Extraction of Organohalide Pesticides, PAHs and PCBs from Fish Tissue Using Pressurized Fluid Extraction Technique.

FLORIDA DEPARTMENT OF ENVIRONMENTAL PROTECTION

BUREAU OF LABORATORIES

2600 BLAIR STONE ROAD

TALLAHASSEE, FLORIDA 32399

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

TABLE OF CONTENTS

1 SCOPE AND APPLICATION
2 SUMMARY OF THE METHOD
3 APPARATUS AND EQUIPMENT
4 REAGENTS AND CHEMICALS
5 SAMPLE COLLECTION, PRESERVATION, AND HANDLING
6 SAMPLE HOMOGENIZATION
7 SAMPLE EXTRACTION
8 SAMPLE CONCENTRATION
9 CLEANUP PROCEDURES-PAHs
10 CLEANUP PROCEDURES-PESTCIDES AND PCBS
11. ELECTRONIC SAMPLE PREPARATION LOG
12. SAMPLE ANALYSIS PROCEDURE
13. QUALITY CONTROL
14. SAFETY/HAZARDOUS WASTE MANAGEMENT
15 REFERENCES

APPENDIX 1: TROUBLESHOOTING A GPC INSTRUMENT

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

1. SCOPE AND APPLICATION

1.1 This method describes the extraction of organochlorine, organonitrogen, organophosphorus pesticides, Polycyclic Aromatic Hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) from fish tissue.

1.2 This procedure can be used to isolate the analytes listed in the following analysis SOPs:

- Organochlorine pesticides and PCB congeners by GC/MS/MS- SOP GC-033-1 (see ref 15.13)
- Toxaphene by GC/MS/NCI - SOP GC-034-1 (see ref 15.14)
- Polycyclic Aromatic Hydrocarbons (PAHs) by GC/MS/MS- SOP SV-020-1 (see reference 15.4)

1.3 This procedure is to be used for all samples with one or more of the following Laboratory Information Management System (LIMS) test IDs: T-PCBCL-TQ, T-PCB-TQ, T-CT-CI, and T-PAH

1.4 The sample extraction, concentration and cleanup steps in this method are essentially the same as in EPA Methods 3630C (see 15.2), 3545A (see 15.3), 3640A (see 15.5), 3620C (see 15.6) and 3665A (see 15.7). Elements of NOAA procedure are incorporated into the PAHs method (ref 15.15 and 15.16).

1.5 All pesticides/PCBs tests share the same preparation procedure. The same extract can be run for different tests. PAHs extraction is completely separate.

2. SUMMARY OF THE METHOD

2.1 For pesticides/PCBs analysis five (5) grams of ground fish tissue sample is mixed with Dionex ASE prep DE Diatomaceous Earth in an ASE350 extraction cell and left at room temperature to dry. It is then extracted in ASE 350 machine for a total time of 21 minutes using two extraction cycles. The extract is concentrated to a volume of about 5 mL and filtered through a short pad of sodium sulfate and glass wool to remove water and the excess fat floating on the surface. The extract is then further concentrated and brought down to a volume of 2 ml.

2.2 For PAHs analysis five (5) grams of ground fish tissue sample is mixed with Dionex ASE prep DE Diatomaceous Earth in a ASE350 extraction cell and left at room temperature to dry. The sample is then extracted in ASE 350 machine for a total time of 21 minutes using two extraction cycles. The extract is concentrated by nitrogen blowdown and brought to a volume of 4 mL or more.

2.3 For pesticides/PCBs analysis 1mL aliquot of the extract is cleaned up with GPC followed by 1 g Florisil and submitted for analysis. 1mL aliquot of the original extract may also be cleaned up with concentrated sulfuric acid and Florisil (optional) for the PCBs and sulfuric acid-resistant chlorinated pesticides analysis.

2.4 For PAHs analysis he concentrated extract (the entire 4 mL volume) is then cleaned-up by passing through 10 g of silica and eluting with methylene chloride

3. APPARATUS AND EQUIPMENT

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

3.1 Mason jars 8, 16 and 32 ounce with screw cap. K-mart brand or any other supermarket brand

3.2 I Chem 60 mL jars with screw caps

3.3 Beakers, 400 mL

3.4 Blender, 12 Speed, Osterizer

3.5 Blade, Teflon O-ring, and screw cap assemblies for blender

3.6 Top Loading Balance, accurate to 0.1 g Adventure SL, AS153 SN 8026461189.

3.7 Round-bottom flasks, 500 mL.

3.8 S-EVAP-KD® (Organomation Associates, Inc): Consists of heated water bath (O A SYS® Heating System) and water-cooled condensers; functions as a large volume K&D concentrator, capable of concentrating eight sample extracts simultaneously.

