Tissue Preparation and Homogenization

References: This standard operating procedure (SOP) is a performance-based method. This SOP describes the procedure as developed by Alpha Analytical.

1. Scope and Application

Matrices: This method is applicable to the preparation and homogenization of plant and animal tissue including: mammals (mice or shrew etc.), fish (whole body and fillets), mollusks (mussels or clams, etc.), crustaceans (lobster or shrimp, etc.), reptiles and amphibians (frogs or turtles, etc.) macro invertebrates (benthic worms, eels, insects and other biota), and vegetation (coastal and wetland grasses)

Definitions: Refer to Alpha Analytical Quality Manual.

This preparation and homogenization procedure may be used prior to the extraction or digestion of the matrices listed above, for the ultimate detection of organic and inorganic analytes. Because this procedure is performance based, it should only be used for compounds where studies have assessed the precision, accuracy, and sensitivity of the technique relative to the project specific goals.

This method is intended to describe the preparation and homogenization procedures to be followed prior to the extraction, digestion and/or clean up of sample extracts or digestates. This procedure uses a variety of cutting and grinding equipment for size reduction, compositing and homogenization. See Section 7 for Equipment and Materials. This method is applicable to the matrices described above. The final determinative analytical methods contain the lists of potential target compounds. Applicable extraction, digestion, and cleanup methods include:

- *Microwave Assisted Acid Digestion of Sediments, Soils, Tissues and Waters* (MP-003),
- *Gel Permeation Chromatography* (OP-006),
- *Sulfuric Acid Cleanup - Method 3665A* (OP-010),
- *Microscale Solvent Extraction (OP-016),*
- *Tissue Extraction (OP-018),*
- *Silica Cleanup (OP-014),*
- *Alumina Column Cleanup (OP-009).*

Data derived from the analysis of tissue samples is generally used to determine if human health, and/or ecological risk criteria have been exceeded.

The data report packages present the documentation of any method modification related to the samples tested. Depending upon the nature of the modification and the extent of intended use, the laboratory may be required to demonstrate that the modifications will produce equivalent results for the matrix. Approval of all method modifications is by one or more of the following laboratory personnel before performing the modification: Area Supervisor, Department Supervisor, Laboratory Director, or Quality Assurance Officer.

This method is restricted to use by or under the supervision of experienced analysts.

2. Summary of Method

This method describes the tissue processing and homogenization procedures to be used prior to the extraction/digestion and analysis of the sample. Samples are best processed when partially frozen. Samples may be re-frozen after processing pending extraction or digestion.

Fish tissue samples (whole bodies, carcass or fillets) are weighed and the weights are recorded following project specifications. Measurements may be taken as needed depending upon the project specifications. The fish may be processed with the skin on or off, depending upon the project specifications. If fillets are to be removed and processed separately, this is generally done after the removal of the skin, however fillets can be processed with the skin on if requested. If compositing is required, the identified samples for composite are filleted or skinned prior to compositing homogenization. The carcass of the fish (after removal of the fillet) may be maintained for separate homogenization and analysis if requested.

Mammals such as mice, shrew or other rodents, should be prepared in a glove box if one is available, due to the potential health hazards associated with mammal tissue. All project specific sample preparation (weighing, skinning, compositing and homogenization) should be performed in the glove box. Waste from the processing must be containerized and treated with bleach before disposal. The outside surfaces of the sample containers must be disinfected before removal from the glove box, or any other work area.

Mollusks, crustaceans and other like invertebrates are measured and weighed prior to processing. Mollusks must be removed from their shells before processing. Due to the low weight of a single mollusk, crustacean or invertebrate, these sample types are generally composited with others of the same species and/or sampling area prior to homogenization. Gender determination may need to be performed with larger crustaceans such as lobsters. This is done prior to any processing and recorded. Additionally, lobsters are usually dissected, and the edible meat (tail and claw) is removed for homogenization. Certain internal organs such as the hepatopancreas may need to be processed separately. If crabs are being processed, the legs, claws and body cavity are generally homogenized together.

Reptiles and amphibians are generally processed as whole body samples. Depending upon the size, the specimen may need to be cut into small pieces and processed in part, then re-combined as a single sample. Due to the thickness of the skin of most reptiles, such as frogs, it is recommended that these be processed without the skin. If the skin must be processed, ensure that the grinder or processor blades are sharpened before use. The blades may need to be re-sharpened between every few samples as needed. Turtles must be removed from the shell prior to processing by digging out the head and legs, and as much of the body as feasible.

Macro invertebrates such as worms, eels, insects or benthic biota are generally processed as whole body samples. Depending upon the size, the specimen may need to be cut into small pieces and processed in part, then re-combined as a single sample. Due to the low weight of a single invertebrate, these sample types are generally composited with others of the same species and/or sampling area prior to homogenization.

Plants are rinsed prior to processing to remove soil, silt, small insects or other debris. Depending upon the size of the plant and the leaves, the sample may be processed mechanically, or may have to be cut into small pieces by hand. Plants can be processed either wet or dry, depending upon project specifications.

After tissue processing, organic samples will be extracted and the extracts cleaned if needed, then analyzed by the determinative analytical procedure. Inorganic digestates do not require further clean up and will only undergo analysis by the determinative analytical procedure.

2.1 Method Modifications from Reference

None.

3. Reporting Limits

Not applicable to this method. Refer to the analytical method SOPs.

4. Interferences

Solvents, reagents, processing equipment and glassware may introduce interferences. These must be demonstrated to be free of interferences by the analysis of a method blank. See the Alpha Analytical SOP *Reagent, Solvent and Standard Control* (G-008) and *Laboratory Glassware Cleaning* (G-002), for additional details.

Field Blanks are recommended to ensure that the field sample packing materials are not a potential source of contamination. This can be done by pouring contaminate free water over the sample collection material and collecting the water in an appropriate container with preservative as needed (*i.e.,* 1L glass amber bottle for organic and a 500mL polyethylene bottle with 1:1 HNO_3 preservative for metals).

Equipment used to process samples for *organic* analyses should be made of stainless steel, Teflon, ceramic, or PTFE. Tissue should be removed with clean, high-quality, corrosion-resistant stainless steel, ceramic or titanium instruments, knives and blades. Homogenates must be stored in borosilicate glass, quartz, or PTFE containers with PTFE-lined lids.

Many interferences can be removed by sample cleanup. The organic cleanup methods performed by Alpha Analytical include those listed in Section 1. Only appropriate cleanup techniques must be performed based on the suspected interference and the compounds of interest. For example, sulfuric acid cleanup is not applicable to samples requiring pesticide analysis because this rigorous cleanup will destroy the majority of pesticides.

