Standard Operating Procedure For:

Extraction of Base/Neutral and Acid Organic Analytes From Water, Sediments and Wastes

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Table 1: BNA Analytes

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Extraction of Base/Neutral and Acid Organic Analytes From Water, Sediments and Wastes

1 SCOPE AND APPLICATION

1.1 This SOP details the extraction of a select group of base-neutral and acid extractable (BNA) analytes from water, sediments and wastes matrices for subsequent analysis by high resolution Gas Chromatography and Mass Spectrometry (GC/MS) following EPA Method 8270E and EPA Method 625.1. These analytes are listed in Table 1. This SOP is used for all samples with the LIMS test ID W-BNA, W-BNA-625, W-BNA-R, W-DPGBE, W-PAH, W-PAH-R, W-PAH-SIM and W-PAH-PA for water, S-BNA and S-PAH, S-PAH-SIM, S-PAH-PA, S-PAH-E-PA and S-PAH-EXT for sediments, WAS-BNA, WAS-PAH and WAS-PAH-PA for wastes. Liquid-liquid extractions are performed following EPA 1311 and EPA 1312 leaching procedures by Inorganic Group for the tests TCLP-BNA and SPLP-BNA.

1.2 FDEP Laboratory Test IDs and equivalent methods reported by the SOP

Laboratory Test Name	Test Description
W-BNA-625	EPA Method 3510C preparation/625.1 analysis, water sample with separate extraction/analysis
W-BNA-R	EPA Method 3510C preparation/8270E analysis, water sample
W- BNA	EPA Method 3510C preparation/625.1 analysis, water sample
W-PAH	EPA Method 3510C preparation/625.1 analysis for PAHs only, water sample
W-PAH-SIM	EPA Method 3510C preparation/8270E analysis for PAHs only using SIM, water sample
W-PAH-R	EPA Method 3510C preparation/8270E analysis for PAHs only, water sample
W-PAH-PA	EPA Method 3510C preparation/8270E analysis for PAHs only, water sample profile analysis
S- BNA	EPA Method 3550C preparation/8270E analysis, soil sample
S- BNA	EPA Method 3545A preparation/8270E analysis, soil sample
S-E8270	EPA Method 3550C preparation/8270E analysis for PAHs only, soil sample
S-PAH	EPA Method 3545A preparation/8270E analysis for PAHs only, soil sample
S-PAH-SIM	EPA Method 3550C preparation/8270E analysis for PAHs only using SIM, soil sample
S-PAH-SIM	EPA Method 3545A preparation/8270E analysis for PAHs only using SIM, soil sample
S-PAH-EXT	EPA Method 3550C preparation/8270E analysis for PAHs extended list, soil sample (homolog)
S-PAH-EXT	EPA Method 3545A preparation/8270E analysis for PAHs extended list, soil sample (homolog)
S-PAH-PA	EPA Method 3550C preparation/8270E analysis for PAHs only, soil sample (profile analysis)
S-PAH-PA	EPA Method 3545A preparation/8270E analysis for PAHs only, soil sample (profile analysis)
S-PAH-E-PA	EPA Method 3550C preparation/8270E analysis for PAHs extended list, soil sample (profile analysis)
S-PAH-E-PA	EPA Method 3545A preparation/8270E analysis for PAHs extended list, soil sample (profile analysis)
WAS-BNA	EPA Method 3580A preparation/8270E analysis, waste sample
WAS-PAH	EPA Method 3580A preparation/8270E analysis for PAHs only, waste sample
WAS-PAH-PA	EPA Method 3580A preparation/8270E analysis for PAHs only, waste sample profile analysis
SPLP-BNA	EPA Method 3510C preparation/8270E analysis of SPLP extract
TCLP-BNA	EPA Method 3510C preparation/8270E analysis of TCLP extract

1.3 BNA extractions are used to isolate up to 150 compounds with a wide range of boiling points and chemical properties. Many of these analytes are extremely sensitive to light, heat and active sites on glassware, etc. Analyte recovery may be significantly reduced if this SOP is not followed closely and carefully.

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1.4 For water this procedure is based on EPA methods 625.1 [10.2] and 3510C [10.3], except for the continuous liquid-liquid extraction portion of this procedure, which is based on EPA methods 625.1 and 3520C[10.4]. Acid/base indicator (pink color at pH 4 and yellow color at pH 9) is used to adjust the pH during the extractions. Excess use of acid and base for pH adjustments can cause low recovery for some sensitive analytes.

1.5 For sediments extraction EPA method 3550C[10.5] using Horn Sonicators.

1.6 Alternatively for sediments EPA method 3545A[10.15] (Accelerated Solvent Extraction using Dionex ASE-350) is used. This method is more efficient and cost effective than the horn tip.

1.7 Sediment extraction procedure is for the samples, which expected to contain low concentrations of organics (<20 mg/kg). If high concentrations are expected, follow the preparation of waste samples.

1.8 For waste this procedure is adapted from EPA Method 3580A[10.6].

1.9 For oil pattern identification, following ASTM Method D 5739-06[10.7], a suspected oil sample is diluted and then fractionated on a silica gel column.

2 SUMMARY OF THE METHOD

2.1 A measured volume of water is serially extracted with methylene chloride at acidic pH 4 and again at basic pH 9 using separatory funnel technique. The resulting methylene chloride extract is concentrated to a final volume of 1 mL or less. If the resulting sample extract contains high levels of high molecular weight compounds, it may be cleaned up using Gel-Permeation Chromatography (GPC) and analyzed by GC/MS. Quantitation by internal standard technique is used. For W-BNA-625 extraction, the base and acid extracts are concentrated and analyzed separately. TCLP and SPLP extracts prepared by EPA 1311 and EPA 1312 methods are treated the same as water samples.

2.2 A measured weight of sediment is serially extracted with 1:1 methylene chloride: acetone using Horn Sonicators. The resulting extract is concentrated to a final volume of 1 mL. Samples are then ready for either a Gel Permeation Chromatography (GPC) cleanup, or for analysis by GC/MS. Quantitation by internal standard technique are used.

2.3 A measured weight of sediment is serially extracted with 3:1 methylene chloride: distilled hexane using Accelerated Solvent Extraction (ASE). The resulting extract is concentrated to a final volume of 0.25 mL. Samples are then ready for either a Gel Permeation Chromatography (GPC) cleanup, or for analysis by GC/MS. Quantitation by internal standard technique are used.

