Standard Operating Procedure

Continuous Liquid-Liquid Extraction by EPA Method 3520C

DEQ08-LAB-0057-SOP
Version 4.1

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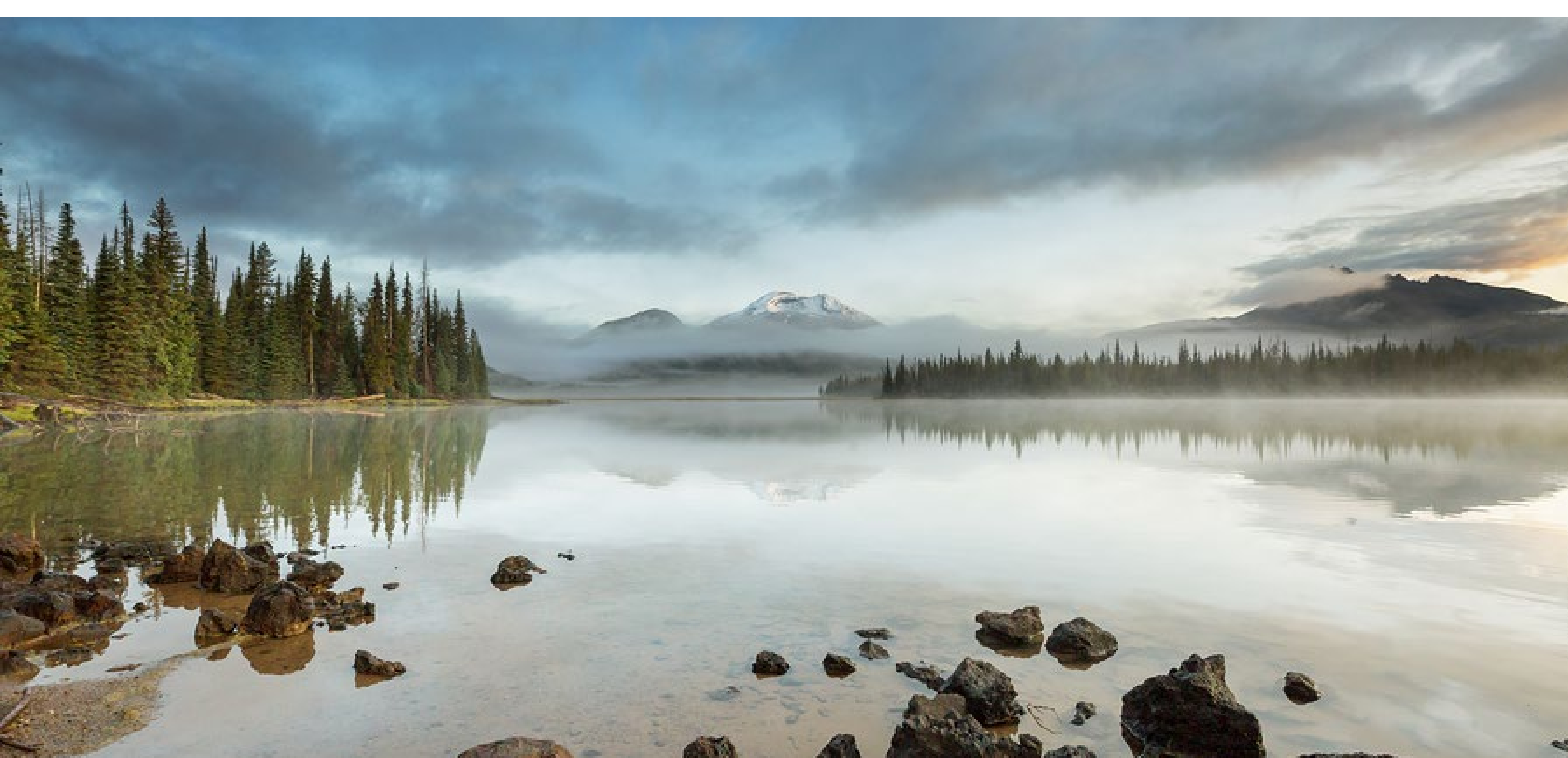

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1. Scope and Application

This SOP is for Continuous Liquid-Liquid Extraction set up by EPA Method 3520C (and compatible with EPA 625). This method is for isolating organic compounds from aqueous samples. The process uses continuous extraction for preparing samples for analysis. This method is applicable to the isolation and concentration of water-insoluble and slightly water-soluble organics in preparation for the following chromatographic procedures: EPA 8270E, EPA 8321, EPA 1613, EPA 1614, EPA 1668, EPA 1698, and Polychlorinated Naphthalenes (PCN). The method uses either a water bath concentration, or nitrogen evaporation to bring the sample to a final volume. This is a general extraction method that is referenced by the specific analytical methods that utilize it.

1.1 Applicable Matrices

This method is applicable to following matrices:

- Ground water
- Source water
- Wastewater
- Aqueous
- Saline/Estuarine

1.2 Detection Limits

See the appropriate analytical method of analysis for detection limits.

2. Summary

A one-liter sample is placed into a continuous liquid-liquid extractor and extracted with organic solvents for 18-24 hours. The extract is then dried using a sodium sulfate funnel, macro concentrated by water bath or on an N-EVAP. After concentration, the sample is ready for further cleanup steps, if necessary, or to be brought to final volume for analysis.

3. Personnel/Qualifications

The analyst should meet the minimum qualifications for a Chemist 1 position. A chemist who has previously demonstrated their proficiency at performing the method should train the analyst. An Initial Demonstration of Capability (IDOC) is to be conducted and passed prior to reporting data as required by the DEQ Laboratory Quality Manual (LQM).

4. Interferences

Organic and soap residues on glassware can pose possible contamination. Soap residue (e.g. sodium dodecyl sulfate), which results in a basic pH on glassware surfaces, may cause degradation of certain analytes. Aldrin,

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heptachlor, and most organophosphorus pesticides will degrade in this situation. This problem is especially pronounced with glassware that may be difficult to rinse. To avoid this, all glassware should be washed and rinsed thoroughly prior to being kiln fired in a 450 ºC oven overnight. The exception to this would be volumetric glassware, which should not be kiln fired. Only high-purity solvents should be used to minimize the introduction of contaminants during extraction.

Avoid use or contact with plastics during the procedure to avoid phthalate ester contamination.

The decomposition of some analytes has been demonstrated under basic extraction conditions required to separate analytes. Organochlorine pesticides may dechlorinate, phthalate esters may exchange, and phenols may react to form tannates. These reactions increase with increasing pH and are decreased by the shorter reaction times available in Method 3510. Method 3510 is preferred over Method 3520 for the analysis of these classes of compounds. However, the recovery of phenols may be optimized by using Method 3520 and performing the initial extraction at the acid pH.

5. Safety

The analytes and solvents mentioned in this method-be they detected in samples, used in extraction, or used to make standards-are hazardous and every effort should be made to avoid contact with them. A laboratory coat, organic-impervious gloves and a fume hood are all good preventatives in this effort. In general, disposable Nitrile gloves provide effective protection from the solvents and analyte standards used in this method as well as possible biological hazards from the field samples themselves. If any solvent or standard contacts the gloves, the analyst should immediately remove the gloves and replace them with new.

Health and Safety has an online database for SDS sheets. Please refer to the Workplace Safety Home page on QNet for accessing this online database.

Analysts working in the LEAD facility must review the laboratory's Chemical Hygiene Plan / Laboratory Safety Plan (DEQ04-LAB-0006-SFTY) and the Emergency Operation Plan (EOP/DEQ04-LAB-0050-SFTY).

Please refer to the attached Job Safety Analysis for further safety information when performing this analytical method.

6. Equipment and Supplies

- Continuous Liquid-Liquid Extractor (CLLE) with glass joints
- Condensers
- N-EVAP- Nitrogen Evaporation (Temperature verified daily using a NIST thermometer stored in the extractions room and recorded on bench sheet.)
- Büchi Syncoreplus Analyst (Must be calibrated annually against the Fluke Thermocouple primary reference thermometer stored with QA, as per DEQ08-LAB-0006-SOP Temperature Monitoring and Thermometer Calibration SOP and documented in logbook. Actual temperature, correction factor and adjusted temperature must be recorded on bench sheet.)
- Büchi Extraction Tubes, 450 mL

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- RapidVap Concentrator (Must be calibrated annually against the Fluke Thermocouple primary reference thermometer stored with QA, as per DEQ08-LAB-0006-SOP Temperature Monitoring and Thermometer Calibration SOP and documented in logbook. Actual temperature, correction factor and adjusted temperature must be recorded on bench sheet.)
- RapidVap Extraction Tubes, 450 mL
- Teflon Caps for the RapidVap Tubes
- Muffle furnace capable of maintaining 600 ºC.
- Kiln capable of maintaining 450 ºC.
- Boiling Chips – Must be cleaned in a soxhlet in DCM overnight prior to use.
- Glass Funnel – Should be cleaned according to procedure.
- Glass Wool – Must be cleaned in a soxhlet in DCM overnight prior to use.
- Glass Stirring Rod
- Heating Mantle
- Top-loading balance, 0.01 g capacity
- Thimbles/Concentrator Tube
- Capillary Tubes
- Pasteur pipettes (glass)
- 250 mL flat bottom flasks
- Aluminum foil
- 1 L Amber jars with Teflon lined caps
- pH Strips (0-14, 7.0-14, and 1.7-3.8)
- Volumetric flasks, 1 mL, 2 mL
- Gas tight syringes (various capacities)
- Auto-sampler vials, caps, and inserts

*Note: Additional equipment and supplies are listed in the specific work instructions for Silica Gel/Florisil (1613, 1614, 1668), Basic Alumina (1614, 1668), Reverse Carbon (1613), Pesticides by GC QQQ (8270E Modified)

7. Reagents

Reagents may contain elemental or compound impurities which might affect analytical data. Only high-purity reagents that conform to the American Chemical Society specifications should be used whenever possible. If the purity of a reagent is in question, analyze for contamination.

- Methylene Chloride (Dichloromethane or DCM), Chromatography grade: boiling point 39 ºC
- Hexane, Chromatography grade: boiling point 68.7 ºC
- Phosphoric Acid: Concentrated
- Hydrochloric Acid: Concentrated
    - Hydrochloric acid solution (1:1 v/v), slowly add 50 mL of concentrated HCl to 50 mL of organic-free reagent water
- Sulfuric Acid: Concentrated
    - Sulfuric acid solution (1:1 v/v), slowly add 50 mL of concentrated H_2SO_4 to 50 mL of organic-free reagent water
- Acetonitrile: boiling point 81.6 ºC
- Acetone: boiling point 56.2 ºC
- DI Water

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- EDTA (Ethylenediaminetetraacetic acid, disodium salt dihydrate), Biotechnology grade
- Sodium Chloride – purified by heating to 600 ºC in a muffle furnace.
- Potassium dihydrogen citrate
- Sodium Sulfate (granular, anhydrous) – purified by heating to 450 ºC in a kiln.
- Sodium Hydroxide: Sodium hydroxide solution (10 N), NaOH. Dissolve 40 g NaOH in organic-free reagent water and dilute to 100 mL. Other concentrations of hydroxide solutions may be used to adjust sample pH, provided that the volume added does not appreciably change (e.g., <1%) the total sample volume.
- Nonane for HRMS (EPA 1613, 1614, 1668): n-Nonane (unlabeled), Cambridge Isotope Labs p/n ULM-2323-4x25 or alternatively n-Nonane, Fisher (Thermo, Acros) p/n AC129111000 (100mL) or AC129115000 (500mL). Cost per mL is equivalent; contamination impact is limited more with the Cambridge 25mL bottle size.

8. Standards

See analytical method for specific standards information. Both a surrogate and spike solution will be used. These standards will be logged into Element and assigned an ID number.