3.9 Kuderna-Danish (K&D) concentrator tubes -- 15 mL

3.10 Ground glass stoppers to fit concentrator tubes

3.11 Kuderna-Danish 500 mL evaporative flasks; attach to concentrator tubes with clips

3.12 Snyder columns -- three ball macro or equivalent

3.13 Clips -- used to attach concentrator tube to K&D flask

3.14 Boiling chips - approximately 10/40 mesh.

3.15 Volumetric pipets, Class A - 0.50, 1.0, 2.0, 5.0, 10.0 mL.

3.16 Volumetric flasks, Class A - 10, 25, 50, 100, 200, 250, 500, 1000 mL.

3.17 Disposable Pasteur pipets.

3.18 Gas tight syringes - 50, 100, 250, 500, 1000 µL.

3.19 N-Evap®116(Organomation Associates, Inc): consists of heated water bath and apparatus to further concentrate the extract using nitrogen gas. The nitrogen should be passed through a drying filter to remove traces of water. Can concentrate 34 samples simultaneously.

3.20 Aluminum weighing pans, for dry weight determination (only when required).

3.21 50-mL culture tubes.

3.22 Shimadzu HPLC SYSTEM for GPC clean-ups, consisting of - System controller CBM-2o, LC 20ADVP pump, Autosampler SIL-20A HT, UV detector and Fraction collector Shimadzu FRC-40. The GPC acquisition is controlled by Shimadzu Lab Solutions Software.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

3.23 GPC Column: Phenomenex Phenomenex Phenogel 5um, 100A (350mmx21.2mm) with guard column 1 um, 100A (60mmx21.2mm)–

3.24 Gelman 0.45 µm syringe filters.

3.25 Sample vials - 1, 2 and 4 mL

3.26 60 mL glass columns for silica cleanup procedure.

3.27 Centrifuge.

3.28 High temperature oven - Wilt Industries Inc. All glassware used must be rinsed with tap water, DI water and acetone. Air-dry the glassware, and then bake in wire mesh basket at 460 °C for 6 hours.

3.29 Knives and cutting board

3.30 Restek Resprep FLORISIL plastic tubes 6-mL (1g) (Catalog #24034) or glass tubes 6-mL (1g) (Catalog #26085)

3.31 Saw blades.

3.32 Screw cup centrifuge tubes, 15 mL

3.33 Teflon lined screw cups for 15 mL centrifuge tubes

3.34 Oven- Fisher Scientific. ASE 350 extraction cells, silica, glasswool and diatomaceous earth are stored there at 120°C.

4. REAGENTS AND CHEMICALS

4.1 Solvents: Acetone, hexane, methylene chloride and iso-octane - HPLC grade - Fisher Scientific.

4.2 Diatomaceous Earth (Dionex) Granular, anhydrous. Purified by heating at 700 °C for 12 hours or overnight. It is then stored at 120°C in an oven.

4.3 Sodium Sulfate - (ACS) Granular, anhydrous. Purified by heating at 450 °C for at least 4 hours.

4.4 Concentrated sulfuric acid - reagent grade, Fisher Scientific.

4.5 GPC Calibration Solution (for pesticides/PCBs) - Prepare a solution in methylene chloride that contains the following compounds at the listed concentrations. See preparation details in GPC SOP GC-029-1 (see 15.8):

Analyte	mg/mL
Corn oil	25
Bis-2-ethylhexyl phthalate	1.0
Perylene	0.02
Sulfur	0.08

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

4.6 GPC Calibration Solution (for PAHs) - Prepare a solution in methylene chloride that contains the following compounds at the listed concentrations. See preparation details in GPC SOP GC-029-1 (see 14.5):

Analyte	mg/mL
Biphenyl	0.01
Perylene	0.01

5. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

5.1 Fish tissue samples are collected in glass jars with Teflon-lined screw caps. The samples are maintained at <6°C from the time of collection until receipt at the laboratory, to a maximum time of 24 hrs. If a longer transport time is necessary, the samples are to be frozen. Ideally, fish should be frozen upon collection and shipped to the laboratory under dry ice. Tissue samples are frozen at the laboratory upon receipt and maintained in the dark at less than -10°C. (Ref: 15.11, 15.12)

5.2 Samples must be extracted within 365 days of collection. The extract must be analyzed within 40 days after sample preparation.