2.4 A measured weight of waste is diluted in methylene chloride, and then filtered. Samples are then ready for analysis by GC/MS.

2.5 Suspected oil spill samples may be identified by comparing their GC/MS biomarker patterns to the patterns observed in known oil reference standards. Prepare the suspect oil sample by diluting one gram of each sample into 10 mL of methylene chloride. A portion of this oil solution is solvent exchanged into hexane. Include one sample duplicate for each set and one suspect reference oil standard at 20, 000 µg/mL in extract. Fractionate each sample, reference oil and one method blank in hexane on a five-gram silica gel column independently. Collect 25 mL of hexane fraction I and 25 mL of methylene chloride fraction II for each sample. Both concentrated sample fractions in 1 mL methylene chloride are finally analyzed by GC/MS along with oil reference standards to identify the oil pattern. The oil pattern identification samples may be prepared together with waste

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dilution samples or may even share the same extracts prepared previously for waste dilution (as in step 2.4). For best results do not add any external standards and surrogates to these samples during extraction and GC/MS analysis.

2.6 Method interferences may be caused by contamination in solvents, glassware, reagents or other sample equipment. Phthalates are universal contaminants found in lotions, oils, and plastic and can therefore easily be introduced into the sample extracts. Never touch the interior of any clean glassware. Any aluminum foil used should be used with the dull side facing the sample extract. Never use Para film. Rinse glassware thoroughly with methylene chloride prior to use.

3 APPARATUS AND EQUIPMENT

3.1 Glassware

3.1.1 S-Evap® sample concentration system: Kuderna-Danish (K&D) concentrator tubes -- 15 mL, Ground glass stoppers to fit concentrator tubes, K&D 500 mL evaporative flasks; attach to concentrator tubes with clips, Snyder columns -- three ball macro or equivalent and 5 Clips -- used to attach concentrator tube to K&D flask, and Teflon boiling chips.

3.1.2 Centrifuge tubes, graduated (10-15 mL), with screw caps with Teflon-lined septa (Calibrate tubes to the volume used before use and record in the Log Book)

3.1.3 If performing liquid-liquid extraction with tumblers: Erlenmeyer flasks, 500 mL, with ground glass mouth and stoppers, Glass powder funnels, approximately 10 cm in diameter, with long stems, Glass wool column only for PAH extractions, 1000 mL graduated cylinder and 2-liter separatory funnels with Teflon stopcocks and stoppers

3.1.4 Gas-tight micro-syringes or volumetric pipets, 250 µL, 500 µL and 1000 µL (Required from the manufacturer, the certificate stating that the volume is accurate to < 1%, otherwise calibrate and keep the record of these gas-tight micro-syringes or volumetric pipets every quarterly)

3.1.5 If performing sediment extraction: Beakers, 400 mL, Glass powder funnels, approximately 10 cm in diameter, with long stems, Vacuum filtration flasks, 500 mL, Porcelain Büchner funnels, 9 cm, Whatman #42 filter paper, 9 cm, Metal spatulas and Rubber funnel supports

3.1.6 Sediment extraction by ASE-350: Accelerated Solvent Extraction Cells – 50, 100 and 150 mL volume size, IChem 60 mL jars with screw caps and diatomaceous earth

3.2 Centrifuge to fit 15-mL centrifuge tubes.

3.3 Large volume (200-500 mL) concentration system: S-Evap®: Consists of heated water bath and water-cooled condensers; capable of concentrating eight sample extracts simultaneously.

3.4 Horn Sonicators (Fisher Scientific/Qsonica) FB705/CL334, maximum output 705W, ¾ inch horn tip.

3.5 N-Evap®: consists of heated water bath and apparatus to further concentrate the extract, and Nitrogen gas.

3.6 Top Loading balance, accurate to 0.1 g.

3.7 For liquid-liquid extraction with tumblers: Automatic separatory funnel tumbler

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3.8 Shimadzu HPLC Auto Prep system for GPC cleanup. Phenomenex high performance GPC column. ISCO Foxy 200XT Fraction collector. Mobile phase is methylene chloride.

3.9 Dionex ASE-350 extractor.

3.10 Oven- Fisher Scientific, to store all the required extraction materials used in the preparations at 120°C.

4 REAGENTS AND CHEMICALS

4.1 Methylene chloride, hexanes and acetone, Optima or Pesticide grade or equivalent (Fisher Scientific). For ASE extraction use distilled hexanes to avoid the phthalates contamination.

4.2 See DEP SOP SV-009 for the details of preparation of spike and surrogate standards used in the extraction. Use 1st source standards for the spiking all the QC samples.

Tinted methanol (acid/base indicator for pH adjustments, 2000 µg/mL, Catalog Number 020328-99, 250 mL (O_2Si smart solutions), add 0.5 to 1 mL for each sample for BN & A extractions.

BNA Surrogate Mix (100/200 µg/mL of Base Neutrals and Acids): Spike 0.25 mL for a final volume of 1 mL extraction for Water and 0.50 mL for Sediment samples. For Soil PAHs add 50 µL of 100 µg/mL of BNA surrogate Mix to a final volume of 1 mL.

Spike all the requested analytes for Water and Soil samples extraction. For a 1 mL of final volume extraction add 200 µL each of BNA 625 & 8270 spike mix (50 µg/mL of each) and 0.1 mL of Benzidine mix (200 µg/mL). For Soil PAHs extraction add 0.1 mL of 50 µg/mL of 23 analyte PAH mix.

Internal standard solutions: 50, 10 and 1 µg/mL 1, 4-dichlorobenzene-d4, naphthalene-d8, acenaphthene-d10, phenanthrene-d10, chrysene-d12 and perylene-d12.

4.3 GPC Calibration Solution.

Prepare a solution in methylene chloride that contains the following analytes in the concentration listed below:

Analyte	**mg/mL**
Corn oil	12.5
Bis-2-ethylhexyl phthalate	0.5
Methoxychlor	0.1
Perylene	0.01
Sulfur	0.04

4.4 1N Sodium hydroxide **(prepare in a hood, using a stirring plate to mix!)**: Prepare by dissolving 40g of NaOH in 1000-mL reagent water. Transfer to a Teflon storage container.

4.5 Sulfuric acid, 1M: Slowly add 56 mL of concentrated sulfuric acid (18M) to approximately 950 mL of HPLC water in a 1000 mL volumetric flask. Bring to a final volume of 1000 mL and mix well. Transfer to a bottle.

