TISSUE HOMOGENIZATION PROCEDURE

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TISSUE HOMOGENIZATION PROCEDURE

1.0 SCOPE AND APPLICATION

This standard operating procedure (SOP) is applicable for the homogenization of fish tissues, earthworms, amphibians, small mammals, and other small biota. It can be adapted for muskrats, mink, and other larger biota. In addition, the procedure may be used for the homogenization of plant tissue samples.

2.0 METHOD SUMMARY

Frozen tissue is homogenized with dry ice using a variable speed laboratory blender. After homogenization is complete, the whole contents of the blender (tissue and dry ice) are transferred to clean jars and the dry ice is allowed to sublime in a freezer below -10 degrees Centigrade (^{o}C). Homogenization of larger animal mass is carried out in several steps.

3.0 SAMPLE PRESERVATION, CONTAINERS, HANDLING, AND STORAGE

3.1 Sample Storage and Handling

- Tissue samples (or specimens) must be protected from light and kept frozen below -10^{0}C from the time of collection until the homogenization process.
- Tissue samples and sample homogenates in glass jars must be stored in a freezer free of all potential contaminants.
- Before and after tissue sample homogenization, tissue samples must be protected from light and kept frozen below -10^{o}C for the periods specified by the SERAS Task Leader and/or the Work Assignment Manager (WAM).
- Tissue samples, homogenates, and sample extracts must be stored separately from standards.

3.2 Holding Times

Homogenization of tissue samples received at 4^{o} ± 2^{o}C or above shall be completed within seven (7) days of sampling. Tissue samples received at -10^{o}C do not have any holding time requirements until thawed for processing.

4.0 INTERFERENCES AND POTENTIAL PROBLEMS

Method interferences may be caused by contaminants in solvents, reagents, glassware and sample processing hardware that lead to discrete artifacts and/or elevated baselines in the analytical method used for analysis. All of these materials must be demonstrated to be free from interferences under the conditions of homogenization and/or analysis by analyzing laboratory reagent blanks on a routine basis. Matrix interferences may also be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source.

5.0 EQUIPMENT/APPARATUS

The following equipment/apparatus are required:

- Variable speed laboratory blenders
- Blending containers - stainless steel (SS) with SS lids of various sizes [40, 100, 250, and 500 milliliters (mL)] depending on sample size
- Univex grinder
- Stainless steel and/or Teflon coated forceps, spatulas, spoons, and scoops
- Stainless steel knife, cleaver, and scissors
- Teflon cutting board
- Stainless steel trays
- Freezer, capable of maintaining a temperature of less than (<) -10°C
- Balance, capable of weighing 200 grams (g) to the nearest 0.01 g
- Glass collection jars with Teflon-lined lids (variable sizes)
- Coolers
- Test tube brushes
- TurboVap II concentrator, with rack and concentrator cells (Zymark Corporation or equivalent)

6.0 REAGENTS

- Methylene chloride (DCM), pesticide residue grade or equivalent
- Hexane, pesticide residue grade or equivalent
- Type II deionized water, free of target analytes
- Solid carbon dioxide (CO_2), food grade or equivalent, pellets or "snow", referred to as "dry ice"
- Nitrogen, ultra high purity (UHP) grade or equivalent

7.0 PROCEDURES

7.1 Equipment Decontamination

7.1.1 Washing

1. All equipment must be thoroughly cleaned and dried before and after use.

2. All glassware and sample processing equipment must be washed with a soap (i.e., Contrad 70 solution or equivalent) and hot water solution.

3. All glassware and sample processing equipment must be thoroughly rinsed with tap water followed by a deionized water rinse.

4. Equipment must be oven dried at 105°C (±5°C).

7.1.2 Solvent Rinse

1. Before use, all glassware and sample processing equipment must be rinsed with methylene chloride.

2. After the methylene chloride rinse, all equipment must be air dried in a fume hood.

7.2 Carbon Dioxide/Solvent Blank Check

The CO_2 used for the homogenization process and the solvents used for decontaminating the equipment and subsequent sample extractions must be analyzed as a potential contamination source.