6. SAMPLE HOMOGENIZATION

6.1 Thoroughly clean all glassware by rinsing it with hot tap water, deionized water and acetone, then bake it in an oven at 550 °C for 8 hours. Alternatively, glassware may be thoroughly washed with Alconox and hot water, then rinsed with hot tap water, deionized water, and acetone -- clean anything that cannot be baked, including fillet knives, cutting boards, and blender blade assemblies, in this way. **Pre-rinse all glassware with methylene chloride prior to use!**

Due to the ubiquitous nature of Polycyclic Aromatic Hydrocarbons (PAHs) combined with very low detection limits of the Agilent 7010 GC/MS/MS system some level of contamination is always to be expected. Constant efforts have to be made by all the technicians and analysts to keep the background contamination levels as low as possible. It is highly recommended to keep all the low-level PAH preparation work physically separated from high level PAH analysis. This especially pertains to the oil fingerprinting/total hydrocarbons analyses that deal with very high concentrations and even neat petroleum products. Furthermore, special precautions should be taken when using mechanical devices such as grinders during sample preparation. Mechanical devices use petroleum lubricants that easily contaminate tissue samples.

6.2 Check out the samples from freezer.

6.3 When homogenizing fish, wash the knife and cutting board with Alconox, hot tap water, deionized water and acetone between samples. Change gloves between samples as well. For PAHs, only use Diamond Grip latex gloves from Microflex due to possibility of cross-contamination with petroleum-based residue often found on laboratory gloves. Diamond Grip gloves showed lowest levels of contamination in laboratory tests. The gloves, once on hands, should be washed with soap and water. When using the blender, use a different blade assembly (blade, O-ring, and screw cap) for each sample.

6.4 The amount of the fish sample taken for analysis will differ based on test requirement:

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

6.4.1 If the whole fish is requested cut entire fish, including organs, into small pieces and blend them into a homogenous paste. Take a 5-g portion from each sample for preparation by ASE 350 extraction..

6.4.2 If only the fillet is to be analyzed, cut each fillet into small pieces and blend them into a paste. Take a 5-g portion from each sample for preparation by using ASE 350.

6.4.3 If specific organs are to be analyzed (e.g., gonads, etc.), remove the organs, cut them into small pieces, and then blend them into a paste. Based on the amount of available material small samples may be extracted.

6.4.4 If fat only is to be analyzed, blend the fat from each sample into a paste. Take a 0.5-1.0 g portion of each sample for preparation.

6.5 Weigh 3 aliquots of 5 g pesticides/PCBs free tissue for the Method Blank (MBLK) and two Laboratory Fortified Blanks (LFB). Select one sample and weigh out three portions instead of one -- two of these will be used as duplicate Matrix Spikes. Unless a PAHs free tissue sample can be found and procured to be used as a clean matrix, the Method Blank (MBLK) and the Laboratory Fortified Blank (LFB) are prepared using Dionex Diatomaceous Earth. If none of the samples have enough tissue to prepare duplicate matrix spikes, prepare a single matrix spike or no matrix spike. Make a note in the Preparation Log that there was not enough sample to prepare Matrix Spikes. Additionally, when T-CT-CI test is requested, weigh another 5g aliquot of the analyte free tissue matrix to prepare T-PQL spike as required by EPA-8276.

6.6 Since very few tissue samples are received per year, extract four, instead of two, LFBs and two MDL verifications with a first batch in a calendar year.

6.7 Due to very low detection limits of the analytical methods involved it is virtually impossible to find a clean pesticide free tissue matrix completely free of all the analytes of interests. DDE-p,p' is always present at some, however small level (below the formal MDL), along with, at least some, chlordane peaks. Therefore, it is recommended to additionally prepare a so called "DE Blank" that does not contain any tissue, only Diatomaceous Earth. This blank is used as a reference to confirm very small positives for the above- mentioned analytes, whenever they are also detectable in the tissue blank.

6.8 After taking a portion of each sample for preparation, put the rest of each sample paste into a labeled Mason jar. Cap the Mason jars and store them in the tissue freezer at –20 °C or below.

6.9 Mix the sample portion that was taken for preparation with 5 g of Diatomaceous Earth in a labeled 60 mL I Chem jar. Let the samples sit for a while at room temperature to fully desiccate. Mix frequently to disperse the tissue. Once dried, the samples are ready to be transferred into ASE 350 extraction cell. After allowing the samples to dry in sodium sulfate, blend each sample along with the sodium sulfate into a fine powder with the blender. Remember to use a new blade assembly for each sample.