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4.6 Sodium sulfate, granular and powder: Bake in a muffle furnace at 700 ºC for 8 hours. Allow to cool before using. Store in a large mouth bottle with backed aluminum foil cover in 200 ºC oven. Note: For low level PAH, sodium sulfate was washed with methylene chloride before bake in muffle furnace.

4.7 Diatomaceous Earth (Dionex) Granular (anhydrous). Purified by washing with methylene chloride and heating at 700 °C for 8 hours or overnight. It is then stored at 120°C in an oven. Empty cells are filled with diatomaceous earth, then placed in the ASE-350 machine and further cleaned by running the extraction program. After they are removed from the ASE-350 carousel they are stored in an oven at 120 °C.

4.8 Glass wool (Silane treated): Bake in a muffle furnace at 700 °C for 8 hours. Store in an oven at 120 °C and do not store in plastic.

4.9 Ottawa Sand Standard: Bake in a muffle furnace at 700 °C for 8 hours. Allow to cool before using. Store in a large mouth bottle with backed aluminum foil cover in 120 °C oven.
Note: For low level PAH, Ottawa sand was washed with methylene chloride before bake in muffle furnace.

4.10 Silica cartridges 1g (6 mL) - Sep-Pak Cartridge, J&W brand or equivalent.

4.11 Silica gel (Davisil, grade 644): Rinse with methylene chloride (do this by packing the silica gel in a column and pushing the methylene chloride through the column with nitrogen pressure) and dry (this will take 3-4 hours). Then, bake in an oven at 700 °C for 8 hours. Store silica gel in an oven at 120 °C. Allow to cool before using. If deactivated silica gel is required, deactivate by adding 3% by weight of distilled water. Re-stopper the flask, and shake on a wrist action shaker for at least 1 hour. Allow to equilibrate for 3 or more hours at room temperature.

4.12 Copper metal: Copper metal, fine granular, analytical grade, 3 to 14 mesh, Mallinckrodt (catalog number 4649-04, 500 grams (Fisher Scientific catalog number MK4649-500). Prepare activated copper metal one hour before sulfur cleanup. The copper is activated by covering it with concentrated hydrochloric acid, stirring it with a glass rod, and allowing it to stand for 5 minutes. The copper is then washed three times each with water, methanol, and dichloromethane, in that order. The copper is stored covered with dichloromethane until use to avoid contact with air.

5 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

5.1 Water samples are collected in 1-liter amber glass bottles, transported to the lab on ice and stored at 6 °C or less. All water samples must be extracted within seven days after collection.

5.2 Sediment and waste samples are collected in glass jars, transported to the lab on ice and stored at 6 ºC or less. All sediment and waste samples must be extracted within 14 days after collection.

5.3 Consider TCLP and SPLP extracts as water sample and follow the water samples holding time of 7 days to complete the preparation and consider waste if not clean and if it is a pure waste, then follow waste holding times of 14 days after the initial TCLP and SPLP preparation (extracts from DEP SOPs MT-004 and MT-023). Use TCLP/SPLP matrix spike for QC samples.

5.4 Water, sediment and waste extracts must be analyzed within 40 days of extraction.

6 SAMPLE PREPARATION

NOTE: a second person must witness the addition of spike standard to the proper samples; verify the correct volume, spike LIMS number and concentration, and initial in the logbook.

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Also record bottle number if more than one bottle available, lot numbers of reagents and solvents used, and bath temperature for extracts concentration.

If entire sample is not used for extraction, always mix thoroughly or shake before sub-sampling.

Note: For W-BNA-625 extraction, the base and acid extracts are concentrated and analyzed separately.

6.1 Water Sample Extraction Procedure by Liquid - Liquid Extraction (EPA 3510C) for W-BNA-8270 and W-BNA-625

6.1.1 Check the sample bottles out of the receiving room walk-in cooler.

6.1.2 Remove the BNA Spike Mix for Water and Sediments and the BNA Surrogate Mix for Water and Sediments from the refrigerator and let them warm to room temperature.

6.1.3 Thoroughly clean all glassware by rinsing it with hot tap water, deionized water and acetone, then baking it in an oven at 700 °C for 8 hours. Alternatively, glassware may be thoroughly washed with Alconox and hot water, and then rinsed with hot tap water, deionized water, and acetone. Pre-rinse all glassware with methylene chloride prior to use!

6.1.4 Assemble one of the following pieces of glassware for each sample:

6.1.4.1 2 L separatory funnel with Teflon stopcock and Teflon stopper

6.1.4.2 500 mL Erlenmeyer flask with ground glass mouth and stopper

6.1.4.3 Two-glass powder funnels

6.1.4.4 500 mL Kuderna-Danish flask, column, and concentrator tube or a Turbo-Vap® concentration vessel.

6.1.4.5 Centrifuge tube with Teflon-lined screw cap

6.1.5 Prepare glass powder funnels by placing a small plug of glass wool in the bottom of funnel and topping with approximately 1-3 cm of muffled granular sodium sulfate. Make sure the sodium sulfate has been baked at 550 °C for at least 8 hours prior to use. Let it cool to room temperature before using.

6.1.6 Rinse the funnel assembly with approximately 10-15 mL of methylene chloride and discard rinsate into waste beaker.

6.1.7 Label all glassware with the appropriate sample number, using labeling tape or pre-printed sample bar code labels. (Do not use a permanent marker! Methylene chloride will wash off the sample ID if it contacts the outside of the glassware.) There should be one blank, two laboratory fortified blanks (i.e., reagent water spikes -- use DEP well water) and two matrix spikes (duplicates) for each set of samples extracted, not to exceed 20 samples per set.

6.1.8 Before proceeding, ensure that the samples are at room temperature.

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6.1.9 Before proceeding, ensure that the spike and surrogate mixes are at room temperature. Many compounds easily precipitate out of solution when in the refrigerator or freezer and they need to be re-dissolved into solution by being warmed to room temperature. Also shake the bottle to ensure complete dissolution. You may place the capped bottle of solution in a sonic water bath for several minutes to quickly warm up the solution if you are pressed for time.

6.1.10 Measure the pH of each sample using wide-range pH paper. Be very careful to use a new glass rod or disposable Pasteur pipet for each sample. Record the value in the Extraction Notebook.

N.B.: The Extraction Notebook is a very valuable reference. Care should be taken that entries for each sample are complete and accurate. Any unusual observations made during the extraction process, including sample color or special extraction instructions should be entered in the notebook.