1. Collect a total mass of 500 g of solid CO_2 pellets or "snow" using a representative portion of each CO_2 container in a SS blending container. A separate 500-g portion should be collected for each analysis fraction [i.e., base/neutral/acid extractables (BNA), pesticide/polychlorinated biphenyls (pest/PCB), metals, and mercury].

2. Blend the CO_2 for approximately 1 minute. Then transfer the CO_2 to a clean 32-ounce (oz) glass jar.

3. Add a sufficient amount of solvent (i.e., DCM for BNA, hexane for pest/PCB) to cover the solid CO_2, and then allow the CO_2 to sublime in a fume hood. No solvent is added to any metals and/or mercury fraction.

4. After the sublimation is complete, transfer the remaining solvent to a concentration device (250-mL concentration tube).

5. Concentrate the solvent solution to a 1mL final volume and quantitatively transfer to the appropriate analysis vials (two vials containing 0.5 mL for pest/PCB and 1.0 mL for BNAs).

6. The CO_2/solvent check sample must not contain any target analytes above the reporting limit. If any contamination is detected, the source of the contamination must be investigated and corrected.

7.3 Homogenization of fish tissues, worms, small mammals, amphibians, and other small biota

1 Cut the tissue specimen into small pieces (1- to 2-inch) using a SS knife or scissors and Teflon cutting board. Massive bone structures such as frog skulls may need sectioning.

2 Select appropriate blending container based on sample size. The total sample mass should be 20-25 percent (%) of the volume of the blending container.

3 Pack area below blades with dry ice (NOTE: The blending container should be completely dry to prevent freezing of blade assembly).

4 Transfer tissue sample to blending container and cover with dry ice.

5 Allow the tissue to freeze for approximately 30 seconds.

6 Cover the SS blending container with either a SS cover or Teflon- lined lid, and blend at high speed until the tissue specimen is completely homogenized.

7 Turn off the blender, and tap the container and lid to loosen any adhered tissue on the container walls.

8 Open the blending container to determine if homogenization is complete. If homogenization is incomplete, add a small amount of dry ice and repeat Steps 6-8 above.

9 Transfer the tissue homogenate and dry ice into a glass jar and cover with a Teflon-lined lid.

10 Store the tissue homogenate sample below -10^{o}C with lid loosely attached to allow sublimation of CO_2 (usually overnight).

11 After all CO_2 has sublimed, tighten the lid on the homogenate container, and store in freezer below -10^{o}C. The tissue homogenate is ready for extraction and analysis.

7.4 Homogenization of muskrats, minks, and other larger biota

1 Section sample into 2- to 3-inch sections using a SS knife and a Teflon cutting board. Massive bone structures such as specimen skulls may also need sectioning. A SS cleaver is useful for large specimens.

2 Homogenize the tissue as in Section 7.3, Steps 2-10.

3 Combine the homogenized tissue into a large clean glass collection jar with a Teflon-lined lid. Manually mix the combined tissue homogenates with a SS spoon to ensure tissue homogeneity. Samples which require more than one collection jar should be mixed in a SS tray before storing in jars.

4 Cover the sample homogenate tightly and store in freezer below -10^{o}C. The sample is ready for extraction and analysis.

7.5 Homogenization of various plant tissue specimens

1 Section the plant tissue specimen into small sections (approximately 0.5- to 1-inch lengths, or 1 x 1 inch squares) using a SS knife or scissors.

2 Select the appropriate SS blending container based on sample size. The volume of plant tissue may be large when compared to its total mass (e.g., leaves). Use of the 1-liter (L) container may be necessary to effectively chop the sample.

3 Pack the area below the blades with dry ice. Note: The blending container should be completely dry to prevent freezing of the blade assembly.