7. SAMPLE EXTRACTION

Tissue samples are analyzed in batches of up to 20 samples. In addition to the tissue samples in each batch there are typically one Method Blank, Laboratory Fortified Blanks

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

(LFBs) and Matrix Spikes. Replicate tissue samples may also be analyzed in special cases.

NOTE: A SECOND PERSON MUST WITNESS THE ADDITION OF SPIKE STANDARD TO THE PROPER SAMPLES, VERIFY THE CORRECT VOLUME AND CONCENTRATION, AND ENSURE CORRECT RECORDING.

7.1 Clean the ASE 350 extraction cells.
ASE 350 extraction cells are cleaned by washing with Alconox and water. They are then rinsed with water, DI water and acetone followed by sonication in acetone and drying in the air. Additionally, empty cells may then be placed in the ASE 350 machine and further cleaned by running the extraction program. After they are removed from the ASE 350 carousel, they are stored in an oven at 120 °C.

7.2 Remove the appropriate surrogate and spiking solutions from the refrigerator. Let them warm to room temperature.

7.3 Clearly label the ASE extraction cells and place a glass-fiber filter at the bottom of each of them. Transfer the sample/drying agent mixture to the ASE cell (the ASE funnel may be used here). The bottom of the cell is tapped firmly but carefully on the countertop during the transfer to completely settle the cell contents. The remaining cell volume is then filled with diatomaceous earth.

7.4 Before proceeding, ensure that the spike and surrogate mixes are at room temperature. Many compounds easily precipitate out of solution when in the refrigerator or freezer and they need to be re-dissolved into solution by being warmed to room temperature and sonicated.

7.5 Spike all Samples, Blanks, and QC samples (Matrix Spikes, and Lab Fortified Blanks) with the GC surrogate spike mix. Spike the LFB and Matrix Spikes with the appropriate spiking solution. As follows:
200 ul of Chlorinated pesticides surrogate spike solution (200 ug/L of both Tetrachloro-m-xylene and Decachlorobiphenyl) for tests T-PCBCL-TQ and T-CT-CI

200 ul chlorinated pesticides/PCB congeners spike (200 ug/L for each analyte) for test T-PCBCL-TQ

200 ul of Toxaphene spike at 10 mg/L for test T-CT-CI
200 ul of 10xdil Toxaphene spike (1 mg/L) for test T-CT-CI; PQL sample only
200 uL of PAH surrogate spike mix. Spike the LFB and Matrix Spikes with the appropriate PAH spiking solution (100 uL).

7.6 Clear the cell threads of sample/DE mixture if necessary. Cap the cell firmly but not forcefully.

7.7 The ASE cells and the ASE collection vials are then loaded in the corresponding positions in the ASE carousels. Collection vials for system rinses should be loaded in the rinse vial positions.

7.8 Press the TRAYS button on the ASE panel to lock the carousel into the starting position. Press the RINSE button twice to flush the solvent lines.

7.9 The sample is extracted using an ASE schedule with the RINSE parameter set to [ON], and using a method with the parameter values set as follows:

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

For pesticides/PCBs:
PREHEAT [0] min
HEAT [5] min
STATIC [5] min
FLUSH% [115] vol
PURGE [180] sec
CYCLES [2]
PRESSURE [2000] psi
TEMPERATURE [100] C
SOL A [Mecl2] [100]%
SOL B [other] [0]%
SOL C [other] [0]%
SOL D [other] [0]%

For PAHs:
PREHEAT [0] min
HEAT [5] min
STATIC [5] min
FLUSH% [115] vol
PURGE [180] sec
CYCLES [2]
PRESSURE [2000] psi
TEMPERATURE [100] C
SOL A [MeCl2] [75]%
SOL B [Hexane] [25]%
SOL C [other] [0]%
SOL D [other] [0]%

7.10 After the extraction has finished remove the ASE vials from the ASE carousel. The total volume of extract should be in excess of 60 mL.

8. SAMPLE CONCENTRATION

8.1 Place the K&D condensers on the S-EVAP-KD®, if this has not already been done. Set the temperature of the water bath to 70-75 °C. When concentrating on the K&D concentrator, do not let the temperature of the water bath exceed 75 °C. Be sure that the water level in the bath is not covering the joint between a K&D flask and a concentrator tube, but is high enough that the tube is partially immersed in the water.