6.1.11 Weigh each amber bottle for later determination of volume. Pour the entire sample into the 2-liter separatory funnel. For the laboratory blank and the duplicate laboratory fortified blanks, use 1000 mL of DEP well water. Choose one sample to spike in duplicate and measure 1000 mL into each of two separatory funnels. **NOTE: If there is insufficient sample to extract two 1000 mL sample matrix spikes and still have one liter left over for possible re-extractions (i.e., less than 4 liters of sample), extract one 1000 mL matrix spike and duplicate lab fortified blanks. If only one bottle of sample was received, extract duplicate lab fortified blanks and save 100 mL of each sample in case of future re-extractions. If less than 2 matrix spikes are extracted, note this in the logbook.**

6.1.12 For every 20 samples in the set, prepare one extraction blank and one Lab-fortified blank using DEP well water and one sample duplicate matrix spikes. Add 0.5 to 1 mL of Tinted methanol (Indicator used for exact pH adjustments). Add BNA surrogate spike to all the samples and matrix spikes to QC samples in the set (see sec. 4.2 for details). **Note the amount added and the concentration of analytes in the extraction log book.**

6.1.13 Adjust the pH of each sample to acidic pH of 4 (sample color changes to pink) by adding 1M H_2SO_4 (needs approximately 8 mL), using narrow-range pH paper to determine pH. **Do not add more than required amount.**

6.1.14 Add 60-mL methylene chloride to each sample bottle, and swirl for 30 seconds to rinse the inner surface. Transfer this rinsate to the separatory funnel and swirl each separatory funnel gently to allow the vapors to escape before capping. Stopper each funnel and secure the tumbler lid.

6.1.15 Turn on the tumbler slowly for a half turn, stopping with the funnels upside down. Slowly open the stopcocks to vent the pressure. Close the stopcocks.

6.1.16 Repeat step 6.1.15 at least two more times, turning two full turns then three full turns, before venting. Continue that until the amount of gas escaping is minimal.

6.1.17 Turn on the tumbler again and set the speed to 6 (about 30-35 turns per minute). Tumble for two minutes.

6.1.18 Stop the tumbler with the funnels upright (stopcocks down) and lock it in place.

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6.1.19 Remove the stoppers from the separatory funnels and allow the methylene chloride and water layers to separate for at least 10 minutes.

6.1.20 Drain the bottom layer through the funnel containing glass wool and sodium sulfate into a 500 mL Erlenmeyer flask. Do not allow water to drain through the stopcock. If there is an emulsion, you may add additional sodium sulfate to the filter funnel and carefully drain it through the funnel. This may introduce some water into the sample (see 6.8.1.4 for removing water). If the emulsion is very bad, it may be necessary to follow one or all of these techniques to break it up:

- Allow separatory funnel to stand for several minutes
- Gently agitate emulsion with a long, glass stirring rod
- Remove entire bottom layer of sample and centrifuge to separate water and methylene chloride
- Add more methylene chloride

6.1.21 Rinse the sodium sulfate funnel liberally with methylene chloride after each layer is drained through.

6.1.22 Extract two more times with 60 mL of methylene chloride following the procedures in steps 6.1.14-6.1.21, collecting and combining each methylene chloride layer in the Erlenmeyer flask.

6.1.23 After all of the methylene chloride layers have been collected in the flask, rinse the sodium sulfate in the funnel with 5-10 mL of methylene chloride, draining it into the 500-mL flask. Stopper the flask or cover with aluminum foil (dull side down facing the sample).

6.1.24 Do not use the same sodium sulfate or glass funnel to collect the acid fraction. Discard the sodium sulfate (see 6.1.29) and prepare a new funnel with glass wool and sodium sulfate (see 6.1.5). Otherwise, analytes in the base fraction may be trapped in the sodium sulfate.

6.1.25 Basify the samples with 1N NaOH to pH 9 (color changes to yellow and needs approximately 10 mL), using narrow-range pH paper to check pH. **Be sure to vent thoroughly before beginning the tumbling procedure!** Extract three times with 60-mL methylene chloride as was done for the acidic fraction (see steps 6.1.14-6.1.22). If no water was collected in the Erlenmeyer flask, you may collect the base fraction in the same Erlenmeyer flask as the acidic fraction. If any water or sodium sulfate is in the flask with the acidic fraction, collect the base fraction in a separate Erlenmeyer flask.

6.1.26 After the final extraction, rinse the glass wool/sodium sulfate funnel with 5-10 mL of methylene chloride, letting it drain into the flask.

6.1.27 Discard the remaining water from all of the separatory funnels.

6.1.28 Determine the original volume of sample extracted by weighing the amber sample bottles. Assume that the sample has a density of 1 g/mL. If in doubt as to the sample density, weigh an exact volume (5-10 mL) to determine density. Record sample volume to the nearest 10 mL.

6.1.29 Discard the contents of the funnels in a red metal can with a plastic bag and close the lid tightly. When it's full dispose of as a hazardous chemical waste. (Note: All spent sodium

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sulfate; glass wool filter papers and solvent- extracted solid samples must be treated as hazardous waste. Place them immediately after extraction in designated closed metal containers in laboratory. Later transfer them to a 55-gallon drum in the waste room to be disposed as hazardous waste.). Cap the flasks until ready to concentrate. Store in the refrigerator if concentration will not be performed within the following two hours. To concentrate, proceed to section 6.8.1 for K&D concentration.

6.1.30 Put away all chemicals and wash all glassware so that it is ready for the next set of extractions. Make sure there is enough sodium sulfate for future extractions; prepare more if there is not.

6.1.31 Be sure to store spike and surrogate mixes in the refrigerator when not in use. During use, re-cap bottles of surrogates or spikes as soon as you are done with them in order to minimize evaporation of solvent. Spike mixes are checked by BNA chemists prior to being allowed into use to ensure that the solution was made correctly. If you leave a solution out overnight, or have any other reason to believe that the solution is no longer valid, inform the BNA supervisor so that it can be re-checked and/or re-made.

N.B.: If carrying out extraction for the following water analyses, note deviations:

6.2 Water Sample Extraction Procedure by Liquid - Liquid Extraction (EPA 3510C) for W-BN-8270, W-PAH-PA, W-PAH-SIM and W-PAH-625/8270

6.2.1 Follow procedure outlined in part 6.1, omitting steps involving extraction of the acid fraction.

6.2.2 If only PAHs analysis is required, extract samples at neutral pH with methylene chloride. See sec 4.2 for spike and surrogate standards addition. Pass extract through one-gram silica gel column and N-blow down to required volume in methylene chloride with appropriate amount of BNA internal standard. Proceed with GC/MS. GPC clean up may be required for dirty samples.