9. Sample Collection, Preservation, Shipment, and Storage

Aqueous samples must be collected and stored in a 1-liter amber glass jar certified clean by the manufacturer and sealed with a Teflon-lined lid. Samples must be stored at temperatures ≤ 4 ºC. Extraction must be performed by the hold time specified by the analytical method of interest. Extracts are stored in a refrigerator or freezer in accordance with the method.

10. Calibration and Standardization

All solvent concentration system water bath temperatures must be recorded on bench sheet prior to use. Acceptance criteria for the concentration systems are ±5 ºC from set point, per reference method.

See the analytical method for calibration and standardization information. See Volumetric Ware Calibration & Verification SOP (DEQ07-LAB-0011-SOP) for the method on calibration of pipettes and syringes.

11. Quality Control

No analytical results are valid, and no further analyses should be performed if the QC parameters are not met. For any QC problems, the source must be identified and corrected before any further analysis is done and all analyses performed since the last time all QC parameters were met must either be appropriately flagged or repeated (if possible) as needed.

Quality control (QC) requirements include determination of the Limit of Detection (LOD), Demonstration of Capability (DOC) followed by regular determination of instrument performance and analysis of reagent blanks, field blanks, and spikes.

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All analysts and laboratories using this method are required to operate a formal Quality Control (QC) program. The laboratory is required to maintain performance records that define the quality of the data thus generated.

11.1 Data assessment and Quality Control Acceptance Criteria

Data assessment and QC acceptance criteria are of primary importance in assessing the quality of data resulting from analytical batch. Be especially attentive to analytical bias; negative bias is of equal concern to positive bias. Any samples returning "hits" for any analyte(s) above the upper end of the calibration range must be diluted and reanalyzed so that all detected analytes quantitate within the calibration range. The following data assessment and QC data are required for all analyses:

Table 1: Listing of required quality control elements, including frequency and acceptance limits.

QC Element	Frequency	Acceptance Criteria	Comments
Initial Demonstration of Capability (IDOC)[1] and Continuing Demonstration of Capability.	At end of method development and prior to reporting data, and thereafter yearly.	Defined by analytical method.	
Limit of Detection (LOD or Method Detection Limit, MDL)	Initially and verified annually or with substantial change to method or instrumentation.	40CFR Part 136, Appendix B calculations.	
Laboratory Control Sample (LCS) / Laboratory Fortified Blank (LFB)	At least once per preparation batch of 20 or fewer samples.	Defined by Analytical Method.	
Method Blank (MB) or Laboratory Reagent Blank (LRB)	At least once per preparation batch of 20 or fewer samples.	< ½ LOQ for each reported analyte.	
Field Blank	One per sampling event.	Concentration for each analyte must be < ½ LOQ. (Not required for batch acceptance).	
Matrix Spike (MS) or Laboratory Fortified Sample Matrix (LFM).	At least once per preparation batch of 20 or fewer samples or according to the DQO of the QAPP.	Defined by Analytical Method.	
Matrix Spike Duplicate (MSD) or Sample Duplicate (Dup).	At least once per preparation batch of 20 or fewer samples or according to the DQO of the QAPP.	Defined by Analytical Method.	
Surrogate	Added to all batch samples and QC components unless defined otherwise by Analytical Method, DQO, or QAPP.	Defined by Analytical Method.	

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[1]IDOCs generally consist of 4 LCS @ mid-point concentration. See laboratory SOP on Demonstrations of Capability for more information.

- **Laboratory Control Standard Duplicate:** When sample batches have insufficient sample volume available, or samples are otherwise unacceptable for use to determine precision by analysis of a sample Duplicate or Matrix Spike Duplicate a Laboratory Control Standard Duplicate should be used to assess batch precision.
- **Laboratory Method Blank:** If any of the method analytes are detected above ½ the reporting limit, the data must be appropriately qualified.
- **Sample/Spike Duplicate:** When the analyte concentration is within an order of magnitude of the reporting limit, lower precision can be expected.

11.2 Corrective actions for out-of-control data

Corrective actions must be taken for all data found to be out-of-control, as summarized in the Table: List of corrective actions to be used in addressing out-of-control QC data. Out-of-control QC data can often follow samples that are especially "dirty" or have high concentrations of analytes. If this is suspected, it is often best to stop the analysis, clean the sample introduction system, and reanalyze the samples after they have been diluted.

In some cases, out-of-control data may be indicative of a serious instrumental problem that may require a service visit. When the corrective actions given below fail to correct the identified problem, assess the situation with the senior chemist.

Table 2: List of corrective actions to be used in addressing out-of-control QC data.

Quality Control Element	Corrective Action
Limit of Detection (LOD)	Determine source of problem, correct, and reanalyze.
Demonstration of Capability (DOC)	Determine source of problem, correct, and reanalyze.
Method Blank (MB or LRB)	Reduce background contamination and reanalyze.
Field Blank	Analyze lab blank to determine the source of the problem (field or lab).
Laboratory Control Standard (LCS or LFB)	Determine source of problem, correct and re-extract batch if sample volume and holding time allow.
Matrix Spike (MS or LFM)	Check makeup of Matrix Spike solution. If LCS passes, indicate matrix problem with sample.
Matrix Spike Duplicate or Sample Duplicate (MSD or Dup)	Reanalyze. If LCS passes, indicate matrix problem with sample.

11.3 Contingencies for handling out-of-control data

On rare occasion the analysis cannot be brought under-control. In these cases, contingency plans must be employed. The following list provides a guideline for handling out-of-control data:

- Contact the project manager and verify the importance of the data. If the analyte is of minimal importance, determine if it can be reported as an estimate or not reported at all.
- Determine with the project manager if an alternate sample can be used for their evaluation purposes.

- If it seems appropriate in your best professional judgment, speak with the project manager about obtaining an alternate sample that may provide the required information.
- If QC data suggests the analysis is showing a low bias, report **only** that data that exceeds a regulatory limit. Be sure and clearly comment this information in the LIMS system and in the analytical documentation.
- If QC data suggests the analysis is showing a high bias, report **only** that data that is below the LOQ. Be sure and clearly comment this information in the LIMS system and in the analytical documentation.
- In *every* case, the problem(s) and attempted corrections should be noted in the instrument logbook (when instrumental problems have occurred), with the analytical batch data, and in the LIMS system for final reporting.

12. Procedure

The following is a general overview of the extraction, and concentration processes. See Appendices for specific work instructions.

12.1 Extraction

A one-liter sample is collected at the site. Once received at the lab, the sample is logged in for analysis and placed into the refrigerator.

1. Batch the samples in Element, print the bench sheet, and export the bench sheet as an XLS file to open in Excel.
2. Remove samples from walk-in. If necessary, thoroughly shake each sample to homogenize and pour off and discard enough volume so that there is enough room for reagents/preservatives, while remaining as close to 1000 mL as possible.
3. Open the exported bench sheet in Excel on the computer in extractions. Weigh each sample jar and enter this weight into the spreadsheet under the “Bottle Weight Full” column.
4. Prepare blanks and LCS by filling clean, kilned amber glass jars with close to 1000 mL of DI water
5. Prior to set up, rinse the flat bottom flask and extraction body with 3, 20mL aliquots of extraction solvent and place 2 or 3 cleaned boiling chips in the flat bottom flask. Discard the rinsate into the appropriate waste container. ***Note: This step is not required for the 8270/8321 Methods*

   NOTE: CLLE’s and flat bottoms should be washed and kilned prior to use, however, if that is not possible, they can be thoroughly washed and solvent rinsed as follows: 3, 20 mL rinses of acetone, followed by 3, 20 mL rinses of DCM, collecting all rinsate into an appropriate solvent waste container.
6. Set up the extraction body in the fume hood, placing the flat bottom on a heating mantle, and affixing the body of the CLLE with a chain clamp so that it is upright and secure. Add 150 mL of methylene chloride to the extractor.
7. Add spike and surrogate to sample and QC jars using a volumetric syringe according to the specifications in the analytical SOP of interest. To remain flexible, exact volumes are not specified in this SOP. Spiking solutions and volumes are identified on the preparation bench sheet(s) and can be traced in that way. In addition, each analytical method has standard preparation spreadsheet saved on the shared organic drive (\\deqlab1\sp-organics\Standard Preparation), which includes spike

concentration and volumes. All spikes and surrogates must be added to the sample bottle prior to transfer to the continuous liquid-liquid body.

8. If necessary, modify the sample and QC jars by acidifying, basifying, or preserving the jars as specified in the work instructions for each method. After pH adjustment, verify the pH is correct by drawing up a small amount of sample with a clean Pasteur pipette or capillary tube and dispense on pH paper. Quantitatively transfer the sample to the extractor making sure not to pour sample down the side arm or otherwise spill it. Add approximately 20 mL of methylene chloride to the sample jar using a tilting repeating dispenser, cap the jar, shake well and add this solvent to the extractor. Repeat the rinsing two more times for a total of approximately 60 mL of rinsate.
9. Rinse the CLLE ground glass neck using a DI water squeeze bottle to assure all the sample reaches the extraction body.
10. Cap CLLE body with a condenser. Turn on condenser water so there is an appropriate flow rate and set the heating mantles to an appropriate level. Once the samples have all started to boil, cover the flat bottoms with foil and extract for 18 to 24 hours at a condenser drip rate of approximately 1 drop per second. Leave the sample jars uncapped in the hood until dry.
11. If extraction is for BNA 8270 or extraction by EPA 625 is otherwise required, it is necessary to verify the pH is unchanged after both the acid and base-neutral extractions. This is done by drawing up a small amount of sample with a clean Pasteur pipette or capillary tube, dispensing on pH paper, and checking against the appropriate pH. Indicate discrepancies on the bench sheet.
12. Once dry, weigh the empty jars and enter the weight into the Excel spreadsheet in the “Bottle Weight Empty” column; this will automatically calculate the initial volume. Save the spreadsheet and close it. Import the spreadsheet back into Element to populate the initial volume for each sample.
13. When the extraction is complete, turn off the heating mantles, remove the foil, and allow the flat bottoms to cool until they are cool to the touch. Remove condenser and turn off condenser water. Remove the chain clamp from the CLLE and carefully lift the CLLE and flat bottom, tipping the whole assembly slightly forward so that the DCM in the bottom of the CLLE is collected into the flat bottom then tip the whole assembly backward so that no water spills into the flat bottom. Gently twist the flat bottom to remove it from the CLLE, rinsing the joint of the CLLE with DCM, catching the rinsate in the flat bottom.
14. Tip CLLE forward and drain the residual DCM from bottom of CLLE body into a solvent waste container and then pour water from CLLE into carboy to be purged overnight before being disposed of down the drain. The solvent waste container is emptied into a large separatory funnel, where it is allowed to settle until the DCM and water have separated. Then the DCM is drained from the bottom into solvent waste, and the water can be added to the carboy.
15. Proceed to sample concentration steps as specified in the work instruction for the analysis of interest.

12.2 Concentration with Büchi

Before concentration using Büchi, ensure that full method validation has been completed.

1. Gather required glassware and equipment: Büchi tubes, tube racks, and sodium sulfate funnels.
2. 3x DCM rinse the Büchi tubes, label with the sample IDs using a sharpie, and top with sodium sulfate funnels that have been rinsed with DCM.

