances, there may be instances in which Ecology uses an alternative methodology, procedure, or process.*

SOP Revision History

Revision Date	Rev number	Summary of changes	Sections	Reviser(s)
09-30-2011	0.1			

Washington State Department of Ecology

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Standard Operating Procedure for Collecting Oil Spill Shellfish Tissue Samples

**1.0 Purpose and Scope**

1.1 This document is the Spill Prevention, Preparedness, & Response (Spills) Program Standard Operating Procedure (SOP) for collecting oil spill shellfish tissue samples.

1.2 **Objectives** – 1) To determine the concentration of oil compounds in tissue. 2) To document the aerial extent and duration that the area was exposed to the spilled material. Bivalves uptake oil quickly, depurate them slowly, and can be used as "composite" samplers. 3) To assess the risk to humans and predators from consuming contaminated shellfish. 4) To document the bioavailability and exposure pathways of the spilled material. 5) To maintain the integrity of the sample(s) during sampling, transport, and storage.

1.3 The main purpose for collecting oil spill tissue samples is to document the concentrations of oil compounds in tissue and the exposure of natural resources to determine if the resources have been injured. If areas are closed to shellfish harvesting due an oil spill, it will probably be necessary to analyze tissue samples to determine when the areas can be re-opened. To be legally valid, samples must be carefully collected to prevent contamination and strict chain-of-custody must be maintained throughout collection, transport, and analysis.

1.4 Composite tissue samples are usually collected for oil spills, analyzing the samples for Polynuclear Aromatic Hydrocarbons (PAHs). PAHs are very persistent and cause most of the chronic toxicity, and are therefore the preferred analysis for NRDA injury assessment. Also, since most of the toxicity in oil is due to the PAHs, it is the preferred analysis for assessing ingestion risk (for humans or wildlife).

**2.0 Applicability**

2.1 This procedure is based on protocols developed by Research Planning, Inc. (<http://www.researchplanning.com/services/nrda.html>) for NOAA for oil spill Natural Resource Damage Assessments (NRDA). The protocols were based on sampling and analytical methods developed for the National Status and Trends Program. These protocols are widely accepted and used by oil handling companies, consultants, and other trustees and should be used for all oil spill related sampling.

**3.0 Definitions**

3.1 NOAA – National Oceanic and Atmospheric Administration.

3.2 GC/FID – Gas Chromatograph/Flame Ionizing Detector

3.3 GC/MS – Gas Chromatograph/Mass Spectrometer

- 3.4 TOC – Total Organic Carbon
- 3.5 TRAP – Trustee Resource Assessment and Protection.
- 3.6 NRDA – Natural Resource Damage Assessment.
- 3.7 SOSC – State on Scene Coordinator

#### **4.0 Personnel Qualifications/Responsibilities**

- 4.1 Any Ecology employee that expects to collect oil spill related samples must attend appropriate training from a Spills Program Sampling Specialist. Oil spill tissue sample collection can be conducted by any employee that has completed basic and intermediate sampling training and has collected at least one quantitative field tissue sample under the supervision of an experienced sampler. All Spills Program employees (including managers and administrative staff) are encouraged to attend basic sampling training. Due to safety requirements, at least two people are recommended for any field sampling and anyone that has taken the training, with or without field experience, can assist an experienced sampler.

#### **5.0 Equipment, Reagents, and Supplies**

##### **5.1 Equipment**

- 5.1.1 Field samples should be collected in certified clean glass containers or wrapped in solvent cleaned foil and placed in Ziploc bags.
- 5.1.2 Dredges, tongs, or grabs are used to collect shellfish from subtidal areas. Shovels or clam rakes are used to dig up infaunal shellfish from intertidal areas.
- 5.1.3 Field samples are transported to a sample processing center (laboratory or clean-room) where the tissue is removed from the shells and homogenized. Small meat grinders work well for homogenizing the tissue. The laboratory that performs the analyses may also homogenize the tissue (usually for an additional fee).
- 5.1.4 Homogenized tissue samples are transferred into 4 oz. pre-cleaned wide-mouth glass jars with Teflon lined caps. The laboratory will use a portion of each sample to determine lipid and water content; a separate sample is not necessary.
- 5.1.5 GPS (set datum to WGS-84, Latitude/Longitude to decimal degrees).
- 5.1.6 Digital camera (synched to GPS time).
- 5.1.7 Ice chests with wet or blue ice (preferably equipped with chain of custody security cables).

##### **5.2 Reagents**

- 5.2.1 Capillary GC Pesticide Residue Grade or equivalent acetone and hexane for decontamination.