Soapy residue may result in basic conditions on glassware and may cause degradation of the pesticides Aldrin and Heptachlor, some organophosphorous pesticides, and can cause metals instrument interferences. All glassware must be rinsed thoroughly with deionized water and solvents/nitric acid to remove soapy residue. See the Alpha Analytical SOP (G-002) *Laboratory Glassware Cleaning*, for additional details.

5. Health and Safety

The toxicity or carcinogenicity of each reagent and standard used in this method is not fully established; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. A reference file of material safety data sheets is available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available in the Chemical Hygiene Plan.

All personnel handling environmental samples known to contain or to have been in contact with municipal waste must follow safety practices for handling known disease causative agents.

6. Sample Collection, Preservation, Shipping and Handling

6.1 Sample Collection

As guidance, a minimum of 50 grams of sample must be collected for organic analyses, and 5 grams for metals analyses, in a glass jar with a Teflon or PTFE-lined screw cap. The amount of sample needed, will depend upon the project DQOs, such as reporting limits and the need for MS/MSD and/or duplicate analyses. Extra sample must be collected, if possible, to allow the laboratory adequate sample volume in case re-preparation and re-analysis is needed. Large whole individual fish, fillets, or vegetation may be wrapped in plastic or aluminum foil depending upon the requested analyses. (See Section 4 or additional details about allowable materials). Large crustaceans, reptiles or amphibians may be individually packed in well-labeled Styrofoam coolers.

6.2 Sample Preservation

It is recommended that samples are preserved by freezing them with dry ice at $\leq$ -20°C. If samples are not shipped frozen, they will be stored in freezers at Alpha Analytical upon arrival, and until processing. The samples must remain frozen and maintained at $\leq$ -20°C $\pm$ 10°C until processing. After processing, individual sample homogenates must also be stored at $\leq$ -20°C $\pm$10°C until extraction/digestion and analysis.

6.3 Sample Shipping

Refer to Section 6.2.

6.4 Sample Handling

Sample processing and extraction/digestion hold times are suspended by freezing the sample. Hold time monitoring is resumed when samples are removed from freezers for processing and then returned to freezers pending extraction/digestion. Movement of samples into and out of freezers is tracked through LIMS. The organic hold time is 14 days from sample collection to extraction, and 40 days from extraction to analysis. The metals hold time is six months from sample collection to digestion and analysis. If mercury is to be determined, the hold time is 28 days from sample collection to digestion and analysis.

7. Equipment and Supplies

7.1 Cutting board: Made of either glass or Teflon.

7.2 Food processor: Black & Decker with titanium cutting blade (small).

7.3 Dissection Tools: Tools may include the following utensils: knives/blades (ceramic, stainless steel, or titanium), stainless steel picks, spatulas (stainless steel or Teflon-coated stainless steel), stainless steel scissors/snips, stainless steel tweezers. (Refer to Section 4.0 for interferences and/or contamination associated with different materials.)

7.4 Pliers: Stainless Steel

7.5 Balances: Analytical Balance with precision to 0.0001g; Top loading balance with precision to 0.01g; Top loading balance with precision to 0.2g.

7.6 Grinding unit: Omni-GLH, electric, custom fitted with stainless steel or titanium interior saw tooth probes (10mm, 20mm, 45mm), or equivalent.

7.7 Tissuemizer: *Janke & Kunkel* IKA - Labortechnik Ultra Turrax T25, stainless steel

7.8 Grinder: LEM electric meat grinder, stainless steel (or equivalent)

7.9 Multi-hazard glove box: Labconco

7.10 Bench liner material

7.11 Latex Gloves – Powder Free

7.12 Glass weighing dish/jar, wax paper, aluminum foil, plastic wrap.

7.13 Camera

7.14 Ruler

7.15 Paper towels: Kim Wipes

8. Reagents and Standards

Use reagent grade or trace metals grade chemicals for all reagents. Deionized (DI) water or reagent water is ASTM Type II laboratory reagent grade water. Other grades may be used.

All reagents are stored at room temperature in flammable cabinets, unless otherwise noted. All reagents expire upon manufacturer's expiration date or one year from date of opening, whichever is sooner.

8.1 Methylene Chloride: ACS approved, Pesticide grade, see ALPHA ANALYTICAL SOP *Reagent, Solvent and Standard Control* (G-008) for additional details regarding solvent purity.

8.2 Methanol: ACS approved, Purge & Trap grade, see ALPHA ANALYTICAL SOP *Reagent, Solvent and Standard Control* (G-008) for additional details regarding solvent purity.

8.3 Hexane: ACS approved, Pesticide grade, see ALPHA ANALYTICAL SOP Reagent. *Solvent and Standard Control* (G-008) for additional details regarding solvent purity

8.4 Acetone: ACS approved, HPLC grade, see ALPHA ANALYTICAL SOP *Reagent, Solvent and Standard Control* (G-008) for additional details regarding solvent purity.

8.5 Nitric acid 50% (1:1): Add 500 mL concentrated HNO_3 to 400 mL of reagent water and dilute to 1 liter in an appropriate beaker or flask. For 25% HNO_3 solution: add 250 mL of concentrated HNO_3 to 400 mL of reagent water and dilute to 1 liter in an appropriate beaker or flask. Store in a corrosion-resistant cabinet.

8.6 10% Bleach solution: Add 100 mL of commercial bleach to 500 mL of reagent water and dilute to 1 liter in an appropriate beaker or flask. Prepare fresh each day of use.

8.7 Alconox cleaning solution. No special storage requirements. No expiration requirements.

9. Quality Control

The laboratory must maintain records to document the quality of data that is generated. Ongoing data quality checks are compared with established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

The following quality control samples mayor may not be included with each processing batch. If not included in the tissue processing steps, they must be included in the extraction/digestion batches that follow processing, or as needed, depending upon project specifications.

9.1 Blank(s)

9.1.1 Rinseate Blank/Equipment Blank or Process Blank

Rinseate/Equipment or Process blanks are generated using contaminate-free reagent (DI) water to rinse all processing equipment after completion of the cleaning procedure (see Section 10.1). The volume of water used will be based on project-specific volume requirements for requested analyses.

All processing equipment is rinsed with pre-determined volume of reagent water (DI) into a collection vessel. All rinse water is transferred from collection vessels to larger glass carboy.

Homogenizer/Generator probes are immersed in a pre-determined volume of DI water. The Homogenizing Unit will be turned on and the probe will process the DI water for a pre-determined time, based on project specifications. The DI water will then be transferred into a larger glass carboy and combined with DI from other processing equipment.

After all rinseates are collected into the glass carboy (or appropriate container), mix the DI water using a large glass stirring rod or by swirling the DI water. Transfer the water into the appropriate pre-prepared sample containers.

9.1.2 Method Blank

Not applicable to this method. Refer to analytical SOPs.