4 Transfer the plant tissue sample to the container (up to approximately 25% of the container volume) and cover with dry ice. Large sample volumes may require adding the sample in portions to the container and homogenizing.

5 Allow the tissue to freeze for approximately 30 seconds.

6 Cap the blending container with the SS cover and blend at high speed for 1-2 minutes. Note: A Teflon -lined lid may be held over the small opening in the cover to contain any escaping CO_2 or small sample particles.

7 Vary the blending speed between intermediate and high speeds, occasionally tapping the container sides with a metal spoon or equivalent to loosen any material adhering to the side. Blending may take 2-3 minutes to completely homogenize the sample.

8 Open the container to check if the homogenization is complete. If the homogenization is incomplete, add additional dry ice, and if necessary, use a SS spoon to position any large particles between the blades. Repeat steps 6-8 above. Note: Additional portions of the same sample may be added at this time for homogenizing the sample as a whole mass.

9 When the homogenization is complete, transfer the homogenized tissue and dry ice mixture into a glass sample jar and cover loosely with a Teflon-lined lid.

10 Store the homogenate sample below -10^{o}C with the lid loosely attached to allow the CO_2 to sublime (usually overnight).

11 After all the CO_2 has sublimed, tighten the lid on the sample container and store below -10^{o}C. The tissue homogenate is ready for extraction and analysis.

8.0 CALCULATIONS

This section is not applicable to this SOP.

9.0 QUALITY ASSURANCE/QUALITY CONTROL

The following procedures will be used.

9.1 Method Interference

1 Interference may be caused by contaminants in solvents, reagents, glassware and sample processing hardware. The potential for contamination from these sources may be minimized by thoroughly cleaning all glassware and processing equipment with soap (Contrad 70 solution or equivalent) and hot water, tap water rinse, deionized water rinse, and oven drying. In addition, just before the homogenization process, the equipment should be rinsed with solvent and air-dried.

2 All solvents used in the preparation of processing equipment must be of pesticide residue analysis grade or equivalent.

9.2 Dry Ice

The quality of dry ice used for the homogenization process must not contain any target analytes above the reporting limit. Use of a commercial source of food grade quality dry ice is acceptable.

1. Preparation of a CO_2/solvent blank is required. A portion of CO_2 from each dry ice container (500 g total mass) should be mixed and homogenized with a stainless steel laboratory blender. The homogenized dry ice is then extracted with the appropriate solvent [DCM or hexane], the CO_2 sublimed and the solvent concentrated to 1 mL. The CO_2/solvent blank must be analyzed for semi-volatiles and pesticide/PCB to demonstrate its purity. No solvent is added to CO_2 blanks to be used for TAL metals or mercury analysis.

2. If the CO_2/solvent blank check exceeds the analysis method contamination limits, the source of the contamination must be investigated and corrective action taken.

10.0 DATA VALIDATION

No specific analytical data are generated by this sample preparation method, however, the quality control requirements described in Section 9.0 must be met.

11.0 HEALTH AND SAFETY

When working with potentially hazardous materials or samples, refer to U.S. EPA, Occupational Safety and Health Administration (OSHA) and corporate health and safety practices. More specifically, refer to SERAS SOP #3013, *SERAS Laboratory Safety Program*. In addition to hazardous chemical contamination, tissue samples (animal or plant) have the potential for biohazards (e.g., bacterial, viral, poisons, toxins) and should be treated accordingly. Samples should be processed in a properly operating biosafety cabinet. This is especially important when the specimens being processed have the potential to carry Hantavirus.

12.0 REFERENCES

United States Environmental Protection Agency, 1980. *Interim Methods for the Sampling and Analysis of Priority Pollutants in Sediments and Fish Tissue*, 600/4-81-055, Environmental Monitoring and Support

Laboratory.

United States Environmental Protection Agency, 1974. *Analysis of Pesticide Residues in Human and Environmental Samples, Section 2*, Health Effects Research Laboratory.

13.0 APPENDICES

This section is not applicable to this SOP.