6.2.3 For PAH analysis using SIM mode follow step 6.2.2 with the exception of addition of 0.05 mL PAH matrix spike (1 µg/mL) and 200 µL of BNA surrogate spike (5 µg/mL). The final volume of the extract is 1mL.

6.2.4 For PAHs/Homologues and petroleum profile analysis, do not add any surrogates or spikes during extraction. Solvent exchange the concentrated extract into hexane and proceed with five gram silica gel fractionation.

6.3 TCLP/SPLP Extracts Extraction by Liquid-liquid extraction (EPA 3510C)

TCLP-BNA and SPLP-BNA for TCLP and SPLP extracts are extracted following EPA 1311 and EPA 1312 methods by Inorganic Group (DEP SOPs MT-004 and MT-023). Follow the procedure outlined in 6.1 above and use 625 spike for spiking. Do one method blank, two lab-fortified blanks and matrix spikes if extra TCLP/SPLP extracts are available.

6.4 Sediment Sample Extraction Procedure (EPA 3550C) for S-BNA-8270, S-AE-8270, S-PAH-8270, S-PAH-PA, S-PAH-E-PA, S-PAH-EXT and S-PAH-SIM.

6.4.1 Check the samples out of the receiving room walk-in cooler.

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6.4.2 Remove the BNA Matrix Spike Mix for Water and Sediments and the BNA Surrogate Mix for Water and Sediments from the refrigerator and let them warm to room temperature.

6.4.3 Thoroughly clean all glassware by rinsing it with hot tap water, deionized water and acetone, then baking it in an oven at 550 °C for 8 hours. Alternatively, glassware may be thoroughly washed with Alconox and hot water, and then rinsed with hot tap water, deionized water, and acetone. Pre-rinse all glassware with methylene chloride prior to use!

6.4.4 Label all glassware with the appropriate sample number, using labeling tape or pre-printed sample bar code labels. (Do not use a permanent marker! Methylene chloride will wash off the sample ID if it contacts the outside of the glassware.) There should be one blank (use Ottawa sand), two laboratory fortified blanks (i.e., Ottawa sand spikes) and two matrix spikes (duplicates) for each set of samples extracted, not to exceed 20 samples per set.

6.4.5 Note the appearance of each sample in the logbook (e.g., presence of foreign materials, variable particle size, etc.).

N.B.: The Extraction Notebook is a very valuable reference. Care should be taken that entries for each sample are complete and accurate. Any unusual observations made during the extraction process, including sample color or special extraction instructions should be entered in the notebook.

6.4.6 Do not decant standing water, large rocks, and other foreign material from the samples at any point and treat as whole sample. The analyst must consult his/her supervisor for direction if obvious non-representative material is found in the sample. For most samples, you will need to remove the sample from the jar and place it on a sheet of aluminum foil (dull side up, shiny side down) in order to homogenize it fully. Do not use aluminum foil if the sample is to be analyzed for metals. If the samples have not already been homogenized, homogenize each sample by mixing it thoroughly using a metal spatula.

6.4.7 If any changes to this step (6.4.6), is a non-EPA approved procedure and it is an option which will be performed per our client's request (Ambient samples require lower MDLs). If sample contains % solids measured less than 50%, then this sample cannot meet the required lower MDLs. Centrifuge a homogenized 100 grams of sample at 500 rpm for 15 minutes and discard the water and repeat second time for 10 more minutes. Discard standing water and homogenize. Remove large rocks and any other foreign materials and perform new % solids. Use these values to calculate the results and report with comment "% Solid determined after sample centrifugation 19.0 ". If %solid measured greater than 50% then centrifugation is not required and use original % solids to calculate results for reporting. Make a note in the extraction book.

6.4.8 Perform a dry weight determination on all samples, unless this has already been done:

6.4.8.1 Weigh 5 g of each sample into a labeled, pre-weighed aluminum-weighing dish Record the weight of the dish and the exact weight of the wet sediment in the Dry Weights Notebook. And dry it overnight at 105 °C. The next day, allow it to cool in a desiccator before weighing it a second time. Record the exact weight of the dry sediment in the dry weights notebook.

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6.4.8.2 A sample's percent solids is equal to the dry sediment weight (not including the dish weight) divided by the wet sediment weight (not including the dish weight) and multiplied by 100%. Enter the wet and dry weights into the computer generated extraction log sheet, which will calculate and display the percent solids.

6.4.8.3 Remember to upload the percent solids of all samples into LIMS.

6.4.9 Using a metal spatula, weigh approximately 30 g of each sample into properly labeled 400 mL beakers. Record the weight in the Extraction Notebook to the nearest 0.1 g. Weigh out a 30 g portion of Ottawa Sand Standard into each of three 400 mL beakers -- these will serve as a laboratory blank and two laboratory fortified blanks. Select one sample and weigh out three 30 g portions instead of one -- two of these will be used as duplicate matrix spikes. There will generally be enough sediment in a sample to prepare duplicate matrix spikes, but if none of the samples have enough, prepare a single matrix spike or no matrix spike along with duplicate LFBs and a blank. Be sure to note this in the notebook.

6.4.10 Before proceeding ensure that the spike and surrogate mixes are at room temperature. Many compounds easily precipitate out of solution when in the refrigerator or freezer and they need to be re-dissolved into solution by being warmed to room temperature. Also shake the bottle to ensure complete dissolution. You may place the capped bottle of solution in a sonic water bath for several minutes to quickly warm up the solution if you are pressed for time.

6.4.11 Add BNA Surrogate and required spikes as in sec 4.2. Note the amount added and the concentration of analytes in the extraction log book.

6.4.12 Add approximately 60 g of sodium sulfate powder to each beaker and mix it into the sediment with a metal spatula until the sediment appears sandy and free flowing. If the sample is very wet, it will be necessary to mix it repeatedly, at intervals, until it becomes free flowing.

6.4.13 Add 100 mL of 1:1 methylene chloride: acetone to each beaker.

6.4.14 Sonicate for three minutes using horn sonicators. The sonicators are programmed to run at 100% amplitude, with duty cycle set at 50% (1s on, 1s off).