8.2 For each sample, assemble a clean K&D apparatus by attaching a 15-mL concentrator tube to a 500-mL evaporator flask, using clips. Label each K&D apparatus with a sample label.

8.3 Transfer each extract from the ASE 350 extract collection bottle (or round bottom flask in the case of Soxhlet extraction) into the appropriate K&D flask using methylene chloride to rinse it three times and add the rinsates to K&D flask. If any extract contains solid particles (such as sodium sulfate or fish tissue), filter it through a short pad of sodium sulfate and glass wool; otherwise pour the extracts directly into the K&D flasks. Add one or two clean boiling chips to each K&D flask.

8.4 For each sample, pre-wet a Snyder column by rinsing it with approximately 1 mL of methylene chloride. Ensure that all balls in the column are moving freely.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

8.5 Place each K&D apparatus on the hot water bath. Attach Snyder columns to the flasks, and the condensers to the Snyder columns.

8.6 At the proper rate of concentration the balls of the column will actively chatter, but the chambers will not flood with condensed solvent. **It is extremely important that the samples are not concentrated to dryness; if this occurs, a significant portion of the lighter molecular weight compounds of interest will be lost.**

8.7 Remove the K&D apparatus from water bath when there is only approximately 5 mL of extract left in the concentrator tube. **Do not let the tube go to dryness.** Let the vapors in the condensing column cool and drain into the tube, then remove the Snyder column and rinse the inside of the K&D flask with a few mL of methylene chloride. Allow this to drain into the concentrator tube.

8.8 Remove the concentrator tube from the K&D flask. There may be water on the outside of the flask -- do not allow it to drip into the sample (The water can be removed with a Kim wipe). Rinse the joint of the K&D flask with methylene chloride into the concentrator tube.

8.9 Transfer the contents of each concentrator tube into a labeled 15-mL centrifuge tube using a disposable Pasteur pipet. Rinse the inside of the concentrator tube three times with 1 mL of methylene chloride and add the rinsates to the centrifuge tube. Most of the ASE 350 extracts will, at this point, contain chunks of fat and droplets of water floating on the surface of methylene chloride. In order to remove those the content of each concentrator tube is passed through a small column containing a plug of glass wool on the bottom covered by a layer of granular sodium sulfate, a layer of sodium sulfate powder and again a layer of granular sodium sulfate.

8.10 Set up N-Evap® 116 by turning on nitrogen gas, heater (30 °C bath temperature), and attaching clean needles.

8.11 Open valves for each needle and check for required flow of nitrogen with a blank solvent in a centrifuge tube.

8.12 Place concentrator tubes into N-Evap®116 tray, then carefully pull needles down to approximately 2 inches above the solvent level. Make sure N_2 flow is adequate but be careful NOT to blow N_2 too vigorously into sample, causing splashing.

8.13 Concentrate and make up to 2 mL (for pesticides/PCBs) or 4 mL (for PAHs volume. Higher volumes may be necessary depending on lipids content.

9. CLEANUP PROCEDURES-PAHs

9.1 All PAHs tissue samples should be cleaned by passing through 10g Silica Gel column followed by Gel Permeation Chromatography (GPC cleanup). For GPC CLEANUP find the procedure in the Lab SOP for GPC cleanup GC-029-1 (see 15.8).

9.2 10 g Silica Gel/5 g alumina cleanup

9.2.1 1-5 g silica/ may be used for clean samples including method blanks and LFBs

9.2.2 Place 10 g of silica into 60 mL glass column plugged with glasswool and ½ inch layer of granular sodium sulfate. Cover with another ½ layer of granular sodium

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

sulfate on the top. Carefully add enough methylene chloride to completely cover the silica layer. Wash the silica gel with additional 15 mL of l methylene chloride.

9.2.3 Transfer the concentrated extract (4mL) to the column and elute with 25 mL of methylene chloride.

9.2.4 Using Nitrogen blow-down system concentrate the eluent to 1 mL. 0.8 mL is then injected into a GPC system for further cleanup

10. CLEANUP PROCEDURES-Pesticides and PCBs

10.1 All tissue sample extracts are subject to cleanup with sulfuric acid and/or GPC and 1 g FLORISIL. Sulfuric acid cleanup can be performed on the original sample extracts, prior to GPC. It is considered a preferable procedure for PCB congeners Toxaphene and resistant chlorinated pesticides. The GPC cleanup procedure is described in detail in DEP SOP GC-029-1 (see 15.8 After GPC proceed to one-gram Florisil cleanup (See this SOP 10.2).