3. For each sample, pour the contents of the flat bottom through the sulfate funnel, collecting in the Büchi tube. Rinse the flat bottom 3x with small aliquots of DCM and pour rinsate through funnel into the Büchi tube after each rinse.
4. Rinse sulfate funnel into the Büchi tube with an additional 20mL aliquot of DCM using a tilting repeating dispenser.
5. Turn on Büchi concentrator, vacuum pump, and chiller. Fill cells with DI water to the max fill line (**Do not operate Büchi without water in each cell**). Preheat water bath and the top heated vacuum plate to the appropriate temperature. Set the chiller to the appropriate temperature.
6. Record the temperature setpoint and actual temperature with correction factor on the bench sheet. Acceptance criteria for the Büchi concentration system is ±5°C from set point, per reference method.
7. Place Büchi tubes into the Büchi cells, making sure they are pushed down all the way and seated correctly in the cell. Place the top heated vacuum plate over the Büchi tubes and fasten to finger tight with the screw nuts to ensure that a seal has been formed at the top of the tubes with the vacuum plate.
8. On the Büchi touch screen select the appropriate program from the “Favorites” or “Methods” menu and press Start. (See work instructions for specific programs)
    - If at the end of the program the samples require further concentration to reach the desired volume, use the “manual” or “timer” settings to set the Büchi parameters (pressure, temperature, rotation) to the same as the last step in the program and concentrate until the desired volume is reached.
    - The Büchi concentrator operates in such a way that the solvent will be retained in the nipple for a short period of time before completely evaporating (~5-10 minutes) due to the cold zone around the nipple. Some samples may reach the desired volume before the rest of the samples in the Büchi concentrator. If this happens, pause the program by pressing the “Aerate” button, turn off the rotation, remove the finished sample(s), and either plug the vacuum holes with the provided plugs or place an empty Büchi tube in place of the removed sample(s). In either case make sure that the top vacuum plate is screwed down so that the pressure is evenly distributed across the plate and a seal is formed around the remaining tubes (e.g. two tubes, one in each middle position or four tubes, one in each corner position). To restart the program press “Hold off” and turn the rotation on to the appropriate setting.
9. After samples reach just below the top of the nipple (~1mL), remove them from the Büchi concentrator.
10. Using a disposable pipette, transfer sample to a 2mL volumetric flask, rinse the Büchi tube 3 times using DCM; use each rinsate to bring sample to the final volume, 2mL.
11. Transfer sample to labeled amber autosampler vial.
12. Place vials in the “Molybdenum” fridge, in the appropriate section.
13. Empty the primary and secondary collection flasks on the Büchi into the chlorinated waste.

12.3 Concentration with RapidVap (alternate method)

Before concentration using the RapidVap, ensure that full method validation has been completed.

1. Prepare sodium sulfate funnels by forming a small, cleaned glass wool plug and inserting it into a clean, kilned funnel. Fill the funnel appr.1/3 full with sodium sulfate that has been kiln fired and kept in a 140 °C drying oven. Allow sodium sulfate to cool, and rinse thoroughly with DCM, collecting the rinsate for proper disposal.
2. Rinse the RapidVap tubes 3 times with small amounts of the extraction solvent and discard the rinsate.
3. Label the RapidVap tubes with sample ID's and place a white Teflon cap and a sulfate funnel onto each one.
4. Pour the extracted sample through the sulfate funnel into the tube.
5. Rinse the flat bottom three times with small amounts of extraction solvent taking care to thoroughly rinse the walls and pour through sulfate funnel. Finally, rinse the sulfate funnel once with approximately 20 mL of extraction solvent.
6. Allow all rinsate to drain into RapidVap tube.
7. Turn on the RapidVap and pre-heat it to 40 °C for DCM or 70 °C for Hexane. Read and record the temperature setpoint and actual temperature with correction factor of the RapidVap heat block on the bench sheet. Acceptance criteria is ±5°C from set point, per reference method.
8. Turn on the nitrogen to the instruments.
9. If solvent exchange is necessary, add the volume of transfer solvent specified in the work instructions to the RapidVap tubes.
10. Carefully place the RapidVap tubes into the RapidVap, taking care to not spill or drop the samples.
11. Set the operating speed, temperature, and number of samples for the RapidVap. **Note**: Operating speed is dependent on the volume of sample and the size of sample tube. Assign speed according to manufacturer's instructions in the included tables (assigned speeds are sometimes too aggressive, check to be sure that no sample volume is lost to spillage). See method work instructions for method specific programs.
12. Once all RapidVap parameters are set, press start and adjust the nitrogen flow to 10 psi or as specified on the method (see specific work instructions).
13. When samples are above 400 mL, let the RapidVap run with no oscillation until they are below 400 mL to keep sample from spilling in the RapidVap.
14. Rinse the RapidVap tubes with extraction solvent when the solvent level reaches $\frac{1}{3}$ bevel.
15. At this point samples may be concentrated to approximately 1 mL and carried thru further cleanup steps if specified in method work instructions. If no further cleanup is required samples should be concentrated for final volume. Final volume may vary based on method, so work instructions should be consulted. If the final volume is 1 mL, remove the extract from the RapidVap at approximately 1 mL, allow sample to air evaporate until the sample reaches approximately 0.7 mL. Do not allow the extract to go below 0.5 mL because the more volatile analytes may be lost.
    - ****Note:** Steroid and Hormone extracts (EPA 1698) are taken to dryness
    - **Note:** For all other methods, if the sample extract goes dry, make a note on the bench sheet and consult with lead worker or manager to determine corrective actions.
16. For a 1 mL final volume using a volumetric flask, pipette the sample into the clean volumetric flask and bring to volume with three rinses of the RapidVap tube with extraction solvent.

17. Once at the proper volume, transfer to a labeled auto sampler vial and place in the freezer for analysis. If the samples are dark colored, they may be brought to higher final volumes after consulting with the analyst. Record the final volume in Element.

12.4 Clean-ups

See Appendices for specific clean-up instructions. See SOP DEQ03-LAB-0053-SOP for the procedure for Gel Permeation Clean-ups.

Table 3: Specific Extraction Conditions

EPA Method	Initial Extraction pH	Secondary pH	Extraction Solvent	Transfer Solvent
8321B	≤2	none	DCM	Acetonitrile
8270E (PSP/toxics)	≤2	none	DCM	none
8270E (acid/base)	<2	>11	DCM	none
1698	as received	none	DCM	Hexane
1613, 1614	as received	none	DCM	Hexane
1668	as received	none	DCM	Hexane
8270E (QQQ)	as received	none	DCM	Hexane

Two sample blocks are available for the RapidVap N2. Recommended speeds are as follows:

Table 4: Rapid Vap Recommended Operating Speeds

Tube Size	Sample Volume	Vortex Speed
600 mL	50	90%
	100	90%
	200	76%
	300	58%
	400	40%
	450	36%
170 mL	50	100%
	75	95%
	100	98%
	125	70%

**see work instructions for operating speeds that are recommended, as the manufacturers speeds can be too aggressive, leading to loss of sample.

Evaporation Time in Upper Portion of Sample Vial (600 mL sample Tubes)(Time in Minutes)

Table 5: RapidVap Evaporation Time

Solvent	Methylene Chloride	Ethyl Acetate	Hexanes	Acetone	Toluene	Water
Block Preheat & Block Set Point Temperature:	40 °C	75 °C	70 °C	55 °C	100 °C	95 °C
Starting Volume:						
450 mL	91	82	54	90	109	623
400 mL	80	65	52	75	80	531
350 mL	69	56	35	64	69	437
300 mL	59	48	29	54	60	379
250 mL	51	40	22	45	50	323
200 mL	43	33	19	38	41	270
150 mL	36	26	16	31	32	221
100 mL	28	19	11	23	23	173
50 mL	18	12	7	14	13	106

13. Calculations

See the appropriate analytical method for information on calculations.

14. Records Management

For detailed instructions on how to generate and review data electronically, please refer to DEQ24-LAB-0007-SOP, Generating Instrument Data Packages Electronically.

For batches prepared prior to July 15, 2024, official extraction and bench sheet records are paper. Beginning July 15, 2024, once reviewed, paper bench sheet and extraction records are scanned and converted to electronic records which become the official record; the original reviewed paper documents are stored in extraction binders as convenience copies and will be periodically purged.

All data, notes, and comments recorded on the paper bench sheet will also be recorded in the LIMS.

After extraction, the paper bench sheet and other supporting documents (both paper and electronic) are then turned in for peer review. When the peer review is completed, the peer reviewer shall scan all paper documents and attach them, along with any other supporting electronic data/documents, to the batch in the LIMS. The peer reviewer shall then update the status of the samples in the batch to Prepared/Extracted in the LIMS if the status has not already been updated by an analyst.

All records are managed in accordance with the state record retention schedule, regardless of format. Access to the data is limited to DEQ employees since the facility is secured. Electronic documents are stored on internal servers prior to being transferred to the State Archives. Electronic documents are archived using the

Oregon Records Management System. ORMS is a statewide, cloud-based system for governing records and information management.

15. Method Performance

Method performance documentation is located on the Quality Assurance shared drive in the folder, under the specific analytical method or personnel. (\\deqlab1\QA\MethodValidationPersonnelIDOC)

16. Maintenance

All glassware must be inspected prior to use and chipped or broken glassware removed from work area. The electrical set up should be inspected for loose wires or tubing before being turned on.

17. Pollution Prevention

Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

For information about pollution prevention that may be applicable to laboratories and research institutions, consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington D.C. 20036, 202.872.4477.

18. Waste Management

The Oregon Department of Environmental Quality requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions.

Recycling is the first course of action for waste management in the laboratory. Since quantitative limitations combined with laboratory efficiency specify that all samples requiring volatile analysis should be collected in clean, un-used 40 mL glass vials, the laboratory has made a concerted effort to recycle these vials through the recycling program. All packing materials (i.e., cardboard boxes) and wastepaper are to be recycled.

Waste Disposal: All samples, standards and sample extracts that are no longer of use and that meet the criteria to be defined as hazardous waste must be treated and/or disposed of as such following all appropriate procedures/protocols in the Lab .

The aqueous liquid that remains in the sample vials following analysis should be removed from the vials and the vials can then be recycled. The liquid should be sparged with air in a fume hood until the contaminants are driven off and it can then be disposed of in the sink.

For further information on waste management consult *The Waste Management Manual for Laboratory Personnel*, available from the American Chemical Society.

19. Definitions

Standard Definitions applicable to laboratory quality systems can be found on in the Laboratory Quality Manual, DEQ91-LAB-0006-LQM.

20. Deviations from Referenced Methods

None.

21. References

SW-846 Chapter Four: *Organic Analytes*, Revision 4, February 2007

EPA Method 625.1: *Base/Neutrals and Acids by GC/MS*; 40CFRPart 136 Appendix A, December 2016

EPA Method 3500C, *Organic Extraction and Sample Preparation*, Revision 3, February 2007

EPA Method 3520C, *Continuous Liquid-Liquid Extraction*, Revision 3, December 1996.

EPA Method 3640A, *Gel Permeation Clean-up*, Revision, 1, September 1994

EPA Method 8000C*, Determinative Chromatographic Separations*, Revision 3, March 2003

EPA Method 8141B, *Organophosphorus Compounds by Gas Chromatography* Revision 2, February 2007

EPA Method 8151A, *Chlorinated Herbicides by GC Using Methylation or Pentafluorobenzylation Derivatization* Revision 1, February 2007

EPA Method 8270E, *Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)* Revision 6, June 2018

EPA Method 1613, *Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS*, October 1994.

EPA Method 1614A: *Brominated Diphenyl Ethers in Water Soil, Sediment and Tissue by HRGC/HRMS*, May 2010.

EPA Method 1668C: *Chlorinated Biphenyl Congeners in Water, Soil, Sediment, Biosolids, and Tissue by HRGC/HRMS,* April 2010.