### 5.3 **Supplies**

- 5.3.1 Sample tags and labels
- 5.3.2 Field notebook
- 5.3.3 Chain-of-custody seals
- 5.3.4 Chain-of-custody forms
- 5.3.5 Nitrile disposable gloves
- 5.3.6 Ziploc bags
- 5.3.7 Paper towels
- 5.3.8 Stainless steel spoons or spatulas
- 5.3.9 Liquinox or equivalent soap
- 5.3.10 Aluminum foil
- 5.3.11 Cleaning brush
- 5.3.12 Distilled water

## 6.0 **Summary of Procedure**

### 6.1 **Sample Collection Procedure**

- 6.1.1 Safety is of greatest concern. Be aware of physical and chemical hazards at the site. Get a safety briefing before entering the area. Do not enter confined spaces unless they have been determined to be safe. Use recommended safety equipment and procedures.
- 6.1.2 Develop a sampling plan. Identify the number and type of samples that are to be collected, the type of containers that will be used, and unique identifiers for each sample. Make a drawing of the area being sampled, including points of reference and the location of each site where samples are collected so the sites could be located later if necessary. Identify the sampling procedure(s) that will be used. Include any preservation techniques used for the samples, the type of analyses that will be done, and the laboratory that the samples will be sent to. The plan can be recorded in a field notebook or on a separate piece of paper; there is no required form or specific format that must be followed. Each plan will be different depending on the number and type of samples being collected and the complexity of sampling site.
- 6.1.3 Attached organisms are pried away from the substrate with a knife, trowel, etc. Infaunal samples should be rinsed with site water to remove sediments. Collect primarily live animals (shells intact and tightly closed). Note the condition of dead animals if they are appropriate for collection. Photograph all collection sites prior to and during sampling.
- 6.1.4 The sampler handling the shellfish should wear disposable gloves and change gloves after each sample. Record observations of any external evidence of contamination.
- 6.1.5 Composite samples are recommended to provide enough sample weight to meet detection limit objectives and to average out the variations at a location among individual organisms. If uncertain about the number of individuals needed to meet

minimum weight requirements, open, shuck, and weigh the tissue from a few individuals for calibration (these animals should be discarded and not included in the sample).

- 6.1.6 Individuals should be of the same species and about the same shell or body size. Record the size range collected or save shells for later measurement.
- 6.1.7 Collect approximately 30 grams wet weight (composite of about 20 individual animals, depending on size).
- 6.1.8 Shellfish should not be opened in the field to minimize the risk of contamination. Rather, sets of whole organisms are placed in large clear wide-mouth jars or wrapped together in clean aluminum foil and placed in double Ziploc bags. Rinse the foil with acetone, then rinse again with hexane (Capillary GC Pesticide Residue Grade or equivalent). Collect solvent rinsate for proper disposal.
- 6.1.9 Wipe oiled shells with sorbent pads, wipes, etc. If heavily oiled, use a solvent on the wipe.
- 6.1.10 Sample control and least oiled areas first, then sample the more contaminated areas.
- 6.1.11 Label each container with an adhesive label directly applied to the container and with a paper tag attached to the container with an elastic band or placed between the two Ziploc bags. Use a waterproof pen or marker to record the project or incident name, a station identifier, a unique sample identifier, the collection date and time, the desired analysis type (PAH), and the sampler's name or initials on each label. The adhesive label should be filled out and applied to the container prior to sample collection.
- 6.1.12 Use packing material around containers to prevent breakage during handling and shipping.
- 6.1.13 Shellfish collected in jars or wrapped in foil are transported to a sample processing center (laboratory or clean-room) where the tissue is removed from the shells, homogenized, and transferred into 4 oz. pre-cleaned wide-mouth glass jars with Teflon lined caps.
- 6.1.14 Immediately place all samples in cooler and keep at 4°C. Freeze as soon as possible. Whole animals or homogenized tissue can be frozen and held for up to 1 year without loss of sample integrity (the samples should be protected to minimize desiccation).
- 6.1.15 Fill out the chain-of-custody form; be sure to record all information for each sample. Record the same information in a field notebook and add specific information about where each sample was collected from, including latitude-longitude and a map if necessary. Also include the sampling devices used, container sizes and types, if any preservative was used, and sampler name.

6.1.16 Make special notation on the chain-of-custody form about any problems or observations during sampling that might affect the integrity of the samples, such as potential sources of contamination, etc.

6.1.17 Maintain strict chain-of-custody during sample storage and transportation.

6.1.18 Tissue samples for PAH analysis can be held at 6°C in the dark for up to 14 days without loss of sample integrity. If PAH samples are extracted within 14 days, the holding time for the extract is 40 days. The holding time for frozen PAH tissue samples is 1 year if held at -18°C. Tissue for lipid content analysis should be analyzed within 14 days or frozen.

### 6.1.19 **Other Sampling Considerations**

6.1.19.1 Temperature can have a very large impact on shellfish physiology. Some animals stop feeding or even passing water over their gills at low or high temperatures. Be aware of these differences when selecting species for monitoring and comparing results among species. Uptake and depuration rates vary widely among species. Depuration usually takes weeks; thus, shellfish sampling should be initiated within 1-2 weeks after maximum exposure.

6.1.19.2 For mapping exposure, it is best to sample species with wide distribution in the study area. For ingestion risk assessment, target key food species.