10.2 ONE GRAM FLORISIL CARTRIDGE CLEANUP

10.2.1 Clean VACUUM MANIFOLD valves and needles with acetone.

10.2.2 Place One Gram Florisil Cartridge with proper label into the vacuum manifold for each sample.

10.2.3 Wash each cartridge with 6 mL of hexane.

10.2.4 Apply and adjust vacuum if needed, and do not let the cartridges go to dryness (USUALLY NO VACUUM IS NEEDED).

10.2.5 Place a rack containing labeled 15-mL centrifuge tubes inside the manifold, and place back the manifold top. (MAKE SURE THAT EACH CENTRIFUGE TUBE IS PLACED INSIDE UNDER THE APPROPRIATE CARTRIDGE).

10.2.6 Transfer 1 mL of extract to the top frit of the appropriate Florisil cartridge. Also prepare a Florisil blank.

10.2.7 Add 3 x 3 mL of hexane - acetone (90:10) followed by 2 x 1 mL hexane to the original sample tube and transfer each time to cartridge as it elutes.

10.2.8 Concentrate the eluent and solvent exchange and make up to 1 mL in iso-octane using N-blow down for PCB's and Chlorinated pesticides analysis.

10.2.9 Vortex for few seconds and proceed with GC/MS analysis.

10.3 SULFURIC ACID CLEANUP

10.3.1 Take 1 mL aliquot of tissue extract into a 15 mL screw cup centrifuge tube and thoroughly solvent exchange into 2 mL of iso-octane removing other solvents present in the extract.

10.3.2 Add 8 mL of concentrated sulfuric acid and shake for 30 seconds, allow to settle, and centrifuge for 5 minutes.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

10.3.3 Transfer the supernatant into a new clean centrifuge tube. Wash the sulfuric acid 3 times with 1 mL of iso-octane and add to the same new centrifuge tube. If the supernatant is not colored proceed to step 10.3.4. If supernatant is colored repeat steps 10.3.2 and 10.3.3. Be very careful not to transfer sulfuric acid into the cleaned extract. This will destroy the GC column.

10.3.4 N2-blow down to 1 mL and proceed to 1g Florisil Cleanup prior running GC/MS analysis (that makes sure no sulfuric acid remains in the extract).

11. ELECTRONIC SAMPLE PREPARATION LOG

Each sample preparation must be recorded by the preparation team in the electronic Prep Worksheet Manager module of LIMS. . The template name is identical with LIMS prep ID:

SOP-GC-004 (TQ) - (GC/MS tissue tests)

Many fields in both the header and the grid are prefilled and seldom change. They need, however, to be always checked for correctness.

Header fields in the electronic preparation logs:

- **Worksheet ID#:** *this is assigned automatically by LIMS. Example SOP-GC-004 (TQ)_41*
- **Prep ID:** *LIMS prep ID defined in LIMS and connected with test ID. Each test ID may only be connected to one Prep ID, but many test IDs may use the same prep method.*
- **SOP #**
- **Prepared By**:
- **Prep Start Date/Time**: *This will be the prep date in LIMS reports*
- **Spike Witness**;
- **Prep Completion Date/Time**:
- **Date/Time Reviewed**:
- **Reviewed By**:
- **Balance ID**: *pre-entered , usually the same*
- **Spike 1:** *Std tracker SN; this is usually surrogate*
- **Spike 2:** *Std tracker SN; this is the first of the spikes used*
- **Spike 3:** *Std tracker SN; this is the second of the spikes used*
- **Spike 4:** *Std tracker SN; this is the third of the spikes used*
- **Spike 5:** *Std tracker SN; this is the fourth of the spikes used*
- **Spike 6:** *Std tracker SN; this is the fifth of the spikes used*
- -different numbers of spikes may be applicable for specific extractions
- ***Equipment ID:*** *ASE 350 SN: 100704459 and other equipment that may be used*
- **Water bath temp:** *in deg C*
- **Final Extract Solvent:** *Isooctane_ lot number*
- **Solvent 1** *Methylene Chloride_ lot number*
- **Solvent 2** *Acetone_ lot number*
- **Solvent 3** *Hexane_ lot number*
- **Solvent 4** *Isooctane_ lot number*
- Different numbers of solvents may be used in different procedures
- **Reagent 1-** *any reagent that may be used in the preparation*