EPA Method 1698*: Hormones in Water, Soil, Sediment, and Biosolids by HRGC/HRMS*, December 2007

22. Revision History

Revision	Date	Changes	Editor
1.0	02/2008	Developed Method	HAN
1.1	12/02/2009	Changed Formatting and Updated Corrections	HAN
	03/01/2010	Reviewed & updated formatting	SLK
1.2	12/20/2010	Added RapidVap Concentration Formatted for easier Reading	DLK
2.0	08/03/2011	Reviewed & removed outdated work instructions Added work instructions for 8270/8321, 8270-BNA, Dx/PAH, PCN. Pesticides (8081), PCB (8082), 1698, 1613, 1614, 1668 and associated cleanups.	ASN ASN
2.1	03/06/2015	Added Work instructions for 1699 and Amino cleanup. Updated procedures to reflect RapidVap and Element changes.	JU
2.2	03/22/2016	Minor editorial changes. Minor changes to work instructions Updated blank requirement to be < ½ LOQ	JU
2.3	12/07/2016	Updated 1698 work instructions, Appendix G & H, to note the 6 mo. expiration for pyridine after opening a new bottle. Updated Appendix K to current procedure.	JU
2.4	12/22/2017	Minor editorial changes. Minor changes to work instructions; added deviation for EPA 625.1, requirement to check the pH after extraction when extracting samples by 625.	CBT NAF
2.5	08/21/2018	Updated JSA to include condenser maintenance. Updated links, added reference to 625.1	CD
2.6	10/12/2020	Updated template, minor editorial changes	NAF
2.7	11/03/2021	Minor editorial changes. Added work instructions for 8270E QQQ Modified. Fixed Section 11.2 title, updated references to reflect change to 8270E.	ENF JU
2.8	11/10/2022	Clarified 12.1 Step 5, 12.2 Step 14, 12.2 Step 15	CS
3.0	12/04/2023	Replaced “fired” with “kilned” throughout. Added electronic access to SDSs in Safety section. Added capillary tubes to Equipment and Procedure sections. Corrected spelling of “pipette” in Procedure section. Updated Procedure sections to reflect current practices. Updated Appendices B, C, D, E, F, G, H, I, M, N to reflect current practices. Removed references to and instructions for EPA 1699 (no longer in use) and renamed appendices accordingly. Updated language in Records Management section to align with the Chromatography Data Review SOP. Minor editorial edits. Updated location of method performance data to the QA drive.	JC NM
	01/24/2024	Conversion to new template	Ryan Lagazon

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	02/02/2024	Additional edits made after conversion to the new template: Replaced “round bottom” with “flat bottom” throughout. Removed work instructions and references for methods no longer in use: NWTPH-Dx and PAH (8270 SIM), Polychlorinated Naphthalenes (PCN), Pesticides (8081) / PCB Aroclors (8082). Added the following items to Equipment and Supplies section: Condensers, Pasteur pipettes (glass), 250 mL flat bottom flasks, Aluminum foil, 1L Amber jars with Teflon lined caps, pH Strips (0-14, 7.0-14, and 1.7-3.8), Volumetric flasks, 1mL, 2mL, Gas tight syringes (various capacities), Auto-sampler vials, caps, and inserts. Added a note in Section 6 referring to additional supplies used specific work instructions. Added the following items to Reagents section: Acetone: boiling point 56.2°C, DI Water, Potassium dihydrogen citrate.	JC
3.1	02/04/2025	Added calibration verification, acceptance criteria and documentation information for concentration systems – Sections 6.0, 10.0, 12.2, Appendices B-K Added Buchi concentration procedure – Section 12.2 and Appendix B Added details for electronic records process that replaced the historic paper process – section 14.0 Minor editorial changes throughout. Removed hyperlinks. Added nonane for HRMS to Reagents list	ENF, BT
4.0	03/17/2025	Added specifics for Buchi concentration in section 12 and Appendix B. Added Table 6. Büchi concentration program "PSP" in Appendix B. Replaced “CLE” with “CLLE” throughout.	JC
4.1	05/06/2025	Fixed pagination error.	SLK

Appendix A: Job Safety Assessment

DEQ Hazard Analysis	Activity:	Organics Extraction
	Program/Location	DEQ Laboratory
	Position # (s):	0339, 3009
	Analysis by:	LSO
	Date:	11/30/2020

Required PPE:

Gloves - Nitrile	Gloves - Thermal	Gloves – Cut Resistant	Safety Glasses	Safety Goggles	Blast Shield	Lab Coat

Required/Recommended Trainings:

1. Review of Chemical Hygiene Plan
2. SIM-plicity Training
3. Review of relevant lab SOPs
4. Compressed Gas Safety Training
5. PPE Policy Review

TASK	HAZARDS	SEVERITY	CONTROLS
1. Computer Use/Data Entry	Repetitive motion injuries	Low	Follow ergonomic recommendations
2. Sample removal from refrigerators	(E) – Wet and cold temperatures	Medium	Gloves
	(CW) – Glass shards from broken sample vials; unknown samples and sample preservatives	Medium	Inspect extracts for hazards prior to removing contents

3. Sample Extraction	(CW) – Glass shards from broken sample vials, glass pipettes or needles on microsyringes.	High	Inspect glassware and extract vials prior to handling. Store syringes in a safe manner to prevent accidental punctures. Handling condensers requires the use of cut-resistant gloves.
	(E) – Solvent, acid or heavy metal exposure, unknown sample contaminant exposure; Compressed gases and cryogenics.	High	Always work in approved hood wearing appropriate PPE: lab coat, safety glasses/goggles, gloves suitable for chemicals in use. Some procedures may require using a blast shield. Proper storage/use of gas cylinder & use proper technique when transporting cylinders. Use only compatible regulators with proper fittings. Test all connections for leaks. Wear thermal gloves when handling liquid nitrogen
	(CW) – shock hazard from electrical components	Low	Ensure instruments are turned off and unplugged when performing any work which may result in a shock hazard, such as board replacement or repair.

* Codes for Potential Hazards

(BIO) Biological		(CO) Caught On		(FS) Fall – Same Level
(CB) Contacted By		(CW) Contact With		(OE) Overexertion
(CBT) Caught Between		(E) Exposure		(SA) Struck Against
(CI) Caught In		(FB) Fall To Below		(SB) Struck By

Risk Severity Level Key	Low	Medium	High	Very High

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Appendix B: Work Instruction: Pesticides by GC/MS & LC/MS/MS (8270/8321)

CLLE Set-up/ Sample Preparation

- Batch the samples in Element, print out the bench sheet, and export the bench sheet as an XLS file to open in Excel.
- Fill 3 x 1 L DCM-rinsed amber jars with 1 L of DI water for QC.
- Shake samples and pour off to as close to 1000 mL as possible, while still leaving room for reagents/preservatives; discard extra sample down drain.
- Open the exported bench sheet in Excel on the computer in extractions. Weigh each sample jar and enter this weight into the spreadsheet under the “Bottle Weight Full” column.
- Spike appropriate QC samples (8270 Blank Spike & Matrix Spike, 8321 Blank Spike & Matrix Spike) and add surrogate to all QC and samples in the amber jars using syringes.
- Salt samples and QC with PSP salt mixture:
    - 0.5 g EDTA
    - 9.4 g Potassium dihydrogen citrate
    - 60 g NaCl
- Acidify all samples and QC to pH ≤ 2 with ~3 mL concentrated Phosphoric Acid.
- Check pH using a few drops of sample removed with a pipette/capillary tube, on a narrow range pH test strip. If a sample requires additional H_3PO_4 record additional mL in the ‘comments’ section on the bench sheet.
- Set up CLLE’s with flat bottoms, adding 150 mL of DCM to each set-up:
    - To set up CLLE’s, place 2-3 boiling chips into the flat bottom (labeled with sample ID) and affix the flask to the bottom ground glass joint of the CLLE, twisting slightly to seat flat bottom securely. Place CLLE upright in hood with the flat bottom flask resting in a heating mantle and back of CLLE resting against chain clamp. Chain CLLE in place, making sure the CLLE is as straight as possible. Pour 150 mL of DCM into the top opening of the CLLE.
- Label the flat bottoms with the corresponding sample or QC ID.
- Pour samples into CLLE’s, rinsing the amber jar 3x with 20 mL aliquots of DCM. Rinse CLLE ground glass joint with DIW squeeze bottle.
- Adjust solvent level in flat bottoms by tilting the CLLE or adding DI water to the CLLE; solvent level should be even with heating mantle.
- Top with condensers, turn on water and heat (should be about one drip per second for each sample).
- Once solvent is boiling, cover flat bottoms with aluminum foil and allow to cycle for 18-24 hours. Record start/end time on bench sheet.
- After 18-24 hours, turn off heat and allow flat bottoms to cool; remove condensers and turn off water.
- Remove flat bottoms from CLLE’s, rinsing CLLE joint (rinsate into sample) with DCM.
- Weigh the empty jars with original caps and enter the weight into the Excel spreadsheet in the “Bottle Weight Empty” column; this will automatically calculate the initial volume. Save the spreadsheet and close it. Import the spreadsheet back into Element to populate the initial volume for each sample. Print and attach to bench sheet.

Buchi/ 8270 Final Volume – Concentration Procedure

Before concentration using the Buchi, ensure that full method validation has been completed.

- Gather required glassware and equipment: Büchi tubes, tube racks, and sodium sulfate funnels.
- 3x DCM rinse the Büchi tubes, label with the sample IDs using a sharpie, and top with sodium sulfate funnels that have been rinsed with DCM.
- For each sample, pour the contents of the flat bottom through the sulfate funnel, collecting in the Büchi tube. Rinse the flat bottom 3x with small aliquots of DCM and pour rinsate through funnel into the Büchi tube after each rinse.
- Rinse sulfate funnel into the Büchi tube with an additional 20mL aliquot of DCM using a tilting repeating dispenser.
- Turn on Büchi concentrator, vacuum pump, and chiller. Fill cells with DI water to the max fill line (**Do not operate Büchi without water in each cell**). Preheat water bath to 30°C and the top heated vacuum plate to 25°C. Set the chiller to 0°C.
- Record the set temperature and the adjusted temperature with correction factor on the bench sheet. Acceptance criteria for the Büchi concentration system is ±5°C from set point, per reference method.
- Place Büchi tubes into the Büchi cells, making sure they are pushed down all the way and seated correctly in the cell. Place the top, heated vacuum plate over the Büchi tubes and fasten to finger tight with the screw nuts to ensure that a seal has been formed at the top of the tubes with the vacuum plate.
- On the Büchi touch screen select the "**PSP**" program from the "Favorites" or "Methods" menu and press start. The program will run for 110 minutes.
    - If at the end of the program the samples require further concentration to reach 1mL, use the "manual" or "timer" settings to set the Büchi parameters (pressure, temperature, rotation) to the same as step 5 in the program (see Table 6) and concentrate until the desired volume is reached.
    - The Büchi concentrator operates in such a way that the solvent will be retained in the nipple for a short period of time before completely evaporating (~5 minutes) due to the cold zone around the nipple. Some samples may reach ~1mL before the rest of the samples in the Büchi concentrator. If this happens, pause the program by pressing the "Aerate" button, turn off the rotation, remove the finished sample(s), and either plug the vacuum holes with the provided plugs or place an empty Büchi tube in place of the removed sample(s). In either case make sure that the top vacuum plate is screwed down so that the pressure is evenly distributed across the plate and a seal is formed around the remaining tubes (e.g. two tubes, one in each middle position or four tubes, one in each corner position). To restart the program press "Hold off" and turn the rotation on to the appropriate setting.
- After samples reach just below the top of the nipple (~1mL), remove them from the Büchi concentrator.
- Using a disposable pipette, transfer sample to a 2mL volumetric flask, rinse the Büchi tube 3 times using DCM, adding rinsate to the flask; use the last rinse to bring sample to the final volume, 2mL.
- Transfer sample to an 8270 labeled amber autosampler vial.
- Place vials in the "Molybdenum" fridge, in the 8270 section.
- Empty the primary and secondary collection flasks on the Büchi into the chlorinated waste.

Table 6. Büchi concentration program "PSP"

Step	Vacuum (mbar)	Timer (minutes)	Rotation (rpm)	Heating bath (°C)	Chiller (°C)
1	1000-750	3	200	30	0
2	750	2	200	30	0
3	750-500	5	200	30	0
4	500-350	10	225	30	0
5	250	90	250	30	0

RapidVAP/ 8270 Final Volume – Alternative Concentration Procedure

Before concentration using the RapidVap, ensure that full method validation has been completed.