9.2 Laboratory Control Sample (LCS)

Not applicable to this method. Refer to analytical SOPs

9.3 Initial Calibration Verification (ICV)

Not applicable to this method. Refer to analytical SOPs.

9.4 Continuing Calibration Verification (CCV)

Not applicable to this method. Refer to analytical SOPs.

9.5 Matrix Spike

Not applicable to this method. Refer to analytical SOPs

9.6 Laboratory Duplicate

Not applicable to this method. Refer to analytical SOPs

9.7 Method-specific Quality Control Samples

Not applicable to this method. Refer to analytical SOPs

9.8 Method Sequence

Not applicable.

10. Procedure

The procedures described below are general cleaning and pre-processing procedures that are to be followed regardless of the type of tissue being processed. Samples are prioritized by the Department Manager or Team Leader based on hold time and client due date. All weights, measurement and other project required observations are recorded on the Tissue Prep Log sheets.

10.1 Equipment Set-up

10.1.1 Wash all utensils, generator probes, sample processor (blades, blade post, cup and lid) and the cutting board(s) with an alconox solution and a sponge. Rinse thoroughly with tap water, then with DI water and allow to dry. Equipment may be dried with a paper towel, if needed.

10.1.2 After drying the equipment, rinse all utensils, processor parts and surfaces with Acetone followed by a rinse with methylene chloride.

10.1.2.1 For *metal analyses only*, rinse all plastic and ceramic utensils with 25% HNO3 followed by another rinse with DI water. Rinse processor parts and surfaces with the alconox solution, followed by a tap water and a DI water rinse. Any metal or titanium surfaces must not come into contact with the 25% HNO3 solution as this may strip some metal alloys from these surfaces and introduce contamination.

10.2 Initial Calibration

Not applicable.

10.3 Equipment Operation and Sample Processing

10.3.1 Gloves must be worn when handling tissue samples.

10.3.2 Tissue samples should be partially thawed before starting, to the point where it becomes possible to make an incision in, or cut through, the flesh.

10.3.3 Note any morphological abnormalities on the processing records.

10.3.4 Fish Tissue Preparation

10.3.4.1 Determine the wet weight for each individual fish using a calibrated balance and appropriate weighing dish. Follow project specifications for alternate sample weight determinations.

10.3.4.2 Determine the length of each fish using a ruler, and record with the weight. Some measurements may, or may not be, a part of the project specifications. Additionally, a picture with a ruler in the foreground may be required. Follow project specifications.

10.3.4.3 Removal of Scales or Skin

10.3.4.3.1 If required by project specifications, the scales and/or skin of the fish will be removed prior to filleting. Clean all glassware and utensils as described in Section 10.1.

10.3.4.3.2 Rinse the fish with DI water and dry using a paper towel. Lay the fish on the cleaned, and/or lined, cutting board

Scrape the fish from tail to head using the blade edge of a cleaned stainless steel, ceramic or titanium knife, to remove the scales. Continue until all scales are removed.

10.3.4.3.3 Depending upon the outward condition of the samples, the sample may be rinsed with DI water and pat dry with paper towel. Place the fish on a clean cutting board, for filleting or skinning.

10.3.4.3.4 To skin the fish: Using a stainless steel knife, cut the skin behind the operculum (gill cover). Using the knife blade, pliers or other cleaned utensil, pull the skin off towards the tail of the fish. If necessary, cut lightly along the inside of the skin, slowly separating the skin from the muscle tissue. Removing the skin may require cutting the skin along the backbone or underbelly of the fish. If necessary follow project specifications for weight determinations.

10.3.4.4 Filleting the Fish

10.3.4.4.1 Using fresh gloves and the specified knife, make a cut behind the entire length of the operculum (gill cover), making sure to cut through the skin, if still attached, and the flesh, as close to the bone as possible. Note: If the fish samples are small, and it appears difficult to fillet, or if the amount of the fillet appears to be insufficient for the analysis requested, consult the Department Manager and/or Project Manager prior to filleting. In some cases it may be necessary to homogenize the whole body.

10.3.4.4.2 Make a cut across the caudal peduncle (the base of the tail fin) keeping as close to the caudal (tail) fin as possible. Continue cutting along the underbelly of the fish, moving from the head to the tail.

10.3.4.4.3 Go back to the cut made at the beginning at the operculum, and slice down the entire length of the fish following along the backbone until reaching the cut previously made across the caudal peduncle.

Gently slide the stainless knife along the backbone of the fish and along the rib cage. Remove the fillet from the fish. Be sure to include the belly flap in each fillet and do not remove the dark muscle tissue in the vicinity of the lateral line from the light muscle tissue that makes up the rest of the muscle tissue mass.

10.3.4.4.4 Remove any bones that may be left attached to the fillet. Repeat the fillet steps 10.3.4.4.1 through 10.3.4.4.3, for the opposite side of the specimen.

10.3.4.4.5 Note in the sample processing records if the internal organs were ruptured during freezing or if inadvertent puncture of the internal organs occurred during the filleting process. If the internal organs did rupture or were punctured, notify project manager for further guidance.

10.3.4.4.6 Place a glass plate on the balance. Tare the balance and record the appropriate weights in the appropriate spreadsheet or logbook as determined from the project specific QAPP. This may include weighing the fillet(s), carcass or skin.

10.3.4.4.7 If the fillet(s) and/or the carcass are to be homogenized immediately, proceed to Section 10.3.4.5. If not, store in the appropriate container; see Section 4 for allowable materials. Note that it may be necessary to chop the fillet(s) or carcass into smaller pieces, with the appropriately cleaned knife, before storage, and before homogenization, so the entire sample will fit into the storage container or the homogenization vessel. See the project specific QAPP for additional details.

10.3.4.4.8 If the samples will not be homogenized immediately, the samples must be returned to the Sample Management office and placed back into the freezer, until homogenization

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10.3.4.5 Homogenization

10.3.4.5.1 Allow the fillet(s), carcass or whole body to partially thaw if previously frozen.

10.3.4.5.2 Fillets/Skin/Whole Body: Weigh a glass jar on the balance and record the weight. Tare the same glass jar. Be sure the jar is large enough to allow headspace for freezing after sample homogenization. While wearing the appropriate gloves, place the sample on the cutting board. Using the appropriate knife, slice and cut the sample into small chucks, preferably 1” squares or less. Add the sample to the appropriate size glass container for homogenization. Record the pre homogenization weight or follow project specific QAPP. Immerse the sample into the pre-cleaned generator probe (see section 10.1). Homogenize the sample until it appears fully and consistently homogenized tuning into a fine paste. This procedure may require mixing the sample during the homogenization process with a stainless steel spatula, ensuring all sample is equally processed and no sample remains on the side of the jar.