6.4.15 Decant and filter the extracts through Whatman No. 42 filter paper in a Büchner funnel into a clean, labeled 500 mL vacuum filtration flask.

6.4.16 Repeat the extraction (steps 6.4.13-6.4.15) two more times with two additional 100 mL portions of solvent (1:1 methylene chloride: acetone). Sonicate for only 3 minutes in each of these two extractions.

6.4.17 After the final sonication pour the entire sample into the funnel. Rinse the beaker three times with methylene chloride, pouring the rinsate into the funnel. Rinse the funnel cake and the sides of the funnel with methylene chloride.

6.4.18 Discard the contents of the funnels in a red metal can with a plastic bag and close the lid tightly. When it's full dispose of as a hazardous chemical waste. (Note: All spent sodium sulfate; glass wool filter papers and solvent- extracted solid samples must be treated as hazardous waste. Place them immediately after extraction in designated closed metal containers in laboratory. Later transfer them to a 55-gallon drum in the waste room to be

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disposed as hazardous waste.). Cover the openings of the flasks with aluminum foil until ready to concentrate. Store in the refrigerator if concentration will not be performed within the following two hours. To concentrate, proceed to section 6.8.1 for K&D concentration.

6.4.19 Put away all chemicals and wash all glassware so that they are ready for the next set of extractions. Make sure there is enough sodium sulfate for future extractions; prepare more if there is not.

6.4.20 Be sure to store spike and surrogate mixes in the refrigerator when not in use. Re-cap bottles of surrogates or spikes immediately after use in order to minimize evaporation of solvent. Spike mixes are checked by BNA chemists prior to being allowed into use; this ensures that the solution was made up correctly. If you leave a solution out overnight, or have any other reason to believe that a solution is no longer valid, inform the BNA supervisor so that it can be re-checked and/or re-made.

6.4.21 If only PAH and PAH homolog analysis is required, then extract sample as above with 3 x 100 mL of 1:1 methylene chloride - acetone and concentrate. Add 0.1 mL of BN surrogate spike Mix (50 µg/mL) and 0.1 mL of PAH matrix spike (50 µg/mL) for sediment. Make up to 1 mL in methylene chloride and do one-gram silica gel column clean up. Add 100 µL of 50 µg/mL BNA internal standard to all the samples. Proceed with GC/MS analysis by injecting 1 µL in full scan. Do GPC cleanup for the dirty samples if it is needed.

6.4.22 For PAH's/Homologues and petroleum profile analysis, do not add any surrogates or spikes during extraction. Solvent exchange the concentrated extract into hexane and proceed with a five-gram silica gel fractionation.

6.4.23 If low level PAH analysis is required, then extract sample as above with 3 x 100 mL of 1:1 methylene chloride - acetone and concentrate. Add 0.2 mL of BN surrogate spike Mix (5 µg/mL) and 0.1 mL of 1 µg/mL PAH matrix spike for sediment. Make up to 1- 2 mL in methylene chloride and do one-gram silica gel column clean up. Add 100 µL of 1 µg/mL BNA internal standard to all the samples and make it to 1 mL final volume with methylene chloride. Proceed with GC/MS analysis by injecting 1 µL in SIM mode using MSD5975C.

6.5 Sediment Sample Extraction Procedure (EPA 3545A) for S-BNA-8270, S-AE-8270, S-E8270, S-PAH-PA, S-PAH-E-PA, S-PAH-EXT and S-PAH-SIM.

6.5.1 Follow the section 6.4.1 and 6.4.2

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

6.5.3 Follow the steps from section 6.4.4 to 6.4.8

6.5.4 Using a metal spatula, weigh approximately 7.5 g of each sample into properly labeled 60 mL IChem jar. Record the weight in the Extraction Notebook to the nearest 0.1 g. Weigh out a 7.5 g portion of Ottawa Sand Standard into each of three 60 mL IChem jar -- these will serve as a laboratory blank and two laboratory fortified blanks. Select one

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sample and weigh out three 7.5 g portions instead of one -- two of these will be used as duplicate matrix spikes. There will generally be enough sediment in a sample to prepare duplicate matrix spikes, but if none of the samples have enough, prepare a single matrix spike or no matrix spike along with duplicate LFBs and a blank. Be sure to note this in the notebook.

6.5.5 Add approximately 5 g of Diatomaceous Earth to each labeled 60 mL IChem jar and mix it with a metal spatula until the sediment appears sandy and free flowing. Let samples sit for a while at room temperature to fully desiccate the soil. If the sample is very wet, it will be necessary to mix it repeatedly, at intervals, until it becomes free flowing. Once dried, the samples are ready to be transferred into ASE-350 extraction cell

6.5.6 Before proceeding ensure that the spike and surrogate mixes are at room temperature. Many compounds easily precipitate out of solution when in the refrigerator or freezer and they need to be re-dissolved into solution by being warmed to room temperature. Also shake the bottle to ensure complete dissolution. You may place the capped bottle of solution in a sonic water bath for several minutes to quickly warm up the solution if you are pressed for time.

6.5.7 Add BNA Surrogate and required spikes as in sec 4.2. Note the amount added and the concentration of analytes in the extraction log book. Clear the cell threads of sample/DE mixture if necessary. Cap the cell firmly but not forcefully.

6.5.8 The ASE cells and the ASE collection vials are then loaded in the corresponding positions in the ASE carousels. Collection vials for system rinses should be loaded in the rinse vial positions. Press the TRAYS button on the ASE panel to lock the carousel into the starting position. Press the RINSE button twice to flush the solvent lines.

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

PREHEAT [0] min
HEAT [5] min
STATIC [5] min
FLUSH% [115] vol
PURGE [180] sec
CYCLES [2]
PRESSURE [1550] psi
TEMPERATURE [100] C
SOL A [Distilled Hexane:Mecl2] [25:75]%
SOL B [other] [0]%
SOL C [other] [0]%
SOL D [other] [0]%

6.5.10 After the extraction has finished remove the ASE vials from the ASE carousel. For sample concentration follow section 6.8.

6.5.11 If only PAH and PAH homolog analysis is required, then extract sample as above with 3:1 methylene chloride – distilled hexane and concentrate. Add 0.25 mL of BN surrogate spike Mix (5 µg/mL) and 0.25 mL of PAH matrix spike (5 µg/mL) for sediment. Make up to 1 - 2 mL in methylene chloride and do one-gram silica gel column clean up. Add 25 µL of 10 µg/mL BNA internal standard to all the samples and make it to 0.25 mL final volume with methylene chloride. Proceed with GC/MS analysis by injecting 1 µL in full scan. Do GPC cleanup for the dirty samples if it is needed.