- DCM Rinse and label large RapidVAP (RV) tubes, and top with sulfate funnels that have been rinsed with DCM.
- For each sample, pour the contents of the flat bottom through the sulfate funnel. Rinse the flat bottom 3x with small aliquots of DCM and pour rinsate through funnel.
- Rinse the sulfate funnel with an additional 20 mL aliquot of DCM.
- Preheat RapidVap. Read and record the set temperature and the adjusted temperature with correction factor of the RapidVap heat block on the bench sheet. Acceptance criteria is ±5°C from set point, per reference method.
- Process on RapidVap as follows:
  - 40 ºC and 70 os for ~30-45min, rinsing with DCM when liquid level reaches 1/3 bevel. ***Note**: Nitrogen level on RapidVap should be 10 psi.*
  - 40 ºC and 70 os for 2-5 min, remove when sample is ~ 1 mL.
- Using a disposable pipette, transfer sample to a 2 mL volumetric flask, rinse the tube 3 times using DCM; use each rinsate to bring sample to the final volume, 2 mL.
- Transfer sample to an 8270 labeled auto sampler vial and cap.
- Vials are then placed into the “Molybdenum” fridge, in the 8270 section.

8321 Solvent Exchange/Final Volume

- Prepare small centrifuge tubes, one for each 8321 sample or QC, as follows:
  - Label the tubes with the sample ID’s.
  - Using a 500 µL syringe, put ~350 µL of Acetonitrile into each tube.
  - Mark the height of the Acetonitrile on the side of each tube, using a permanent marker.
- Rinse a 1 mL syringe with DCM and remove exactly 1mL of sample from each of the 8270 labeled auto sampler vials, dispensing into the appropriate centrifuge tube.
  - Rinse the syringe with DCM three times between each sample, discarding rinsate into appropriate solvent waste container.
  - Remaining sample volume in auto sampler vials can now be placed back into sample freezer for 8270 analyses.

- After dispensing all samples, rinse the syringe first with DCM, and then with Acetonitrile. Use 1 mL syringe to add ~2 mL of Acetonitrile to each tube, adding it forcefully enough to mix the sample with the solvent thoroughly.
- Preheat N-evap to 60-65 ºC. Read and record the temperature of the N-Evap water bath using a NIST thermometer stored in extractions on the bench sheet.
- Load the tubes onto N-evap that has been preheated and apply a gentle stream of nitrogen.
    - Monitor the temp, as the DCM will not be removed if the temp falls.
- Allow the samples to reduce until they reach the mark on the tube.
- Using a 500 µL syringe, draw the sample up, rinse the tube 3 times with Acetonitrile, drawing each rinse into the syringe, for a final volume of 500 µL.
- Transfer each sample into an appropriately 8321 labeled auto sampler vial and cap.
- Place vials in the “Molybdenum” fridge, in the 8321 section.

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Appendix C: Work Instruction: Semi-Volatiles, Acid/Base-Neutral by GC/MS (8270)

- Batch the samples in Element, print out the bench sheet, and export the bench sheet as an XLS file to open in Excel.
- Fill 3 DCM-rinsed 1 L amber jars with 1 L of DI water for QC.
- Shake samples and pour off to as close to 1000 mL as possible, while still leaving room for reagents/preservatives; discard extra sample down drain.
- Open the exported bench sheet in Excel on the computer in extractions. Weigh each sample jar and enter this weight into the spreadsheet under the "Bottle Weight Full" column.
- Spike appropriate QC samples (LCS, LCSD, MS, MSD) and add surrogate to all QC and samples in the amber jars.
- Salt samples and blanks with 8270 salt mixture:
    - 0.5 g EDTA
    - 60 g NaCl
- Acidify all to pH 2 with ~1.1 mL of phosphoric Acid.
- Check pH using a few drops of sample removed with a pipette/capillary tube, on a narrow range pH test strip. If a sample requires additional acid, record additional mLs in the 'comments' section on the bench sheet.
- Rinse CLLE's and flat bottoms with 3 aliquots of 20 mL DCM, discarding rinsate in appropriate container.
- Set up CLLE's with flat bottoms, adding 150 mL of DCM to each set-up:
    - To set up CLLE's, place 2-3 boiling chips into the flat bottom and affix flat bottom-to-bottom ground glass joint of CLLE, twisting slightly to seat flat bottom securely, and place CLLE upright in hood, with flat bottom resting in a heating mantle, and back of CLLE resting against chain clamp. Chain CLLE in place, making sure the CLLE is as straight as possible. Pour 150 mL of DCM into the top opening of the CLLE.
- Label the flat bottoms with the corresponding sample or QC ID.
- Pour samples into CLLE's, rinsing the amber jar 3x with 20 mL aliquots of DCM. Rinse CLLE ground glass joint with DIW squeeze bottle.
- Adjust solvent level in flat bottoms by tilting the CLLE or adding DI water to the CLLE; solvent level should be even with heating mantle.
- Top with condensers, turn on water and heat (should be about one drip per second for each sample).
- Once solvent is boiling, cover flat bottoms with aluminum foil and allow to cycle for 18-24 hours. Record start time on bench sheet.
- Weigh the empty jars with original caps and enter the weight into the Excel spreadsheet in the "Bottle Weight Empty" column; this will automatically calculate the initial volume. Save the spreadsheet and close it. Import the spreadsheet back into Element to populate the initial volume for each sample. Print and attach to bench sheet.
- After 18-24 hours, turn off heat and allow flat bottoms to cool; remove condensers and turn off water.
- Remove condensers from CLLE's and check that pH<2; note on bench sheet if not. Basify sample with ~4.4 mL of 10 N Sodium Hydroxide using a new 5 mL disposable pipette for each sample. Use the pipette to mix the sample.

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- Check that pH ≥11, using a few drops of sample on a pH test strip. If a sample requires additional NaOH record additional mLs in the ‘comments’ section on the bench sheet.
- Rinse top joints (rinsate into sample) well with DI water and re-install condensers.
- Run CLLE’s for another 18-24 hours.
- After 18-24 hours, turn off heat and allow flat bottoms to cool; remove condensers and turn off water. Check that pH ≥11; note on benchsheet if not.
- Remove flat bottoms from CLLE’s, rinsing CLLE joint (rinsate into sample) with DCM.

RapidVAP/Final Volume

- DCM Rinse and label large RV tubes, and top with sulfate funnels that have been rinsed with DCM.
- For each sample, pour the contents of the flat bottom through the sulfate funnel, rinsing the flat bottom 3x with small aliquots of DCM.
- Rinse the sulfate funnel with an additional 20mL aliquot of DCM.
- Preheat RapidVap. Read and record the set temperature and the correction factor adjusted temperature of the RapidVap heat block on the bench sheet.
- Process on RapidVap as follows:
  - 40 ºC and 70 os for 30-45 min, rinsing with DCM when liquid level reaches 1/3 bevel. ***Note**: Nitrogen level on RapidVap should be 10 psi.*
  - 40 ºC and 7 0os for 2-5min, remove when sample is ~ 1 mL.
- Let tubes sit in hood until solvent has evaporated to 0.7 mL.
- Using a disposable pipette, transfer sample to a 1 mL volumetric flask, rinse the tube 3 times using DCM, adding to volumetric each time. Add 100 uL of Acetone to the flask and fill any remaining volume with DCM to bring up to 1 mL (Final Volume solvent is 10% Acetone in DCM).
- Dispense into labeled auto sampler vial and cap.

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Appendix D: Work Instruction: Steroids (1698) – Effluent

CLLE Set-up/ Sample Preparation

- Batch the samples in Element, print out the bench sheet, and export the bench sheet as an XLS file to open in Excel.
- Fill 2 DCM Rinsed 1 L amber jars with 1 L of DI water for QC.
- Shake samples and pour off to as close to 1000 mL as possible; discard extra sample down drain.
- Open the exported bench sheet in Excel on the computer in extractions. Weigh each sample jar and enter this weight into the spreadsheet under the “Bottle Weight Full” column.
- Rinse CLLE’s and round bottoms with 3 aliquots of 20 mL DCM, discarding rinsate in appropriate container.
- Set up CLLE’s with round bottoms, adding 150 mL of DCM to each set-up:
    - To set up CLLE’s, place 2-3 boiling chips into the round bottom and affix round bottom-to-bottom ground glass joint of CLLE, twisting slightly to seat round bottom securely. Place CLLE upright in hood with round bottom resting in a heating mantle and back of CLLE resting against chain clamp. Chain CLLE in place, making sure the CLLE is as straight as possible. Pour 150 mL of DCM into the top opening of the CLLE.
- Label the round bottoms with the corresponding sample or QC ID.
- Spike appropriate QC samples (Blank Spike, MS, MSD) and add surrogate to all QC and samples in the amber jars using HRMS syringes (kept in standards prep). Pour samples into CLLE’s, rinse the amber jar 3x with 20 mL aliquots of DCM, adding each rinse into the CLLE. Rinse CLLE ground glass joint with DIW squeeze bottle.
- Adjust solvent level in round bottoms by tilting the CLLE or adding DI water to the CLLE; solvent level should be even with heating mantle.
- Top with condensers, turn on water and heat (should be about one drip per second for each sample).
- Once solvent is boiling, cover round bottoms with aluminum foil and allow to cycle for 18-24 hours. Record start/end time on Bench sheet.
- Weigh the empty jars with original caps and enter the weight into the Excel spreadsheet in the “Bottle Weight Empty” column; this will automatically calculate the initial volume. Save the spreadsheet and close it. Import the spreadsheet back into Element to populate the initial volume for each sample. Print and attach to bench sheet.
- After 18-24 hours, turn off heat and allow round bottoms to cool; remove condensers and turn off water.
- Remove round bottoms from CLLE’s, rinsing CLLE joint (rinsate into sample) with DCM.

RapidVAP

- DCM Rinse and label large RapidVAP (RV) tubes, and top with sulfate funnels that have been rinsed with DCM.
- For each sample, pour the contents of the round bottom through the sulfate funnel. Rinse the round bottom 3x with small aliquots of DCM and pour rinsate through funnel.
- Rinse the sulfate funnel with an additional 20 mL aliquot of DCM.

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- Preheat RapidVap. Read and record the set temperature and the correction factor adjusted temperature of the RapidVap heat block on the bench sheet.
- Process on RapidVap as follows:
    - 40 ºC and 58 os for 30 min ***Note****: Nitrogen level on RapidVap should be 5 cfh.*
    - **Add 10uL of Internal Standard to each sample with a syringe and rinse sides of tubes with DCM.**
    - 40 ºC and 70 os for 15-30min, rinsing with DCM when liquid level reaches bevel.
    - 40 ºC and 70 os until samples are completely dry. Remove each sample from RapidVap as soon as it is dry; do not allow dry samples to cook on RapidVap.
    - If there is dried sample on the bevel of the RV tube, rinse down with DCM and process again to dryness.

Final Volume

- Final Volume should be performed in a hood, as chemicals used are very toxic.
- Carefully add 250 uL of BSFTA to each sample with a syringe, making sure to get it only into the tip of the RV tube. Pour Pyridine into a small, clean test tube, and then add 250 uL of Pyridine to each sample with a syringe. ***Note****: Pyridine has an expiration date of 6 months after opening container, anything beyond that has been deemed unacceptable and should not be used for this test. Verify the Pyridine has been logged in correctly and is not expired.*
    - Both reagents can be found in the standards prep room.
- Vortex each sample for 10 seconds to thoroughly mix the derivatization reagents.
- Let sit covered under tin foil for one hour.
- Add 500 uL of Hexane to each RV tube using a syringe, taking care to add it to the tip of the RV tube.
- Using a long Pasteur pipette gently mix the reagents in the tip of the RV tube and transfer into a labeled pre-kilned auto-sampler vial.