10.3.4.5.3 Large Whole Body/Carcass: Large sample carcasses may need to be homogenized using a hand held grinder/ electric grinder or food processor. Add the pre-sliced sample to the pre-cleaned blender (see section 10.1) and “push” through the auger part of the grinder. Collect the sample into a pre-tared jar or glass plate. Further processing using additional equipment may be necessary to achieve a consistently homogenized sample.

10.3.4.5.4 After homogenization, remove as much sample from the processing equipment as possible using a stainless steel spatula or other utensil and add to the processed sample. Re-weigh the sample and record the post-homogenization weight. Individual homogenates may be processed further to prepare composite homogenates as required by project specifications. All individual weights that make up one composite must be recorded, if required, or one composite weight may be recorded. If individual or composite homogenates were frozen prior to extraction/digestion, these homogenates must be thawed and re-homogenized by hand mixing prior to being extracted or digested.

10.3.4.5.5 Place the individual or composite homogenized samples into the appropriate glass jars to be frozen pending future extraction/digestion. If the samples will not be extracted/digested immediately, the samples must be returned to the Sample Management office and placed back into the freezer, until extraction/digestion. All freezer logbooks must be filled out for hold time tracking purposes. Note the return of the samples to Sample Management must be documented in the LIMS Tracking log.

10.3.4.5.6 All utensils and equipment must be washed in between samples according to the procedures described previously in Section 10.1.

10.3.5 Mollusk Preparation

10.3.5.1 Wash all utensils, the cutting board, and surfaces as previously described in Section 10.1. Note the allowable materials in Section 4. Obtain samples from the Sample Management office and log them out of the freezer logbooks for hold time tracking purposes. Note removal of samples in the LIMS Tracking log.

10.3.5.2 If required by the project specifications, measure and record the length of the sample shell.

10.3.5.3 Cover the balance with the proper material as described in Section 4, and weigh and record the sample weight.

10.3.5.4 Wearing the proper gloves, place the sample on the cleaned cutting board. Samples should be partially thawed. If the sample is frozen, it will be difficult to break open the shell. If the sample is excessively thawed, the internal tissue will become soupy and difficult to remove.

10.3.5.5 If preparing *Bivalve* specimens, use the titanium knife to cut the abductor muscle by sliding the knife through the crevice where the two shells meet. Once the abductor muscle is cut the two shell pieces should come apart easily.

10.3.5.6 Carefully remove the top shell, and scoop out the internal tissue that is resting on the mantle. Be careful not to tip the bottom shell. If the sample is excessively thawed, the sample internal fluids may spill out of the shell. The internal fluids must be retained as part of the sample. If the bivalve is

still partially frozen as suggested, the tissue should easily be removed from the shell in one piece.

10.3.5.7 Cover the balance with the proper material and weigh the amount of tissue obtained from the sample. Record the weight along with the information previously recorded on the processing records. The sample may now be stored pending homogenization in the appropriate jar, see Section 10.3.5.17. If the sample will be homogenized immediately, proceed to 10.3.5.13.

10.3.5.8 If preparing *Gastropod* specimens, a mallet will be necessary to open the shell.

10.3.5.9 Place a paper towel or piece of lab mat over the shell of the Gastropod specimen

10.3.5.10 Holding the shell still with one hand, use the mallet to hit the paper towel that is over the shell, in order to crush the shell.

10.3.5.11 Using the appropriately cleaned tweezers, remove the tissue from the crushed shell pieces.

10.3.5.12 Cover the balance with the proper material and weigh the amount of tissue obtained from the sample. Record the weight along with the information previously recorded on the processing records. The sample may now be stored pending homogenization in the appropriate jar, see Section 10.3.5.17. If the sample will be homogenized immediately, proceed to 10.3.5.13.

10.3.5.13 Since the amount of tissue obtained from one bivalve or gastropod is generally small, several specimens are frequently combined to make one sample. Utensils do not need to be rinsed between the individual samples that comprise one composite, but utensils must always be rinsed in between each composite sample.

10.3.5.14 If several specimens will be composited to make one sample, follow the applicable Sections of 10.3.5.1 through 10.3.5.11, for each of the specimens. The tissue obtained from each specimen may be weighed and recorded individually, then totaled for the composite weight. If only one composite weight is sufficient for the project specifications, weigh the entire composite and record that weight.

10.3.5.15 After the tissue has been removed from all of the specimen shells for one composite or individual sample, place the tissue in the clean small processor with the titanium blade to be homogenized. Grind the sample until it appears to be fully and consistently homogenized and there are no large chunks.

10.3.5.16 Individual homogenates may be processed further to prepare composite homogenates as required by project specifications. Composite homogenates must be prepared from equal weights of individual homogenates. All individual weights that make up one composite must be recorded, if required, or one composite weight may be recorded. If individual or composite homogenates were frozen prior to

extraction/digestion, these homogenates must be thawed and re-homogenized by hand mixing prior to being extracted or digested.

10.3.5.17 Place the processed samples into the appropriate glass jars to be frozen for future extraction/digestion, see Section 4. If the samples will not be extracted/digested immediately, the samples must be returned to the Sample Management office and placed back into the freezer, until extraction/digestion. Record placement of the samples in the freezer, in the freezer storage logbook, for hold time tracking. Note return of the samples to Sample Management in the LIMS Tracking log.

10.3.5.18 All utensils and equipment must be washed in between samples according to the procedures described previously in Section 10.1.

10.3.6 Crustacean Preparation

10.3.6.1 Lobsters

10.3.6.1.1 Wash all utensils, the cutting board, and surfaces as previously described in Section 10.1. Note the allowable materials in Section 4. Obtain samples from the Sample Management office and log them out of the freezer logbooks for hold time tracking purposes. Note removal of samples in the LIMS Tracking log.

10.3.6.1.2 If project specifications require gender determination of lobsters, this must be done prior to dissecting. To determine the gender, hold the lobster by the thorax, and flip it over to examine the underneath abdomen. Just below the legs and where the abdomen division begins, there is a first pair of swimmerets. The first pair of swimmerets is what is used to distinguish the lobster's gender. If the first pair is soft, has small hairs, and the swimmerets are crossed, it is **female**. On a **male** lobster, the first pair of swimmerets is hard and stiff, and generally do not touch.