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6.5.12 If low level PAH analysis is required, then extract sample as above with 3:1 methylene chloride – distilled hexane and concentrate. Add 0.05 mL of BN surrogate spike Mix (5 µg/mL) and 0.025 mL of 1 µg/mL PAH matrix spike for sediment. Make up to 1- 2 mL in methylene chloride and do one-gram silica gel column clean up. Add 25 µL of 1 µg/mL BNA internal standard to all the samples and make it to 0.25 mL final volume with methylene chloride. Proceed with GC/MS analysis by injecting 1 µL in SIM mode using MSD5975C.

6.6 Waste Sample Extraction (EPA 3580A) for WAS-BNA-8270, WAS-PAH-8270 and WAS-PAH-PA

6.6.1 Dilution of Non-Aqueous Wastes

This method is designed for wastes that are soluble in methylene chloride and may contain chemicals at a level greater than 20,000 mg/kg.

6.6.1.1 Check the samples out of the receiving room walk-in cooler.

6.6.1.2 Remove the BNA Matrix Spike Mix for Wastes and the BNA Surrogate Mix for Wastes from the refrigerator and let them warm to room temperature.

6.6.1.3 Thoroughly clean all glassware by rinsing it with hot tap water, deionized water and acetone, then baking it in an oven at 700 °C for 8 hours. Alternatively, glassware may be thoroughly washed with Alconox and hot water, and then rinsed with hot tap water, deionized water, and acetone. **Pre-rinse all glassware with methylene chloride prior to use!**

6.6.1.4 Label all glassware with the appropriate sample number, using labeling tape or pre-printed sample bar code labels. (Do not use a permanent marker! Methylene chloride will wash off the sample ID if it contacts the outside of the glassware.) There should be one blank (methylene chloride), two laboratory fortified blanks (i.e., methylene chloride spikes) and two matrix spikes (duplicates) for each set of samples extracted, not to exceed 20 samples per set.

6.6.1.5 Note the appearance of each sample in the logbook.

N.B.: The Extraction Notebook is a very valuable reference. Care should be taken that entries for each sample are complete and accurate. Any unusual observations made during the extraction process, including sample color or special extraction instructions, should be entered in the notebook.

6.6.1.6 Using a metal spatula, weigh approximately 1 g of each sample into a labeled centrifuge tube. Wipe the mouth of the tube with a clean Kim wipe to remove any sample material. Record the weight of the sample to the nearest 0.1 g in the extraction notebook. Cap the tube before proceeding to the next sample to prevent cross-contamination. Weigh 1 g of methylene chloride into each of three centrifuge tubes. These will serve as a laboratory blank and two laboratory fortified blanks. Select one sample and weigh out three 1 g portions instead of one -- two of these will be used as duplicate matrix spikes. There will generally be enough waste in a sample to prepare duplicate matrix spikes, but if none of the samples have enough, prepare a single matrix spike or no matrix spike along with duplicate LFBs and a blank. Be sure to note this in the notebook.

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6.6.1.7 Before proceeding, ensure that the spike and surrogate mixes are at room temperature. Many compounds easily precipitate out of solution when in the refrigerator or freezer and they need to be re-dissolved into solution by being warmed to room temperature. Also shake the bottle to ensure complete dissolution. You may place the capped bottle of solution in a sonic water bath for several minutes to quickly warm up the solution if you are pressed for time

6.6.1.8 Add 500 µL of the BNA Surrogate Mix (200 µg/mL acids, 100 µg/mL base-neutrals) to all samples, blanks, and spikes. Add 500 µL of the BNA Matrix Spike Mix (50 µg/mL) to the laboratory fortified blanks and matrix spikes.

N.B.: When adding surrogate and spike mixes, be careful that you do not mix the two up; if you are unsure whether you have made a mistake in spiking, it is best to simply re-spike a new sample. If there is no more sample left to re-spike, note the exact amount added so the chemist can make the correct recovery calculations.

6.6.1.9 Dilute the samples to 10 mL with methylene chloride and vortex well to mix, and sonicate for 5 minutes.

6.6.1.10 For each sample, loosely pack a disposable Pasteur pipet with a small plug of glass wool and top with 1/4 inch - 1/2 inch of sodium sulfate. Using a second Pasteur pipet, transfer the sample extract to the top of the sodium sulfate-packed pipet. Allow the extract to filter through the sodium sulfate into a second labeled centrifuge tube until 1 mL has been collected. Leave the remaining extract in the original centrifuge tube. When all extracts have been filtered, mark the meniscus on each tube of unfiltered extract and place the unfiltered extracts in the refrigerator.

6.6.1.11 At this point, the effective final volume is 10 mL. Ten mL should be used as final volume in all calculations.

6.6.1.12 Proceed to section 6.8 for instructions on how to concentrate the filtered extracts and add internal standard.

6.6.2 Extraction of Aqueous Waste

This method is applicable to samples expected to contain high concentrations of organics (>200 µg/L) in an aqueous matrix. These samples are prepared as water samples, as detailed in section 6.1 for Water, with the following exceptions:

6.6.2.1 Before beginning the extraction, consult the chemist who will be performing the analysis to confirm the extraction procedure to be used and the final extract volume.

6.6.2.2 Add 500 µL of the BNA Surrogate Mix (200 µg/mL acids, 100 µg/mL base-neutrals) to all samples, blanks, and spikes.

6.6.2.3 Add 500 µL of the "BNA Matrix Spike Mix (50µg/mL)" to the LFBs and matrix spikes.

6.6.3 Extractions for Solid Wastes

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This method is for samples expected to contain high concentrations of organics (>20 mg/kg). Follow the procedure outlined in 6.5.1 with the following exceptions.

6.6.3.1 Weigh out 2 g of each sample instead of 1 g.

6.6.3.2 For the blank and LFBs use 2 g of Ottawa Sand Standard instead of 1 g methylene chloride.

6.6.3.3 Perform a dry weight determination on all samples, unless this has already been done. See section 6.4.8 for dry weight determination.

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6.6.3.4 Samples that are wet or gummy must be mixed with 2 g or more of sodium sulfate until they are free flowing before the dilution may proceed. If the sample is sandy and free flowing, this step may be omitted.