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Appendix E: Work Instruction: Steroids (1698) –Source and Surface Water

CLLE Set-up/ Sample Preparation

- Batch the samples in Element, print out the bench sheet, and export the bench sheet as an XLS file to open in Excel.
- Fill 2 DCM Rinsed 1 L amber jars with 1 L of DI water for QC.
- Shake samples and pour off to as close to 1000 mL as possible; discard extra sample down drain.
- Open the exported bench sheet in Excel on the computer in extractions. Weigh each sample jar and enter this weight into the spreadsheet under the “Bottle Weight Full” column.
- Rinse CLLE’s and round bottoms with 3 aliquots of 20 mL DCM, discarding rinsate in appropriate container.
- Set up CLLE’s with round bottoms, adding 150 mL of DCM to each set-up:
  - To set up CLLE’s, place 2-3 boiling chips into the round bottom and affix round bottom-to-bottom ground glass joint of CLLE, twisting slightly to seat round bottom securely. Place CLLE upright in hood with round bottom resting in a heating mantle and back of CLLE resting against chain clamp. Chain CLLE in place, making sure the CLLE is as straight as possible. Pour 150 mL of DCM into the top opening of the CLLE.
- Label the round bottoms with the corresponding sample or QC ID.
- Spike appropriate QC samples (Blank Spike, MS, MSD) and add surrogate to all QC and samples in the amber jars using HRMS syringes (kept in standards prep). Pour samples into CLLE’s, rinse the amber jar 3x with 20 mL aliquots of DCM, adding each rinse into the CLLE. Rinse CLLE ground glass joint with DIW squeeze bottle.
- Adjust solvent level in round bottoms by tilting the CLLE or adding DI water to the CLLE; solvent level should be even with heating mantle.
- Top with condensers, turn on water and heat (should be about one drip per second for each sample).
- Once solvent is boiling, cover round bottoms with aluminum foil and allow to cycle for 18-24 hours. Record start/end time on Bench sheet.
- Weigh the empty jars with original caps and enter the weight into the Excel spreadsheet in the “Bottle Weight Empty” column; this will automatically calculate the initial volume. Save the spreadsheet and close it. Import the spreadsheet back into Element to populate the initial volume for each sample. Print and attach to bench sheet.
- After 18-24 hours, turn off heat and allow round bottoms to cool; remove condensers and turn off water.
- Remove round bottoms from CLLE’s, rinsing CLLE joint (rinsate into sample) with DCM.

RapidVAP

- DCM Rinse and label large RapidVAP (RV) tubes, and top with sulfate funnels that have been rinsed with DCM.
- For each sample, pour the contents of the round bottom through the sulfate funnel. Rinse the round bottom 3x with small aliquots of DCM and pour rinsate through funnel.
- Rinse the sulfate funnel with an additional 20 mL aliquot of DCM.

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- Preheat RapidVap. Read and record the set temperature and the correction factor adjusted temperature of the RapidVap heat block on the bench sheet.
- Process on RapidVap as follows:
  - 40 ºC and 58 os for 30 min ***Note****: Nitrogen level on RapidVap should be 5 cfh.*
  - **Add 10 uL of the Internal Standard to each sample with a syringe and rinse sides of tubes with DCM.**
  - 40 ºC and 70 os for 15-30min, rinsing with DCM when liquid level reaches bevel.
  - 40 ºC and 70 os until samples are completely dry. Remove each sample from RapidVap as soon as it is dry; do not allow dry samples to cook on RapidVap.
  - If there is dried sample on the bevel of the RV tube, rinse down with DCM and process again to dryness.

Final Volume

TO BE DONE IN HOOD

- Carefully add 100 µL of BSFTA to each sample directly into the tip of the RV tube using a syringe.
- Add 100 µL of Pyridine to each sample. ***Note****: Pyridine has an expiration date of 6 months after opening container, anything beyond that has been deemed unacceptable and should not be used for this test. Verify the Pyridine has been logged in correctly and is not expired.*
  - Pour Pyridine into a small test tube and syringe it into samples, being mindful of toxic fumes.
- Vortex each sample in the hood for 10 seconds to thoroughly mix the derivatization reagents.
- Let sit covered under aluminum foil for 1 hour.
- Using a long pipette, gently mix the reagents in the tip of the RV tube. Pipette the sample into a labeled (handwritten) auto sampler vial with KILNED inserts.
  - Reagents are located in the standards prep room.
  - Surrogates and spikes are located in the small extractions refrigerator.

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Appendix F: Work Instruction: Dioxins/Furans (1613), PBDE (1614), PCB Congeners (1668)

CLLE Set-up/ Sample Preparation

- Batch the samples in Element, print out the bench sheet, and export the bench sheet as an XLS file to open in Excel.
- Fill 2 DCM Rinsed 1 L amber jars with 1 L of DI water for QC.
- Shake samples and pour off to as close to 1000 mL as possible; discard extra sample down drain.
- Open the exported bench sheet in Excel on the computer in extractions. Weigh each sample jar and enter this weight into the spreadsheet under the “Bottle Weight Full” column.
- Rinse CLLE’s and flat bottoms with 3 aliquots of 20 mL DCM, discarding rinsate in appropriate container.
- Set up CLLE’s with flat bottoms, adding 150 mL of DCM to each set-up:
    - To set up CLLE’s, place 2-3 boiling chips into the flat bottom and affix flat bottom-to-bottom ground glass joint of CLLE, twisting slightly to seat flat bottom securely. Place CLLE upright in hood with flat bottom resting in a heating mantle and back of CLLE resting against chain clamp. Chain CLLE in place, making sure the CLLE is as straight as possible. Pour 150 mL of DCM into the top opening of the CLLE.
- Label the flat bottoms with the corresponding sample or QC ID.
- Spike appropriate QC samples (Blank Spike, MS, MSD) and add surrogate to all QC and samples in the amber jars using HRMS syringes (kept in standards prep). Pour samples into CLLE’s, rinse the amber jar 3x with 20 mL aliquots of DCM, adding each rinse into the CLLE. Rinse CLLE ground glass joint with DIW squeeze bottle.
- Adjust solvent level in flat bottoms by tilting the CLLE or adding DI water to the CLLE; solvent level should be even with heating mantle.
- Top with condensers, turn on water and heat (should be about one drip per second for each sample).
- Once solvent is boiling, cover flat bottoms with aluminum foil and allow to cycle for 18-24 hours. Record start/end time on Bench sheet.
- Weigh the empty jars with original caps and enter the weight into the Excel spreadsheet in the “Bottle Weight Empty” column; this will automatically calculate the initial volume. Save the spreadsheet and close it. Import the spreadsheet back into Element to populate the initial volume for each sample. Print and attach to bench sheet.
- After 18-24 hours, turn off heat and allow flat bottoms to cool; remove condensers and turn off water.
- Remove flat bottoms from CLLE’s, rinsing CLLE joint (rinsate into sample) with DCM.
- Add Clean-up spike to each flat bottom flask. **Note:** 1613/1314/1668 each has their own clean-up spike that should be added prior to the RapidVap section.

RapidVAP

- DCM Rinse and label large RapidVAP (RV) tubes, and top with sulfate funnels that have been rinsed with DCM.
- For each sample, pour the contents of the flat bottom through the sulfate funnel. Rinse the flat bottom 3x with small aliquots of DCM and pour rinsate through funnel.

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- Rinse the sulfate funnel with an additional 20 mL aliquot of DCM.
- Add 30 mL Hexane to each sample and cap with a Teflon lid.
- Preheat RapidVap. Read and record the set temperature and the correction factor adjusted temperature of the RapidVap heat block on the bench sheet.
- Process on RapidVap as follows:
    - 40 ºC and 70 os for 40-60 min, rinsing with Hexane when liquid level reaches 1/3 bevel. ***Note****: Nitrogen level on RapidVap should be 10 psi.*
    - 70 °C and 70 os for 2-5 min, remove when samples are completely exchanged into Hexane and ~1 mL.
- Proceed to multi-layer silica gel and florisil cleanup work instructions (**Appendix G**).
    - If samples are not to be put through multi-layer silica gel and florisil cleanup columns immediately, pipette samples into labeled 4 mL amber glass vials, rinsing the RV tube 3x with Hexane, and adding rinsate to vial.

Appendix G: Work Instruction: Clean-up Procedure, Silica Gel/Florisil (1613, 1614, 1668)

Additional Equipment & Supplies

- Pre-activated Florisil,
- Vacuum Adapter
- Ring Stand, Clamp
- Multi-Layer Silica Column
- 6.35mm x 10mm reducing union
- 250 mL separatory funnel
- 24mm/24mm PP viton connector
- Boling tube
- Erlenmeyer flask
- 250 mL flat bottom

Column Set-Up

- Take a clean florisil tube, break open an ampule of Pre-Activated Florisil and pour it into the tube.
- Using a 6.35mm x 10mm reducing union, insert the small end of the Florisil tube into the reducing union on the top. Attach a vacuum adapter with a stopcock into the bottom or large end of the reducing union. Tighten both ends of the reducing union, being careful to check for glass-on-glass contact between the Florisil tube and the vacuum adapter.
- Attach another reducing union on the top of the Florisil tube and tighten.
- Set up the tube on a ring stand in the hood, clamping it at the bottom of the reducing union, so that a small beaker will fit under the column.
- Attach a 6.35 mm Multi-Layer Silica Gel column on the top of the upper reducing union and tighten. Affix a 250 mL separatory funnel with a stopcock on the top of the column using a 24mm/24mm PP Viton Connector, threaded bottom of the separatory funnel to the top of the silica column.

Column Conditioning

- Place a small beaker under each column.
- Add 50 mL Hexane to each separatory funnel and adjust the top stopcock to drip slowly but steadily.
- Stop by closing stopcock when the Hexane level is about 1cm above the bed of the multi-layer silica column.

Loading Samples

- Remove the separatory funnel and connector.
- Remove the waste beakers and dispose of the hexane waste in the appropriate container.
- Place clean 250 mL flat bottom flasks labeled with PCB and sample ID under each column, lowering the column to fit inside of the joint of the flat bottom.
- Pipette the sample into the top of the column. Turn the bottom stopcock on to a steady drip and rinse the vial 3 times with Hexane, adding rinsate to column with a pipette.

- Turn the stopcock off when the liquid level is just above the top of the silica bed. Be careful not to let bed go dry after the conditioning step.

Column Elution (PCB Fraction)

- Add 5 mL hexane to the top of the column. Open the stopcock to a steady drip and allow about half of the hexane to pull through the column.
- Reattach the separatory funnel and connector and add 170 mL Hexane to the separatory funnel.
- Adjust top stopcock so that upper drip is about the same as lower drip.
- Allow solvent to be gravity fed through the column until ~1 cm above the silica bed, then turn off the bottom stopcock.

A. PBDE Fraction

- Loosen the reducing union between the florisil and silica columns and remove the silica column and separatory funnel, repositioning it over a 125 mL flask labeled with PBDE and sample ID.
- Add 100 mL 30:70 DCM:Hexane to the separatory funnel and adjust the stopcock to a steady drip.
- Allow it to drain until all the liquid is pulled through the column. Remove sample from under column and cap for refrigerated storage or proceed with concentration.

PBDE Fraction Concentration

- Preheat N-evap to 38 °C. Read and record the temperature of the N-Evap water bath using a NIST thermometer stored in extractions on the bench sheet.
- Pour ~50 mL of sample into a clean, labeled Boling tube and reduce on an N-evap set at 38 °C using gentle stream of nitrogen. Add the remainder of the sample to the Boling tube as it is reduced.
- Rinse sample flask 3 times with Hexane and add rinsate to the Boling tube.
- Continue reducing sample till it is approximately 1 mL and remove from N-evap.
- Proceed with samples to Basic Alumina Column cleanup (**Appendix H**).