10.3.6.1.3 If the hepatopancreas of the lobster samples is to be analyzed, the samples should be received alive. If the samples are frozen prior to dissection the hepatopancreas could burst upon thawing making it difficult to remove. To remove the hepatopancreas, the live lobster should be placed on a cleaned cutting board. Wearing the proper gloves, one analyst holds the two chelipeds (claws) out in front of the lobster, while also holding down the lower abdomen and telson (tail). The second analyst takes a knife, and places it on the grove in the carapace (outer shell), just behind the head region. Keeping the knife at an angle, the second analyst must push down and forward to remove the head. Once the head is removed the hepatopancreas can be seen lying just under the carapace and running the length of the thorax. The hepatopancreas is generally a greenish-yellow color, but there may be some variation. Scoop the hepatopancreas out gently trying not to break it into pieces. Cover the tray of the balance with the proper material, and weigh and record the weight of the hepatopancreas on the processing record, and place it into an appropriate sample jar for freezing and future extraction/ digestion.

10.3.6.1.4 To remove the edible meat, remove the two chelipeds from the body of the lobster at the joint. Place a piece of lab mat or paper towel over the cheliped and pound with a mallot. Once the shell is crushed remove the meat, using the appropriately cleaned tweezers or other tool, making sure to get all the meat in the joints and arms. Cover the balance tray with the appropriate material and weigh and record the total tissue weight obtained from the two chelipeds and arms. Record this weight with the previously recorded information on the sample processing record.

10.3.6.1.5 Remove the abdomen and telson from the rest of the carapace by pulling the lobster apart. Using the titanium coated knife, cut through the center underside tissue of the lobster and laterally along the exoskeleton of the tail. Once the abdomen and tail have been cut open, separate the shell from the edible meat using cleaned utensils. Any eggs found in the female lobsters will have to be removed and discarded. Cover the balance tray with the appropriate material, and record the weight of the tissue obtained from the abdomen and telson on the processing record. The sample may now be stored pending homogenization in the appropriate jar.

10.3.6.2 Crabs

10.3.6.2.1 If removing tissue from *crabs* break off all legs and claws. Squeeze, pull, cut or pick all the tissue out of the legs and chelipeds. Pull apart the carapace. The carapace should be easy to remove by pulling up on the holes left from when the legs were broken off. Scoop out the tissue. Cover the balance tray with the appropriate material and record the weight of the tissue obtained from the legs, claws, and carapace on the processing record. The sample may now be stored pending homogenization in the appropriate jar, see Section 10.3.6.7. Any eggs found in the female crabs will have to be removed and discarded.

10.3.6.2.2 If the hepatopancreas of the crab samples is to be analyzed, the samples should be received alive. If the samples are frozen prior to dissection the hepatopancreas could burst upon thawing, making it difficult to remove. In order to remove the hepatopancreas of a frozen crab, remove the legs and claws, and then the top shell can be removed by cutting along the outside edge of the top shell. The top shell can then be removed. It is best if the crab(s) are chilled live in a refrigerator for 30-60 minutes, prior to removal of the hepatopancreas, to slow the crab's movements. To remove the hepatopancreas, the live crab should be placed on a cleaned cutting board. Wearing the proper gloves, the analyst must hold the crab still, with the claws facing away from the analyst. Then grab the back of the top shell with fingers or cleaned pliers, and pull the back shell from the crab. Once the back shell is removed the hepatopancreas can be seen lying inside the body cavity. The hepatopancreas is generally a greenish-yellow color, but there may be some variation. Scoop the hepatopancreas out gently trying not to break it into pieces. Cover the tray of the balance with the proper material, and weigh and record the weight of the hepatopancreas on the processing record. Place it into an appropriate sample jar for freezing and future extraction/ digestion.

10.3.6.3 Since the amount of tissue obtained from one crustacean may be small, several specimens may be combined to make one sample. Utensils do not need to be rinsed between the individual samples that comprise one composite, but utensils must always be cleaned and rinsed in between each composite sample.

10.3.6.4 If several specimens will be composited to make one sample, follow the applicable Sections of 10.3.6.1.1 through 10.3.6.1.5 for lobsters, or 10.3.6.2.1 through 10.3.6.2.2 for crabs for each of the specimens. The tissue obtained from each specimen may be weighed and recorded individually, then totaled for the composite weight. If only one composite weight is sufficient for the project specifications, weigh the entire composite and record that weight.

10.3.6.5 After the tissue has been removed from all of the specimen shells for one composite or individual sample, grind the sample until it appears to be fully and consistently homogenized and there are no large chunks. This procedure may require mixing the sample during the homogenization process with a stainless steel spatula, ensuring all sample is equally processed and no sample remains on the side of the jar.

10.3.6.6 Individual homogenates may be processed further to prepare composite homogenates as required by project specifications. All individual weights that make up one composite must be recorded, if required, or one composite weight may be recorded. If individual or composite homogenates were frozen prior to extraction/digestion, these homogenates must be thawed and re-homogenized by hand mixing prior to being extracted or digested.

10.3.6.7 Place the processed samples into the appropriate glass jars to be frozen for future extraction/digestion, see Section 4 for allowable materials. If the samples will not be extracted/digested immediately, the samples must be returned to the Sample Management office and placed back into the freezer, until extraction/digestion. Record placement of the samples in the freezer, in the LIMS, for hold time tracking. Note return of the samples to Sample Management in the LIMS Tracking log.

10.3.6.8 All utensils and equipment must be washed in between samples according to the procedures described previously in Section 10.1. If any processing equipment comes in contact with a crab that is not going to be included in the composite, the equipment must be washed as described in section 10.1 before continuing.

10.3.7 Mammals (Mice and Shrew)

10.3.7.1 Wash all utensils, the cutting board, and surfaces as previously described in Section 10.1. Note the allowable materials in Section 4. Obtain samples from the Sample Management office and log them out of the freezer logbooks for hold time tracking purposes.

10.3.7.2 Place the first five, partially thawed samples to be processed, and all equipment needed into the glove box on a freshly laid out lab mat. Equipment needed includes:

- Empty and pre-labeled glass sample containers for the processed homogenate,
- PVC gloves or Latex gloves,
- 10% Bleach solution, 25% HNO3 and methylene chloride, methanol and hexane in squirt bottles,
- Omni grinding unit,
- Balance,
- Nylon bristled brushes,
- Ceramic, titanium,or stainless steel (organic compounds only) knives, spatulas and/or other utensils,
- Cutting board (s),
- DI water in a squirt bottle and Kim wipes,
- Laboratory waste bottles with caps.

10.3.7.3 Once all materials are in the glove box and set up for use, seal the transfer box and ensure the motor blower is on. Over tightening of the outer or inner door knobs is not necessary to achieve a good seal. Place your hands into the gloves attached to the glove ports and place PVC or Latex gloves over the glove port gloves for use. The outer PVC or Latex gloves will need to be changed in between each sample.