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

- **Cleanup procedures:** list *GPC File: 100914GPC_3*
- **Prep comments:** *enter other chemicals/supplies here with lot numbers; example Diatomaceous Earth lot#, Florisil lot# etc. ASE extraction filters cellulose 34.66, 100 ml ASE 350/150 cell lot #.*
- **Internal standard:** *enter the ISTD information like this: ISTD SN: 53520 100uL, 10-10-2014 IC*

Grid Fields in the electronic preparation Logs

Prep Batch ID: ***consecutive "P" number assigned by LIMS in the form of P123456. The same number has to be used for sample reporting in QC manager.*** **Sample ID**: *the sample IDs are selected in LIMS when the worksheet is created.; for QC samples those rows are typically pre-filled in the template*

- **Sample ID**; *the laboratory sample Id assigned in LIMS*
- **Test ID**: *The analysis requested for the sample.*
- **Matrix:** *matrix assigned in LIM*
- **Sample wet Wt (g)**/: M*ass of the sample used for analysis e.g. usually 5g of tissue*
- **Final extract Volume (mL**: The volume of the sample extract after full concentration step; 2 mL for regular tissue sample extraction
    - **Sample Clean-up**: *The clean-up procedure used, if applicable. Write down anything done differently than SOP requirements.*
    - **Spike 1:** *volume of surrogate spike added*
    - **Spike 2:** *volume of the first spike added*
    - **Spike 3:** *volume of the second spike added*
    - **Spike 4:** *volume of the third spike added*
    - **Spike 5:** *volume of the fourth spike added*

      The number of spikes used for a specific preparations may vary depending on what tests are required.
- **Comments:** *This section should include any observations regarding unusual appearance or odor of the sample, any dilutions made during preparations or any other remarks about the preparation*.

12. SAMPLE ANALYSIS PROCEDURE

See analysis SOPs GC-033-1 (see ref 15.13), GC-034-1 (Ref 15.14 and SOP SV-020-1 (ref 15.4)

13. QUALITY CONTROL

See QA/QC SOP GC-001-2 (see 15.9).

14. SAFETY/HAZARDOUS WASTE MANAGEMENT

14.1 Wear safety glasses, gloves and a lab coat at all times while performing sample preparation! Methylene chloride will burn any skin it contacts; so wear nitrile gloves (Latex gloves will not stand up to methylene chloride.).

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

14.2 Handle all the sample extraction, solvent transfer, concentration, sample preparation, and clean up in a hood at all times. Avoid breathing methylene chloride or other solvent vapors. All extractions MUST be prepared in a fume hood.

14.3 Discard all methylene chloride waste in the appropriate Chlorinated Waste bottles. Keep chlorinated and non-chlorinated waste solvents separate. If mixed, record the volume of each and dispose with chlorinated waste.

14.4 Dispose of leftover and expired neat standards into a sealed, labeled container.

14.5 Dispose of all the stock standards, working standards and used sample extracts into a labeled chlorinated waste container. For PCB standards use a special waste container labeled “PCB Wastes”.

14.6 Dispose of all used GC vials containing standards or samples into a labeled chlorinated waste container.

14.7 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; 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.

14.8 A reference file of material data handling sheets is kept in the office area, across from the entrance to the tech room (room A316J). Refer to this file for any material you handle. Update this file upon receiving new chemicals or reagents. The MSDS for many chemicals can also be viewed on-line, at http://hazard.com/MSDS/

14.9 Primary standards of these toxic compounds should be prepared in a hood. A NIOSH/MESA approved toxic gas respirator should be worn when an analyst handles high concentrations of these toxic compounds.

14.10 Dispose of all unwanted, broken glassware into a broken glassware disposal box. Inspect every piece of glassware. Do not use any that are chipped, cracked, etched, or scratched. Glassware with minor damage should be stored for repair.

14.11 See Safety-Waste SOP OG-001-3 (see 15.10) for further details.

15. REFERENCES

15.1 DEP SOP CM-031-1- . Glassware Cleaning and Muffle Furnace Protocols in the Organic Preparations Laboratory.

15.2 EPA 3630C Silica Gel Cleanup: Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods, SW-846; 3rd Edition, Revision 3, December 1996.

15.3 EPA3545A Pressurized Fluid Extraction: Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods, SW-846; 3rd Edition, Revision 1, February 2007.

15.4 .DEP SOP SV-020-1 Analysis of Polycyclic Aromatic Hydrocarbons (PAHs) in Fish Tissue using GC/MS/MS

15.5 EPA 3640A Gel-Permeation Cleanup: Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods, SW-846; 3rd Edition, Update 4, January 3, 2008.