B. PCB Fraction

- Place a clean, empty 6.35 mm column on top of the florisil column, tightening the reducing union. Add 25 mL 2% DCM in Hexane to the empty column.
- Open the stopcock to allow a steady drip of the solvent into the PCB labeled flat bottom.
- Close the stopcock when the liquid level is just above bed.
- Raise the column and remove the flat bottom flask. Cap flask for refrigerated storage or proceed with concentration.

PCB Fraction Concentration

- Preheat N-evap to 60 °C. Read and record the temperature of the N-Evap water bath using a NIST thermometer stored in extractions on the bench sheet.
- Pour ~50 mL of sample into a clean, labeled Boling tube and reduce on an N-evap set at 60 °C using gentle stream of nitrogen. Add the remainder of the sample to the Boling tube as it is reduced.
- Rinse sample flask 3 times with Hexane and add rinsate to the Boling tube.

- Continue reducing sample till it is approximately 1 mL and remove from N-evap.
- Proceed with samples to Basic Alumina Column cleanup (**Appendix H**).

C. Dioxin Fraction

- Place a clean Boling tube labeled with Dioxin and the sample ID under the Florisil column, adjusting the column so that the bottom of the vacuum adapter is inside the top of the Boling tube.
- Add 50 mL of DCM to the top of the empty column and adjust the stopcock to a steady drip.
- Allow it to drain until all the liquid has pulled through the column and has stopped dripping. Remove sample from under column, cap for refrigerated storage or proceed with concentration.

Dioxin Fraction Concentration

- Preheat N-evap to 38 °C. Read and record the temperature of the N-Evap water bath using a NIST thermometer stored in extractions on the bench sheet.
- Place Boling tube containing sample on an N-evap heated to approximately 38 °C. Using a gentle stream of nitrogen, blow the samples down to about 1 mL.
- Add about 5 mL of Hexane to the Boling tube and place back on the N-evap, reducing to approximately 1 mL again and remove from N-evap.
- Proceed with samples to Dual-Layer Carbon Reversible Column cleanup (**Appendix I**).

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Appendix H: Work Instruction: Clean-up Procedure and Final Volume, Basic Alumina (1614, 1668)

Additional Equipment & Supplies

- Special thimbles
- Basic Aluminum Oxide
- 50 mL glass reservoir
- Solid phase extraction manifold
- Boling tubes
- 60 mL VOAs
- Clear autosampler vials
- N-nonane

Apparatus Setup

- Assemble special thimbles with stopcocks. The thimbles should be kilned prior to use.
- Insert glass wool plug and fill with ~3 g Basic Aluminum oxide using a premeasured test tube.
- Place on empty manifold and top each thimble with a 50 mL glass reservoir.

Column Conditioning

- Pour 50mL Hexane into reservoir and adjust stopcock to have a steady drip.
- Allow to drain to ~1 cm above top of bed and close stopcock.

Loading Samples

- Label 1 Boling tube and 1 60 mL VOA for each sample with sample ID and fraction #1 and #2, respectively.
- Load Boling tube set #1 into manifold sleeve, place in manifold and put top with thimbles on it in place.
- Pipette the sample from the Boling tube into the special thimble.
- Turn on stopcock to a steady drip of 1-2 drips per second, and rinse Boling tube 3 times with ~0.2 mL Hexane each rinse, adding each rinse to the special thimble.
- Measure 50 mL of 50:50 DCM:Hexane into a graduated cylinder, and when liquid level is close to top of alumina bed, slowly pour in ~2 mL of the DCM:Hexane, and allow to drain until ~2 cm above top of bed.
- Add the remainder of the 50:50 DCM:Hexane to the reservoir.
- Allow to drain until the liquid level reaches the top of the thimble.
- Turn off stopcocks and remove Boling tube set #1, replacing with VOA set #2 of labeled tubes (be careful that the samples are going into the right tubes).
- Add 25 mL 50:50 DCM:Hexane to each reservoir and turn on stopcocks to steady drip and allow to drip until liquid is pulled through and dripping stops.

Prepping for Final Volume

- Preheat N-evap to 38 ºC. Read and record the temperature of the N-Evap water bath using a NIST thermometer stored in extractions on the bench sheet.
- Load Boling tubes onto N-EVAP set at 38 ºC and blow down until tube #1 and #2 of each sample can be combined into 1 tube.

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- o Quantitatively transfer the contents of tube #2 into tube #1, rinsing tube #2 3 times with DCM.
- o Continue to blow down samples and raise the temperature to Hexane (60 °C).
- o Rinse boling tube with Hexane once it reaches the bevel.
- o Continue blowing down until sample is reduced to about half the Boling tube nipple height then remove the Boling tube and proceed with final volume procedure.

Final Volume

- o Label clear auto sampler vials (ASV) with the sample ID and PCB or PBDE fraction, as appropriate. (Batch number is not necessary at this point)
- o Add inserts to auto sampler vials.
- o Using disposable pipettes, transfer the samples to the corresponding ASV's, rinsing the Boling tube 3 times with small aliquots of Hexane, and adding the rinsate to the ASV. If three rinses will not fit into the ASV, allow the ASV to sit in the hood until sufficient volume has evaporated to allow the rest of the rinses to fit.
- o Leave the ASV's uncapped in the hood to evaporate until reduced by about half. This process will take several hours. Check on samples often to check volume and confirm evaporation process.

For Water Samples

- o Add 20 µL of N-nonane to each vial.
- o When samples have again reduced to between the 1.0 and 0.5 markers on the vials, add the internal standard to each vial. (IS is located in the freezer of the Ozzy fridge, 1668 for PCB, and 1614 for PBDE)
- o Let samples reduce down until approximately 15-25 µL remains (approximately the bottom of the label area on the ASV).
- o Label amber auto sampler vials (handwritten) and add spring inserts.
- o Using a 50 µL syringe, draw up the sample from the vial, and rinse the vial 3x with small amounts of N-nonane and draw these up into the syringe, for a total volume of 40 µL. (Use a 25 µL syringe for the rinses)
- o Dispense the sample into the spring insert of the appropriately labeled amber auto sampler vial, cap and store in the Moly Jr freezer.

For Tissue Samples

- o Add 40 µL of N-nonane to each vial.
- o When samples have again reduced to between the 1.0 and 0.5 markers on the vials, add the internal standard to each vial. (IS is located in the freezer of the Ozzy fridge, 1668 for PCB, and 1614 for PBDE)
- o Let samples reduce down until approximately 35-45 µL remains.
- o Label amber auto sampler vials (handwritten) and add spring inserts.
- o Using a 100 µL syringe, draw up the sample from the vial, and rinse the vial 3x with small amounts of N-nonane and draw these up into the syringe, for a total volume of 80 µL. (Use a 25 µL syringe for the rinses)
- o Dispense the sample into the spring insert of the appropriately labeled amber auto sampler vial, cap and store in the Moly Jr freezer.

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Appendix I: Work Instruction: Clean-up Procedure and Final Volume, Reverse Carbon (1613)

Additional Equipment & Supplies

- Short stemmed stopcock
- 10mm/10mm union
- Dual-Layer Carbon Reversible Column
- 6.35mm/10mm reducing union
- Syringe luer adapter
- Clamp
- Ring stand
- 50 mL Luer lock glass syringe
- Toluene
- N-nonane
- Boling tube

Initial Column Set-up

- Insert Short Stemmed Stopcock into one end of a 10mm/10mm Union.
- Insert the large end of a Dual-Layer Carbon Reversible Tube into the other end of the 10mm/10mm Union.
- Secure the 6.35mm side of a 6.35mm/10mm Reducing Union over the Carbon end (small end) of the Dual Layer Carbon Reversible Tube from step 2.
- Fit the non-lipped end of a Syringe Luer Adapter into the 10mm end of the 6.35mm/10mm Reducing Union from the previous step.
- Clamp assembly, stopcock pointing down, by upper (6.35mm/10mm) union and secure it in the hood.
- Repeat steps 1 – 5 for as many assemblies as needed.
- When needed, the Glass Syringe will be inserted into the ground glass end of the Syringe Luer Adapter.

Column Conditioning

- Open stopcock on the assembly and place a beaker underneath to catch solvent waste.
- Draw up 40 mL of Toluene into the Glass Syringe and secure it in the Syringe Luer Adapter on top of the assembly. Applying pressure to Glass Syringe, elute 5 mL of solvent; allow remaining 35 mL to gravity feed.
- Once all of the Toluene has been eluted, draw up 50 mL of Hexane with the same Glass Syringe, and secure to top of the assembly. Elute the first 5 mL of solvent using pressure and allow remaining 45mL to gravity feed.
- Repeat the previous step once more, using 50 mL of Hexane.
- After all solvent has eluted from the Glass Syringe, remove it from assembly. With solvent still remaining in the Syringe Luer Adapter, close stopcock so the solvent level is at the top of the 6.35mm/10mm Reducing Union.

Reversing Column/Loading Sample

- Remove entire assembly from clamp. Unscrew the top joint of the 6.35mm/10mm Reducing Union and remove the Syringe Luer Adapter; in its place, secure a Stopcock Vacuum Adapter (or Short Stemmed Stopcock).
- Close stopcock on Stopcock Vacuum Adapter and flip entire assembly upside down.
- Remove the 10mm/10mm Union (including the Short-Stemmed Stopcock) from what is now the top of the assembly; replace with a 6.35mm/10mm Reducing Union.
- Clamp assembly by the lower reducing union and secure it in the hood.
- Add a 35 cm Empty Dioxin Column, to top of assembly.
- Pipette sample into top of the Empty Dioxin Column; rinse Boling tube 3x with Hexane, pipetting aliquots into column.
- Open stopcock and add 30 mL of 3% DCM in Hexane to the column; adjust position of beaker to catch solvent waste.
- Once all samples are loaded on to columns, adjust the drip rate by applying vacuum to columns with slow drip rates.
- Elute solvent until it only remains in the upper portion of the Dual Layer Carbon Reversible Tube; remove the 35 cm Empty Dioxin Column from the top of the assembly. Close the stopcock when solvent reaches the top of the 6.35mm/10mm Reducing Union.

Eluting Analytes

- Reassemble columns to configuration during the initial set-up and conditioning steps: remove from clamp, remove top 6.35mm/10mm Reducing Union and replace with 10mm/10mm Union, reattach Short Stem Stopcock (close stopcock), flip entire assembly upside down, remove Stopcock Vacuum Adapter and replace with Syringe Luer Adapter, clamp assembly by upper union and secure in hood.
- Position clean, labeled Boling tubes under correct assemblies to collect eluted solvent.
- Draw up 30 mL of Toluene into the Glass Syringe and secure it in the Syringe Luer Adapter on top of the assembly, open stopcock. Applying pressure to Glass Syringe, elute 5 mL of solvent; allow remaining 25 mL to gravity feed.
- Repeat the previous step once more, using 30 mL Toluene. Total collected elution volume is 60 mL.
- Once 60 mL of Toluene has eluted, remove the Glass Syringe and draw air up to the 20 mL mark; reattach to assembly and gently push remaining liquid from column.
- Cap samples and dismantle assemblies.

Final Volume

- Preheat N-evap to 70 °C. Read and record the temperature of the N-Evap water bath using a NIST thermometer stored in extractions on the bench sheet.
- Load Boling tubes onto N-evap set at 70 °C and blow down until about half the nipple height.
- Label clear auto sampler vials with the sample ID. (Batch number is not necessary at this point)
- Add inserts to auto sampler vials (ASV).
- Using disposable pipettes, transfer the samples to the corresponding ASV’s, rinsing the Boling tube 3x times with small aliquots of Toluene, and adding the rinsate to the ASV. If three rinses will not fit into the ASV, allow the ASV to sit in the hood until sufficient volume has evaporated to allow the rest of the rinses to fit.

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- Leave the ASV's uncapped in the hood to evaporate until reduced by about half. This process will take several hours. Check on samples often to check volume and confirm evaporation process.