10.3.7.4 If the gender of the mouse or shrew needs to be determined, turn the animal over and note the length of the anus and the distance of the anus from the tail. If the anus is elongated in shape and does not touch the base of the tail, testicles and a large genital papilla are visible, and there are no nipples, the animal is **male**. If the anus is round in shape and almost touches the base of the tail and/or there are nipples (up to five sets), the animal is **female**. If the animal is very small, young or immature and a gender determination cannot be made, note that the gender is *un-determined*. Record the gender observations on the processing records.

10.3.7.5 If skinning of the mammal is required, carefully make an incision at the tail end and cut just below the skin along the back, from one hind leg to the other. Make another cut from one hind leg to one front leg, and repeat the cut on the other side of the animal. Starting from the tail, lift the skin flap, and carefully separate the skin from the muscle tissue below. Pull the skin forward from the tail to the head to expose the back tissue of the animal. Repeat the procedure on the stomach side of the animal. Note: it may be very difficult to remove the skin from the legs, head and the tail. If some skin cannot be removed, note this on the processing records.

10.3.7.6 Weigh and record the weight of the mammal on the processing records. Depending upon the size of the mammal, it may need to be chopped into small pieces before being ground. Generally, mice and shrew can be quartered before homogenization if needed.

10.3.7.7 Put the whole body or chopped sample into the cup of the grinding unit. Ensure the sample is in contact with the blades of the unit and place a bag over the entire grinding unit to help contain and minimize splatter on the walls of the glove box.

10.3.7.8 Turn the grinding unit on low speed and gradually increase the speed to homogenize the sample being careful to minimize any splatter or outside contamination. Homogenize until a uniform consistency is achieved.

10.3.7.9 Transfer the homogenized sample from the cup to the pre-labeled sample jar using the appropriate utensil. Carefully clean the threads of the sample jar with a DI water-soaked Kim wipe. Clean the outside of the sample jar with a 10% bleach-soaked Kim wipe. Set the sample jar inside the transfer box and close the transfer box inner door.

10.3.7.10 To clean the grinding unit in between samples, remove as much residual tissue on the blade as possible by operating the unit at low or medium speed with DI water in the sample cup. Keep a bag over the grinding unit as the primary containment for splashing. If necessary, use the nylon brush to gently scrub the exposed surfaces and to dislodge remaining tissue. Repeat as necessary, until the unit appears clean. Any plastic or ceramic parts must now be given a final rinse with 25% HNO3 then DI water when processing samples for metals analysis. If processing for organic compounds only, rinse with DI water, acetone and then the methylene chloride.

10.3.7.11 Repeat steps 10.3.2.3 through 10.3.7.10 until the five samples have been processed and each placed into the transfer box. Ensure the outer Latex or PVC gloves are changed in between each sample.

10.3.7.12 Since the amount of tissue obtained from one mouse or shrew may be small, several specimens may be combined to make one sample, as required by project specifications. Utensils do not need to be rinsed between the individual samples that comprise one composite, but utensils must always be cleaned in between each composite sample.

10.3.7.13 If several specimens will be composited to make one sample, follow the applicable Sections of 10.3.7.3 through 10.3.7.10, for each of the specimens. The tissue obtained from each specimen may be weighed and recorded individually, then totaled for the composite weight. If only one composite weight is sufficient for the project specifications, weigh the entire composite and record that weight.

10.3.7.14 Remove the individual or composite sample jars from the transfer box from the outside of the glove box, and return them to the Sample Management office for storage in the freezers until extraction/digestion. At the same time, obtain the next five samples to be processed and homogenized from the Sample Management office freezers. Movement of samples into and out of freezer storage must be documented in the freezer logbooks and in the LIMS Tracking log.

10.3.7.15 Allow the samples to partially thaw and begin again at 10.3.7.3 through 10.3.7.14 until all samples have been processed and homogenized. Clean the outer surfaces of the homogenate sample jars as described in

10.3.7.10, and remove them from the transfer box. If the samples will not be extracted/digested immediately, the samples must be returned to the Sample Management office and placed back into the freezer, until extraction/digestion. Record placement of the samples in the freezer, in the freezer storage logbook, for hold time tracking. Note return of the samples to Sample Management in the LIMS Tracking log.

10.3.7.16 Before removing any equipment from the glove box, the following disinfection steps must be taken:

- Remove the primary containment bag. Take care not to invert the bag. Place this bag into another bag.
- After the grinding unit, cup and blades have been cleaned with DI water as in 10.3.7.10, rinse the entire unit with the 10% bleach solution. Collect the bleach in a waste bottle.
- Remove the bags that were twist tie secured to the grinding unit, and place them into another bag. Rinse the entire unit again with the bleach solution.
- Roll up the bench liner, and place this into a bag.
- Pour all waste solutions into capped waste bottles. Place these bottles and any other bleach cleaned utensils, into bags, and seal all bags.
- Wipe the inside surfaces of the glove box with Kim wipes soaked in the bleach solution.
- The glove box transfer doors may now be opened to remove the grinding unit and waste. The waste material may be discarded after adding 10% bleach. The utensils and the grinding unit may be re-washed according to the normal cleaning procedures.

10.3.7.17 Individual homogenates may be processed further to prepare composite homogenates as required by project specifications. Composite homogenates must be prepared from equal weights of individual homogenates. All individual weights that make up one composite must be recorded, if required, or one composite weight may be recorded. If individual or composite homogenates were frozen prior to extraction/digestion, these homogenates must be thawed and re-homogenized by hand mixing prior to being extracted or digested.

10.3.7.18 After individual homogenates have been combined to form the final sample composite homogenate, as requested, all utensils and equipment must be washed, in between samples, according to the procedures described previously in Section 10.1.

10.3.7.19 If the final sample composite homogenates will not be extracted/digested immediately, the samples must be returned to the Sample Management office and placed back into the freezer, until extraction/digestion. Record placement of the samples in the freezer, in the LIMS, for hold time tracking. Note return of the samples to Sample Management in the LIMS Tracking log.

10.3.8 Reptiles and Amphibians (Frogs and Turtles)

10.3.8.1 Wash all utensils, the cutting board, and surfaces as previously described in Section 10.1. Note the allowable materials in Section 4. Obtain samples from the Sample Management office and log them out of the freezer logbooks for hold time tracking purposes. Note removal of samples in the LIMS Tracking log.

10.3.8.2 Wearing the proper gloves, place the *turtle* sample on the cleaned cutting board. The turtle should be partially thawed. If the turtle is frozen, it will be difficult to remove the muscle. If the sample is excessively thawed, the internal tissue will become soupy and difficult to remove.

10.3.8.3 Take all project required measurements. The distance between the anterior and posterior edge of a turtle carapace (top of shell) should be measured with a ruler and recorded on the processing records. If the entire mass of the turtle, including the shell, needs to be recorded, cover the balance with the proper material and weigh and record this weight on the processing records.