6.1.19.3 Collect background samples from clean sites representative of pre-oiling conditions, as well as areas not yet oiled but in the potential path of the oil. These data will provide the best evidence of changes in contamination due to exposure to the spilled material.

6.1.19.4 NOAA National Status and Trends, EPA EMAP, or state Mussel Watch programs may have background data for contaminants in shellfish and sampling protocols.

6.1.19.5 Use a physical or mental model of the extent of water and sediment contamination to determine the number and location of samples. Minimum guidelines are at least three samples per area of relatively uniform exposure or distinct waterbody. Also, sample along exposure gradients at regular intervals proportionate to the exposure area so that at least six stations are sampled.

## 6.2 **Analytical Methods**

6.2.1 **Polynuclear Aromatic Hydrocarbons (PAHs).** PAHs are used to characterize the spilled oil, monitor weathering, and predict toxicity. If PAHs are to be measured, it is important that the analytes include the alkyl-substituted PAH homologs, in addition to the standard PAH "priority pollutants." This method is referred to as Modified EPA Method 8270 (NOAA list), because the list of PAHs is expanded to include the alkylated homologs, using GC/MS in the selected ion monitoring (SIM) mode.

6.2.1.1 **Lipid Content.** Lipid content is defined as the percent of sample tissue extracted and remaining after solvent evaporation. It is used to normalize organic contaminants in tissue.

6.2.1.2 **Water Content.** Most results are reported as dry weight, to reduce sample variability.

### 6.3 **Decontamination Procedure**

6.3.1 Wash with soap (Liquinox or equivalent) and water (preferably hot), scrubbing all surfaces thoroughly with the cleaning brush. If the item being cleaned is heavily contaminated (oiled), this step should be repeated after washing to remove gross contamination.

6.3.2 Sampling equipment (shovels, rakes, etc.) should be decontaminated between each site.

6.3.3 Procedure:

6.3.4 Rinse with tap water and then triple rinse with distilled water.

6.3.5 Rinse with acetone.

6.3.6 Rinse with hexane.

6.3.7 Allow to air dry.

6.3.8 If item is not used immediately, wrap in solvent rinsed aluminum foil.

### 7.0 **Records Management**

7.1 Sampling Plan Template – Use this form as an aid for developing a complete and comprehensive sampling plan. This form is not required for developing a sampling plan, but provides ready access to guidelines and reminders.

7.2 Sampling Documentation Form – Use this form as an aid and reminder for recording complete and comprehensive sampling information, and as a single place to record information for multiple samples.

7.3 Both forms are available at:

<http://teams/sites/SPPR/response/trap/Sampling/Forms/AllItems.aspx> (SharePoint)  
or X:\Spills\_Program\Response Section\TRAP-NRDA\Sampling\

7.4 Oil Spill Chain-of-Custody Form (ECY 050-42 (11/01))

### 8.0 **Quality Control and Quality Assurance Section**

8.1 There are no QA/QC requirements specific or unique to this procedure. Field splits should be avoided, unless enough individual animals are collected to make two complete samples per site. If splits are requested, it is best to submit the tissue samples to the laboratory and the laboratory can be asked to split the samples. Typically, the laboratory will extract the samples and then split the extracts. The laboratory will usually provide splits directly to the requester or their laboratory if the sample contains enough material.

## **9.0 Safety**

- 9.1 Sample collection can present some unusual circumstances that could have equally unusual associated safety hazards. Samplers should consult with the Safety Officer or SOSC and review the incident safety plan or Hazard Assessment Worksheet (HAW) prior to developing a sampling plan so known hazards can be avoided. Samplers should also be aware that sampling will often take place in areas that may not be adequately addressed in the safety plan or HAW. If there is any question, then a separate HAW should be prepared prior to sampling. A Sampling HAW is available at: <http://teams/sites/SPPR/response/trap/default.aspx> (SharePoint) or X:\Spills\_Program\ Response Section\TRAP-NRDA\Sampling, that includes action levels appropriate for sampling various petroleum products.
- 9.2 Some hazards that could be associated with sampling are:
- 9.3 Low areas that could collect fumes (vapors) or have reduced oxygen levels (confined space)
- 9.4 Higher concentrations of fumes at ground level where samples are collected
- 9.5 General water hazards when collecting samples on a shoreline or from a boat or dock, etc. including the potential for mobile logs in the surf zone and rogue waves.
- 9.6 Increased risk of slips, trips, and falls
- 9.7 Traffic when sampling near a highway
- 9.8 Dangerous animals
- 9.9 Exposure to elements (hot or cold)
- 9.10 Eye damage from splashes or brush/branches

## **10.0 References**

- 10.1 NOAA, 1993. Sampling and analytical methods of the National Status and Trends Program, National Benthic Surveillance and Mussel Water Projects, 1984-1992. Volume IV, Comprehensive descriptions of trace organic analytical methods. Lauenstein, G.G. and A.Y. Cantillo (eds.). NOAA Tech. Memo NOS ORCA 71, Silver Spring, MD. 181 pp.