15.6 EPA 3620C Florisil Cleanup: Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods, SW-846; 3rd Edition, Update 4, January 3, 2008..

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

15.7 EPA 3665A Sulfuric Acid/Permanganate Cleanup: Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods, SW-846; 3rd Edition, Update 4, January 3, 2008.

15.8 DEP SOP GC-029-1 Gel-Permeation Chromatography (GPC) Cleanup procedure for Pesticides, Polychlorinated Biphenyls (PCBs) and other Organic Analytes.

15.9 DEP SOP GC-001-2 Quality Assurance / Quality Control and Data Review Authorization in the GC Pesticides Laboratory.

15.10 DEP SOP OG-001-3 Safety and Waste Management Practices in the Organic Analysis Group (Volatile, Semi-Volatile and Pesticides).

15.11 EPA 1668C Chlorinated Biphenyl Congeners in Water, Soil, Sediment, Biosolids and Tissue by HRGC/HRMS; April 2010

15.12 EPA 1699 Pesticides in Water, Soil, Sediment, Biosolids and Tissue by HRGC/HRMS; December 2007

15.13 DEP SOP GC-033-1 Analysis of Pesticides and PCBs in Water, Soil and Tissue Samples Using GC/MS/MS

15.14 DEP SOP GC-034-1 Analysis of Chlordane and Toxaphene using GC/MS with Negative Chemical Ionization.

15.15 Extraction, clean-up and gas chromatography/mass spectrometry analysis of sediments and tissues for organic contaminants" NOAA technical Memorandum NMFS-NWFSC-59, March 2004.

15.16 Extraction, clean-up end gas Chromatography/Mass spectrometry Analysis of edible seafood for parent and alkyl polycyclic aromatic Hydrocarbons in response to the Deepwater horizon MC252 Oil spill Event. NOAA Method, July 2010

APPENDIX 1

TROUBLESHOOTING A (GPC) INSTRUMENT

Along with routine sample cleanup, a fully trained analyst should be able to troubleshoot an HPLC instrument for problems that arise in normal operations. All HPLC instruments have service agreements. Always consider calling the technical service department of the company that manufactured your HPLC. While you may not need the service engineer to repair the problem on site, they can be a tremendous source of valuable information and can often diagnose your problem over the telephone. Telephone numbers for each company, service department, can be found in each instrument "Maintenance Log book" or in the instrument manual itself. As always, whenever you have gone through a troubleshooting/ repair episode, record all information in the pertinent HPLC Instrument Maintenance Logbook so information will be available for the next person when he or she (or you, again!) is attempting to diagnose a problem.

And as always, proper routine maintenance of the HPLC can alleviate many major repairs in the future. Some basic maintenance points:

- Clean GPC columns after use by flushing with methylene chloride.
- After every 50-60 samples, the GPC column need back flush. It also depends how dirty are the samples run in the instrument.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

- Back flush procedure for the GPC column: Connect the column in opposite direction. Flush the column with methylene chloride for 1h. Inject 1 mL of THF and run for 5 min, then inject another 1 mL of toluene and let it run for 30 min. Disconnect the column and connect in normal direction and flush the system with methylene chloride for 30 min.

- Replace frits and seals routinely.

- Diagnose and fix high-pressure problems as they occur. Operating the HPLC at higher than normal pressures places stress on all the LC components and can contribute to a more rapid deterioration of components.

- Filter all samples that are injected into the LC.

APPENDIX OF CHANGES

12/27/2013	Added GC/MS tests (T-PCBCL-TQ and T-CT-CI) to the SOP.
12/17/2014	Addition of Electronic Preparation Log
	Addition of DE Blank – see section 6.7
	Addition of T-PQL spike
12/08/2015	Removed GC-ECD tests (discontinued)
04/12/2016	Section 7.5 page 8, specific spiking information added (FDEP response to CERP audit item 3b).
	Typographical error corrected. Section 15.3 page 14. (FDEP response to CERP audit item 3c).
04/06/2017	N-EVAP temperature corrected. Should be 30 C.
04/05/2018	Added Florisil blank. Added the prep SOP number to the prep worksheet header.
03/17/2020	Lab Solutions software for GPC.
03/10/2021	SOP SV-016-1.11 (PAHs) was merged into this SOP. Numerous changes throughout the text. Removed Soxthlet extraction. Updated GPC instrumentation/columns info.
02/09/2022	References updated. Test T-PEST-NP removed.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.