10.3.8.4 Since the plastron (bottom of shell) and carapace are extremely dense and difficult to cut through with normal dissecting tools, the muscle tissue of the turtle must be removed by cutting the body of the turtle away from the shell. Insert a knife, made of the proper material, into the skin of the turtle, close to the shell on the lower half of the body. Slowly, cut along the entire circumference of the shell. Repeat the procedure on the upper half of the body, on both sides of the shell.

10.3.8.5 With dissection scissors, or a ceramic or titanium paring knife of the proper material, remove the skin from the hind limbs, tail, fore limbs and neck.

10.3.8.6 Using the appropriate utensils, remove the muscle tissue from the tail, neck, hind limbs, and fore limbs, including the feet, leaving bone and claws behind. Remove any visible muscle tissue within the carapace. Most of this tissue will be found in the upper portion of the carapace around the pectoral area.

10.3.8.7 Cover the balance with the proper material and weigh the amount of tissue obtained from the turtle sample. Record the weight along with the information previously recorded on the processing records. The sample may now be stored pending homogenization in the appropriate jar, see Section10.3.8.15. If the sample will be homogenized immediately, proceed to 10.3.8.13.

10.3.8.8 If processing *frogs*, allow the frog to partially thaw, take the project specific measurements, and record them on the processing records. The number of frogs required to make up one sample, and the weight and length of the individual frogs, must be taken and recorded, if specified. In all cases, the skin must be removed from the frog prior to processing and chopped into smaller pieces, due to its thickness. It will then be added to the processor with the whole body of the frog, or it may be discarded depending upon the project specifications.

10.3.8.9 To skin the frog, make an incision, using the proper utensils, and cut into an area where there is an excess of skin, most likely around the neck. Slowly, pull the skin off of the frog using dissecting scissors, or a ceramic or titanium paring knife, as needed. Once skin is removed chop it up into tiny pieces using the appropriate knife and set it aside to be processed with the whole frog body.

10.3.8.10 Cover the balance with the proper material and weigh the amount of tissue obtained from the frog sample, if the tissue and not the whole body will be processed. Record the weight along with the information previously recorded on the processing records. The sample may now be stored pending homogenization in the appropriate jar, see Section 10.3.8.15. If the sample will be homogenized immediately, proceed to 10.3.8.13.

10.3.8.11 Since the amount of tissue obtained from one small turtle or frog may be insignificant, several specimens may be combined to make up one sample. Utensils do not need to be rinsed between the individual samples that comprise one composite, but utensils must always be rinsed in between each composite sample.

10.3.8.12 If several specimens will be composited to make up one sample, follow the applicable Sections of 10.3.8.1 through 10.3.8.10, for each of the specimens. The tissue obtained from each specimen may be weighed and recorded individually, then totaled for the composite weight. If only the composite weight is sufficient for the project specifications, weight the entire composite and record that weight.

10.3.8.13 After the tissue has been removed from all of the specimens, homogenize the muscle tissue, and skin if required, by placing it into the small or large food processor fitted with the appropriate blades (stainless steel for the large processor and titanium for the small processor). See Section 4 for allowable materials. The sample may need to be cut into smaller pieces for processing. Grind the sample until it appears to be fully and consistently homogenous. Continue to grind the sample until there are no chunks present in the homogenate.

10.3.8.14 Individual homogenates may be processed further to prepare composite homogenates as required by project specifications. Composite homogenates must be prepared from equal weights of individual homogenates. All individual weights that make up one composite must be recorded, if required, or one composite weight may be recorded. If individual or composite homogenates were frozen prior to extraction/digestion, these homogenates must be thawed and re-homogenized by hand mixing prior to being extracted or digested.

10.3.8.15 Individual or composite samples may be returned to the Sample Management office for further storage in freezers pending extraction/digestion. All processed samples are stored in the proper containers noted in Section 4. All freezer logbooks must be filled out for hold time tracking purposes. Return of samples to Sample Management must be documented in the LIMS Tracking log.

10.3.8.16 All utensils and equipment must be washed in between samples according to the procedures described previously in Section 10.1.

10.3.9 Macro Invertebrates

10.3.9.1 Wash all utensils, the cutting board, and surfaces as previously described in Section 10.1. Note the allowable materials in Section 4. Obtain samples from the Sample Management office and log them out of the freezer logbooks for hold time tracking purposes. Note removal of samples in the LIMS Tracking log.

10.3.9.2 Cover the balance tray with the appropriate material and record the weight of the invertebrate sample. Since the weight obtained from one invertebrate (benthic worms, insects or biota) may be small, several invertebrates may be combined to make one sample. In many cases, several invertebrates of the same species and sample location are delivered to the laboratory in one sample jar. Each specimen from this jar must be weighed, if requested, and composited to form one homogenized and unique sample. If only one composite weight is sufficient for the project specifications, weigh the entire composite and record that weight. Utensils do not need to be rinsed between the individual samples or specimens that comprise one composite, but utensils must always be rinsed in between each composite sample.

10.3.9.3 Invertebrates such as *eels* must be chopped into smaller pieces before homogenization. This is generally due to the length of the specimen and the thickness of the skin.

10.3.9.3.1 For project specifications requiring eel specimens to be skinned prior to homogenization, first secure eel to cutting board using a stainless steel screw. Using a stainless steel knife, cut the skin behind the operculum (gill cover). Using the knife blade, pliers or other cleaned utensil, pull the skin off towards the tail. If necessary, cut lightly along the inside of the skin, slowly separating the skin from the muscle tissue. Removing the skin may require cutting the skin along the backbone or underbelly. If necessary follow project specifications for weight determinations.

10.3.9.4 Place the weighed specimen into the clean small processor with the titanium blade to be homogenized. Process the sample until it appears to be fully and consistently homogenized and there are no large chunks.

10.3.9.5 Individual homogenates may be processed further to prepare composite homogenates as required by project specifications. Composite homogenates must be prepared from equal weights of individual homogenates. All individual weights that make up one composite must be recorded, if required, or one composite weight may be recorded. If individual or composite homogenates were frozen prior to extraction/digestion, these homogenates must be thawed and re-homogenized by hand mixing prior to being extracted or digested.

10.3.9.6 Individual or composite samples may be returned to the Sample Management office for further storage in freezers pending

extraction/digestion. All homogenates are stored in the proper containers noted in Section 4. All freezer logbooks must be filled out for hold time tracking purposes. Return of samples to Sample Management must be documented in the LIMS Tracking log.

10.3.9.7 All utensils and equipment must be washed in between samples according to the procedures described previously in Section 10.1.

10.3.10 Plants

10.3.10.1 Wash all utensils, the cutting board, and surfaces as previously described in Section 10.1. Note the allowable materials in Section 4. Obtain samples from the Sample Management office and log them out of the freezer logbooks for hold time tracking purposes. Note removal of samples in the LIMS Tracking log.

