### Protocols For Collecting NRDA Samples

#### SHELLFISH TISSUES

**Sampling Objectives**
- To document extent and duration of the area exposed to the spilled material. Bivalves uptake oil quickly, depurate them slowly, and can be used as "composite" samplers.
- To determine the spill source via fingerprinting analysis.
- To assess the risk to organisms from consuming contaminated prey.
- To document the bioavailability and exposure pathways of the spilled material.
- To maintain the integrity the sample(s) during sampling, transport, and storage.

**Sample Size (see back page for description of Analytical Methods)**
- PAH by GC/MS-SIM: 30 g wet weight (composite of ~20 individual organisms)
- Lipid and Water Content: A sub-set of the sample is used for these analyses

**Sampling Equipment**
- Dredges, tongs, or grabs are used to collect shellfish from subtidal areas. Shovels are used to dig up infaunal shellfish from intertidal areas. A screen is useful for sieving sediments.
- If oil is present, decon dredges, knives, etc. between samples. First wash with laboratory-grade detergent and clean water, with a triple clean water rinse (distilled water from a local store is OK). If the equipment was obviously contaminated, rinse with methanol or acetone, followed by methylene chloride or hexane (Capillary GC Pesticide Residue Grade or equivalent). Let solvent evaporate before use. Do not work with solvents downwind of exhaust or other airborne hydrocarbon source. Collect solvent rinsate for proper disposal.

**Sample Collection Methods**
- 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 sampling.
- The sampler handling the shellfish should wear surgical gloves and change gloves after each sample. Record observations of any external evidence of contamination.
- 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 individuals of a certain size for calibration.
- Individuals should be the same shell (or body) size. Record size range collected or save shells for later measurement. Same size is not as important if only for fingerprinting.
- Shellfish should not be opened in the field to minimize the risk of contamination. Rather, sets of whole organisms are wrapped together in clean aluminum foil. Rinse the foil with methanol or acetone, then rinse again with methylene chloride or hexane (Capillary GC Pesticide Residue Grade or equivalent). Collect solvent rinsate for proper disposal.
- Wipe oiled shells with sorbent pads, wipes, etc. If heavily oiled, use a solvent on the wipe.
- Place all individuals of the same species from a site in a glass jar or double Ziploc bags.
- A waterproof sample label is placed between the two bags or on the sample jar and lid.
- Sample control and least oiled areas first, then sample the more contaminated areas.
- Use packing material around containers to prevent breakage during handling and shipping.

**Preservation/Holding Times**
- Immediately place all samples in cooler and keep at 4°C. Freeze as soon as possible.

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properly frozen, they can be held for years without loss of sample integrity.

Analytical Methods

- Polynuclear Aromatic Hydrocarbons (PAH). Since most of the toxicity in oil is due to the PAHs, it is the preferred analysis for assessing ingestion risk. The analytes must include the alkyl-substituted PAH homologs, in addition to the standard PAH "priority pollutants". This method is referred to as Modified EP A Method 8270, because the list of PAHs is expanded to include the alkylated homologs, using GC/MS in the selected ion monitoring mode. Detection levels should be 1 ppb for individual PAHs to support injury assessment using toxicity thresholds. PAHs are also used for fingerprint analysis and differentiating between the spilled material and background contamination.

- Lipid Content. Lipid content is defined as the percent of sample tissue extracted and remaining after solvent evaporation using dichloromethane. It is used to normalize organic contaminants in tissues, to aid in spatial and temporal comparisons among samples.

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

Other Considerations

- Temperature can have a very large impact on shellfish physiology. Some animals stop feeding or even pass 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.

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

- Avoid sources of contamination such as exhaust fumes and engine cooling systems on vessels. Work up-wind of any exhausts. Segregate dirty/clean areas. Layout clean substrates to work on and replace frequently.

- 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.

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

- 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.

Key References