For Water Samples

- Add 10 µL of N-nonane to each vial.
- When samples have again reduced to between the 1.0 and 0.5 markers on the vials, add the internal standard to each vial. (IS is located in the Ozzy freezer.)
- Let samples reduce until down to nipple of insert or about 10 µL.
- Label amber auto sampler vials and add spring inserts.
- Using a 25 µL syringe, draw up the sample from the vial, and rinse the vial 3x with small amounts of N-nonane and draw these up into the syringe, for a total volume of 20 µL. (Use a 10 µL syringe for the rinses)
- Dispense the sample into the spring insert of the appropriately labeled amber auto sampler vial, cap and store in the Moly Jr freezer.

For Tissue Samples

- Add 20 µL of N-nonane to each vial.
- When samples have again reduced to between the 1.0 and 0.5 markers on the vials, add the internal standard to each vial. (IS is located in the Ozzy freezer.)
- Let samples reduce down until approximately 15-25 µL.
- Label amber auto sampler vials and add spring inserts.
- Using a 50 µL syringe, draw up the sample from the vial, and rinse the vial 3 times with small amounts of N-nonane and draw these up into the syringe, for a total volume of 40 µL. (Use a 25 µL syringe for the rinses.)
- Dispense the sample into the spring insert of the appropriately labeled amber auto sampler vial, cap and store in the Moly Jr freezer.

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Appendix J: Work Instruction: Semi-Volatiles, Base-Neutral Only (8270)

- Batch the samples in Element, print out the bench sheet, and export the bench sheet as an XLS file to open in Excel.
- Fill 2 new 1 L amber jars with 1 L of DI water for QC.
- Shake samples and pour off to as close to 1000 mL as possible, while still leaving room for reagents/preservatives; discard extra sample down drain.
- Open the exported bench sheet in Excel on the computer in extractions. Weigh each sample jar and enter this weight into the spreadsheet under the “Bottle Weight Full” column.
- Spike appropriate QC samples (LCS, LCSD, MS, MSD) and add surrogate to all QC and samples in the amber jars.
- Salt samples and blanks with 8270 salt mixture:
    - 0.5 g EDTA
    - 60 g NaCl
- Basify all samples to pH >11 with 10 N NaOH.
- Check pH using a few drops of sample removed with a pipette/capillary tube, on a full range pH test strip. If a sample requires additional base, record additional mL in the ‘comments’ section on the bench sheet.
- Rinse CLLE’s and flat bottoms with 3 aliquots of 20 mL DCM, discarding rinsate in appropriate container.
- Set up CLLE’s with flat bottoms, adding 150 mL of DCM to each set-up:
    - To set up CLLE’s, place 2-3 boiling chips into the flat bottom and affix flat bottom-to-bottom ground glass joint of CLLE, twisting slightly to seat flat bottom securely, and place CLLE upright in hood, with flat bottom resting in a heating mantle, and back of CLLE resting against chain clamp. Chain CLLE in place, making sure the CLLE is as straight as possible. Pour 150 mL of DCM into the top opening of the CLLE.
- Label the flat bottoms with the corresponding sample or QC ID.
- Pour samples into CLLE’s, rinsing the amber jar 3x with 20 mL aliquots of DCM. Rinse CLLE ground glass joint with DIW squeeze bottle.
- Adjust solvent level in flat bottoms by tilting the CLLE or adding DI water to the CLLE; solvent level should be even with heating mantle.
- Top with condensers, turn on water and heat (should be about one drip per second for each sample).
- Once solvent is boiling, cover flat bottoms with aluminum foil and allow to cycle for 18-24 hours. Record start time on bench sheet.
- Weigh the empty jars with original caps and enter the weight into the Excel spreadsheet in the “Bottle Weight Empty” column; this will automatically calculate the initial volume. Save the spreadsheet and close it. Import the spreadsheet back into Element to populate the initial volume for each sample. Print and attach to bench sheet.
- After 18-24 hours, turn off heat and allow flat bottoms to cool; remove condensers and turn off water.
- Remove flat bottoms from CLLE’s, rinsing CLLE joint (rinsate into sample) with DCM.

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RapidVAP/Final Volume

- DCM Rinse and label large RV tubes, and top with sulfate funnels that have been rinsed with DCM.
- For each sample, pour the contents of the flat bottom through the sulfate funnel, rinsing the flat bottom 3x with small aliquots of DCM.
- Rinse the sulfate funnel with an additional 20 mL aliquot of DCM.
- Preheat RapidVap. Read and record the set temperature and the correction factor adjusted temperature of the RapidVap heat block on the bench sheet.
- Process on RapidVap as follows:
    - 40 °C and 70 os for 30-45 min, rinsing with DCM when liquid level reaches 1/3 bevel.
      Note*: Nitrogen level on RapidVap should be 10 psi*
    - 40 °C and 70 os for 2-5 min, remove when sample is ~ 1 mL.
- Let tubes sit in hood until solvent has evaporated to 0.7 mL.
- Using a disposable pipette, transfer sample to a 1 mL volumetric flask, rinse the tube 3 times using DCM, adding to volumetric each time. Add 100 uL of Acetone to the flask and fill any remaining volume with DCM to bring up to 1mL (Final Volume solvent is 10% Acetone in DCM).
- Dispense into labeled auto sampler vial and cap.

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Appendix K: Work Instruction: Pesticides by GC QQQ (8270E Modified)

Additional Equipment & Supplies

- Vacuum manifold
- CarboPrep Plus SPE cartridge
- Boling tubes
- Toluene

CLLE Set-up/ Sample Preparation

- Batch the samples in Element, print out the bench sheet, and export the bench sheet as an XLS file to open in Excel.
- Fill 2 DCM-rinsed 1 L amber jars with 1L of DI water for QC.
- Shake samples and pour off to as close to 1000 mL as possible; discard extra sample down the drain.
- Open the exported bench sheet in Excel on the computer in extractions. Weigh each sample jar and enter this weight into the spreadsheet under the “Bottle Weight Full” column.
- Rinse CLLE’s and flat bottoms with 3 aliquots of 20 mL DCM, discarding rinsate in appropriate container.
- Set up CLLE’s with flat bottoms:
  - To set up CLLE’s, place 2-3 boiling chips into the flat bottom and affix flat bottom-to-bottom ground glass joint of CLLE, twisting slightly to seat flat bottom securely.
  - Place CLLE upright in hood with flat bottom resting in a heating mantle and the back of CLLE resting against chain clamp. Chain CLLE in place, making sure the CLLE is as straight as possible.
  - Pour 150 mL of DCM into the top opening of the CLLE.
- Label flat bottoms with the corresponding sample or QC ID.
- Spike appropriate QC samples (BS, MS, MSD) and add surrogate to all QC and samples in the amber jars. Use the designated QQQ syringes kept in standards prep room.
- Pour samples into CLLE’s and rinse the amber jar 3x with 20 mL aliquots of DCM, adding each rinse into the CLLE. Rinse CLLE top glass joint with DIW squeeze bottle.
- Adjust solvent level in flat bottoms by tilting the CLLE or adding DI water to the CLLE.
- Top with condensers, turn on water and heat (should be about one drip per second for each sample).
- Once solvent is boiling, cover flat bottoms with aluminum foil and allow to cycle for 18-24 hours. Record start/end time on the bench sheet.
- The next day, weigh the empty jars with original caps and enter the weight into the Excel spreadsheet in the “Bottle Weight Empty” column; this will automatically calculate the initial volume. Save the spreadsheet and close it. Import the spreadsheet back into Element to populate the initial volume for each sample. Print and attach to bench sheet.
- After 18-24 hours, turn off heat and allow flat bottoms to cool; remove condensers and turn off water.
- Remove flat bottoms from CLLE’s, rinsing CLLE joint (rinsate into sample) with DCM.

RapidVap

- DCM-rinse and label RapidVap (RV) tubes, and top with sulfate funnels that have been rinsed with DCM.
- For each sample, pour contents of the flat bottom through the sulfate funnel. Rinse the flat bottom 3x with small aliquots of DCM and pour rinsate through funnel.
- Rinse the sulfate funnel with an additional 20 mL aliquot of DCM.
- Add 15 mL of Hexane to each RV tube.
- Preheat RapidVap. Read and record the set temperature and the correction factor adjusted temperature of the RapidVap heat block on the bench sheet.
- Process on RapidVap as follows:
    - 40 °C and 70 os for 30-45 min, rinsing with Hexane when liquid level reaches bevel. ***Note****: Nitrogen level on RapidVap should be 10 psi.*
    - 60 °C and 70 os until samples reach 1/3 bevel and rinse with hexane again, then remove from RapidVap when sample blows down to ~1 mL.
- Pipette extract to 4 mL amber glass vials, rinsing the RV tubes 3x with small aliquots of Hexane (<0.5ml) and adding the rinsate to the vial.
- Proceed to CarboPrep Plus SPE clean-up step.

CarboPrep Plus SPE Clean-up

- Set up vacuum manifold by securing CarboPrep Plus SPE cartridge tip in the manifold with the stopcocks open.
- Pipette 10% Acetone in Hexane into cartridge and fill to the top of cartridge. Allow solvent to enter the cartridge bed and once it begins to drip out of the cartridge tip, close stopcocks.
- Allow cartridge to soak for 5 minutes.
- After 5 minutes, open the stopcocks and allow the solvent to steadily drain until the solvent meniscus is slightly above the cartridge bed, then close the stopcocks. The drip rate of the solvent should be at a rate of approximately 1-2 mL/min. **Note:** To establish the desired flow rate, connect the manifold to the vacuum in the hood with a heavy wall gum rubber tube. Turn on vacuum slowly and regulate the flow rate by adjusting the stopcocks.
- Discard the waste solvent from the conditioning step, and place labeled 60 mL boling tubes under each cartridge. Ensure each stopcock is closed.
- Pipette sample extract into the respective cartridge. Open stopcock and let sample flow through the cartridge bed until sample meniscus is just above the top of the cartridge bed. Rinse the extract vials 3x with hexane and add the rinsate to the cartridge. **Note:** The drip rate of the extract and rinsate should drain steadily at approximately 1-2 mL/min. Adjust vacuum and/or stopcocks to regulate the flow rate.
- Close stopcocks when the sample meniscus is slightly above the cartridge bed, ensuring the cartridge does not go dry.

- Add 1 mL of 10% Acetone in Hexane to each cartridge. Open the stopcocks and allow solvent to flow through the cartridge at the same drip rate, until solvent meniscus is slightly above the cartridge bed, then close the stopcocks.
- Measure 8 mL of 10% Acetone in Hexane and add it to each cartridge. Open the stopcocks and monitor the solvent level as it flows through the cartridge, allowing a steady drip rate of 1-2mL/min. **Note:** The cartridge holds approximately 3 ml solvent, so 8 ml of solvent will have to be added in stages.
- After 8ml solvent has been collected from the sample elution step and cartridges are dry, remove collection vessels from manifold.
- Rinse each Boling tube with Toluene and concentrate on the N-evap station at 60 °C until sample extract reaches the bevel.
- Once sample extract reaches bevel, rinse Boling tubes with Toluene and raise temperature on the N-evap to 70 °C . Continue to concentrate until sample extract is ~1/3 nipple of Boling tube.
- Transfer extract to respective labeled (handwritten) clear auto sampler vials with non-spring inserts. Rinse Boling tube with Toluene to ensure complete transfer.
- Allow extract to evaporate to half volume in the insert by leaving the auto sampler vials uncapped in the hood for several hours. To speed the process up, the N-evap can be used with minimal pressure and no heat.
- Label amber auto sampler vials (handwritten) and add non-spring inserts.
- Use the designated QQQ syringes kept in the standards prep room. Using the 100µl syringe, draw up extract from vial, and rinse vial inserts 3x with the 25 µL syringe using small amounts of Toluene, for a total final volume of 100 µL.
- Dispense the sample into amber auto sampler vial with non-spring inserts, cap and store in the Moly Jr freezer.

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