10.3.10.2 Wearing the appropriate gloves, plants must be rinsed with DI water to remove soil, silt, small insects, and other debris. Place the plants in a stainless steel or plastic strainer, depending on the determinative sample analysis, and rinse thoroughly with DI water. If analyzing the sample for metals and organic compounds, rinse the plants carefully over a sink, being sure not to touch the sides of the sink with the plant sample.

10.3.10.3 Depending on the size and texture of the plants, some may be homogenized in the small food processor with the titanium blade. Samples such as long grass will have to be chopped into small pieces (approximately 1/2 inch) using titanium or ceramic knives. Leaves can generally be homogenized in the small food processor without pre-cutting.

10.3.10.4 Some project specifications may require the plants to be dried prior to homogenization. A plastic salad spinner may be used to remove excess water from samples, if organic compounds do not need to be determined. If both metals and organic compounds need to be determined, air drying for 48 hours, or oven drying overnight at low temperatures (S 50°C), can be done. Freeze drying the plant is an additional option for the removal of water and may be employed per project specifications.

10.3.10.5 Cover the balance tray with the appropriate material and record the weight of the plant sample. Since the weight obtained from one plant may be small, several plants may be combined to make one sample. Utensils do not need to be rinsed between the individual samples that comprise one composite, but utensils must always be rinsed in between each composite sample.

10.3.10.6 If several plants will be composited to make one sample, follow the applicable Sections of 10.3.10.2 through 10.3.10.5, for each of the specimens. The weight of each specimen may be recorded individually, and then totaled for the composite weight. If only one composite weight is sufficient for the project specifications, weigh the entire composite and record that weight on the processing records.

10.3.10.7 After the plant weight for one composite or individual sample has been recorded, place the plant(s) in the clean small processor with the titanium

blade to be homogenized, or place them onto the cleaned cutting board to be chopped. Grind or chop the plants until they appear to be fully homogenized.

10.3.10.8 Individual homogenates may be processed further to prepare composite homogenates as required by project specifications. Composite homogenates must be prepared from equal weights of individual homogenates. All individual weights that make up one composite must be recorded, if required, or one composite weight may be recorded. If individual or composite homogenates were frozen prior to extraction/digestion, these homogenates must be thawed and re-homogenized by hand mixing prior to being extracted or digested.

10.3.10.9 Place the homogenized plants into the appropriate glass jars to be frozen for future extraction/digestion, see Section 4. If the samples will not be extracted/digested immediately, the samples must be returned to the Sample Management office and placed back into the freezer, until extraction/digestion. Record placement of the samples in the freezer, in the freezer storage logbook, for hold time tracking. Return of samples to Sample Management must be documented in the LIMS Tracking log.

10.3.10.10 All utensils and equipment must be washed in between samples according to the procedures described previously in Section 10.1.

10.4 Continuing Calibration

Not applicable.

10.5 Preventive Maintenance

Not applicable to this method.

11. Data Evaluation, Calculations and Reporting

The processing bench sheets and other relevant laboratory notebooks must follow the specifications in the Alpha Analytical *Logbook Usage Work Instructions* (WI 108-01), and all record keeping and document control practices. Separate project-specific documents may be used in place of Alpha bench sheets, as necessary.

See the appropriate Alpha Analytical SOPs noted in Section 1, for details on sample analysis, data evaluation, calculations and data reporting.

All results for the organic/inorganic compounds of interests are reportable without qualification if extraction/digestion and analytical holding times are met, preservation (including cooler and freezer temperatures) are met, all QC criteria defined in the table below are met, and matrix interference is not suspected during extraction/digestion and/or analysis of the samples. If any of the below QC parameters are not met, all associated samples must be evaluated for re-extraction and/or re-analysis.

QC Parameter	Acceptance Criteria
Equipment/Processing Blank	< reporting limit
Method Blank	< reporting limit
Laboratory Control Sample	See the applicable ALPHA analytical SOP for acceptance criteria
Matrix Duplicate	See the applicable ALPHA analytical SOP for acceptance criteria
Matrix Spike	See the applicable ALPHA analytical SOP for acceptance criteria
Matrix Spike Duplicate	See the applicable ALPHA analytical SOP for acceptance criteria
Surrogate Recoveries	See the applicable ALPHA analytical SOP for acceptance criteria
Standard Reference Material	See the applicable ALPHA analytical SOP for acceptance criteria

12. Contingencies for Handling Out-of-Control Data or Unacceptable Data

Section 9, Quality Control, defines the preparation and/or analytical corrective actions that must be taken in instances where QC outliers exist.

Section 11 outlines sample batch QC acceptance criteria. If non-compliant organic or inorganic compound analytical results are to be reported, the Department Manager and/or the Laboratory Director, and the QA Manager must approve the reporting of these results. The laboratory Project Manager shall be notified, and may chose to relay the non-compliance to the client, for approval, or other corrective action, such as re-sampling and re-analysis. The analyst or Department Manager performing the secondary review initiates the project narrative, and the narrative must clearly document the non-compliance and provide a reason for acceptance of these results.

13. Method Performance

13.1 Method Detection Limit Study (MDL) / Limit of Detection Study (LOD) / Limit of Quantitation (LOQ) – Not Applicable

Not Applicable

13.2 Demonstration of Capability Studies

Not Applicable

14. Pollution Prevention and Waste Management

Refer to Alpha's Chemical Hygiene Plan and Hazardous Waste and Sample Disposal SOP for further pollution prevention and waste management information.

Once satisfactory organic or inorganic compound results have been generated, the extracts/digestates are held for 30 days, or longer, if specified by a client contract. Then, organic

extracts are discarded into a 55-gallon drum labeled "Vial Waste" and inorganic digestates are poured into a 55-gallon drum marked “Acid/Non-chlorinated" waste.

All solvent or reagent waste generated during processing and/or extraction/digestion must be stored in satellite containers in the preparation laboratories labeled "Organic Solvent", "Acid/Non-chlorinated" or "Bleach".

Once the organic solvent satellite containers are full, they must be emptied into 55-gallon drums marked "Organic Solvent Waste". Cleanup waste from the HPLC fractionators (silica cleanup) or GPC is emptied into the 55-gallon drum marked "HPLC Solvent Waste". Bleach from disinfection is emptied into the 20-gallon drum marked "Bleach", and reagent waste generated during metals analysis is emptied into a 55-gallon drum marked “Acid/Non-chlorinated" waste.

15. Referenced Documents

Chemical Hygiene Plan

SOP/08-05 MDL/LOD/LOQ Generation

SOP/08-12 IDC/DOC Generation

SOP/G-006 Hazardous Waste and Sample Disposal

16. Attachments

None.