6.6.3.5 The combined volume of the sample and sodium sulfate may exceed 10 mL, making it impossible to be confident about your final volume. It may be better to add sufficient solvent to cover the sample, in a measured volume, in series, as is done with soils (see section 6.4), combining the resulting extracts and blowing down to a pre-determined volume before filtering.

6.7 Sample Preparation for Pattern Identification (WAS-PAH-PA)

6.7.1 Prepare 100, 000 µg/mL of MC 252 source oil stock in 10 mL of methylene chloride or hexanes accurately and store at – 10 °C for routine use. Take one gram each of suspect oil samples or tar balls or contaminated soils in 10 mL of methylene chloride (prepare one sample in the batch in duplicate). Sonicate for 15 minutes and vortex to mix, and add sodium sulfate if any water present. Centrifuge and take 0.2 mL each of these concentrated MC 252 source oil and 0.5 mL or more of all the suspect samples (weathered oil samples collected in year 2012 need 2 to 4 mL out of 10 mL of one gram sample) and 0.2 mL of methylene chloride for method blank and solvent exchange into hexane for five gram silica gel fractionation. Waste samples prepared in section 6.7 may be used for pattern identification if the test is required at later stage.

6.7.2 Add 2 mL of hexane and nitrogen blow down to 0.5 mL under gentle stream of nitrogen at 40 °C of bath temperature (section 6.8.2). Add 2nd 2 mL of hexane and blow down to 0.5 mL and repeat 3rd time by adding another 2 mL of hexane and blow down to 0.5 mL. Dilute to 1 mL with hexane and proceed to a five-gram silica gel column fractionation.

6.7.3 Dry pack a five-gram silica gel column (30 cm length x 1.6 cm diameter width) for fractionation. Push a plug of silanized glass wool into the bottom of the column with a long glass rod and pour approximately one-quarter to one-half inch of granular sodium sulfate on top of the glass wool plug. Pour 5 g of silica gel on top of the sodium sulfate. Pour another half inch of sodium sulfate on top of the silica gel. Rinse column with 40 mL of hexane and letting the hexane drip through and discard the solvent.

6.7.4 Transfer extract to the top of the appropriate column and collect in a centrifuge tube labeled for sample. Rinse each tube with 4 times with 3 mL of hexane and add rinsate to column and collect up to 25 mL of hexane as fraction I.

6.7.5 Place another labeled centrifuge tube and elute column with methylene chloride. Collect up to 25 mL of methylene chloride as fraction II.

6.7.6 N-blow down fraction I and II to 0.5 mL and make up to 1 mL in methylene chloride. Final volume of 1 mL for fractions I and II represents the 20, 000 µg/mL concentration

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for the MC 252 source oil. Depending on how much oil left in the real suspect samples, these fractions may be concentrated to lower volume. Go to GC/MS analysis for pattern identification (DEP SOP SV-006).

6.8 Sample Extract Concentration

NOTE: Sample extracts may be concentrated using the S-Evap® System (K&D apparatus) or the Turbo-Vap® System. Store all sample extracts in the refrigerator at 4 °C until you are ready to work with them. If you are not positive that you can get to the samples within 30 minutes of removal from the refrigerator, then leave them there. Extracts can go directly from the refrigerator to K&D or to N_2 blow down without warming to room temperature, so there is no need to take them out until you are ready to pour them into a K&D flask or to place them onto the N_2 blow-down. Keeping them in the refrigerator until just prior to use will also ensure that the samples are exposed to as little light as possible. Many BNA compounds are light sensitive and can photo-degrade.

6.8.1 S-Evap® System Kuderna-Danish (K&D) Concentration

6.8.1.1 K&D water samples between 70 °C and 75 °C and soil samples between 75 °C and 85 °C. It is best to K&D samples to a volume of 3-5 mL instead of to 0.5 mL-1 mL. Concentration can be completed on the N-Evap® (It is too easy to boil samples dry when you have many samples on the S-Evap® at 1 mL and are trying to remove them all before they go dry.). During K&D concentration of samples, it is <u>vitally important</u> that they not be allowed to go dry. If this occurs, **re-extract the entire sample.** It is virtually ensured that the QC will be unacceptable if the sample goes dry even for a second. If the sample goes dry in the refrigerator, it will also have to be re-extracted.

6.8.1.2 An hour or so before concentrating the sample extracts turn on the S-Evap®, setting the dial to 5.5 to heat the water. The water level should be approximately half the depth of the water bath. Be sure the water will not cover the joint between the K&D flask and the concentrator tube. Cover the holes with watch glasses to prevent steam from escaping.

6.8.1.3 Attach the concentrator tube to the Kuderna-Danish flask with a plastic clip and add 2-3 Teflon boiling chips. Label the flask with the sample number.

6.8.1.4 If water or solid particles are present in the water sample extract, remove as follows:

6.8.1.4.1 If there appears to be water in the sample extract flask, add a small amount of granular sodium sulfate to the flask and swirl the flask to allow the sodium sulfate to absorb excess water.

6.8.1.4.1.1 If the water is pH 7 (i.e., it is from condensation on the glass), no other action needs to be taken.

6.8.1.4.1.2 If the water is acidic or basic (i.e., it is part of the pH-adjusted sample that has become mixed with the extract), and both the acid and basic fractions were collected in the same flask, add sodium sulfate and decant the extract into a K&D flask. Then, use 1N NaOH (if the water is acidic), or 1M H_2SO_4 (if the water is basic) to rinse the inside of the flask. Add a small amount of methylene

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chloride and swirl the flask. Then, remove any remaining water with sodium sulfate and decant the methylene chloride into the K&D flask along with the rest of the extract.

6.8.1.4.2 If there is a large amount of water in the extract, or both water and solid particles, place a small plug of silanized glass wool into the bottom of a clean glass powder funnel and pour 2-3 cm of granular sodium sulfate on top of it. Carefully pour the sample extract from the Erlenmeyer flask through the funnel and into the K&D flask. If the water in the flask is not neutral, follow step 6.8.1.4.1.2. Rinse the Erlenmeyer flask three times with 5 mL or so of methylene chloride and add that to the funnel. Rinse the funnel with 5-10 mL of methylene chloride.

6.8.1.4.3 If the extract contains solid particles, but no water, proceed as in step 6.8.1.4.2, but omit the sodium sulfate. Filter the extract through a glass funnel containing silanized glass wool.

6.8.1.4.4 Make sure that there is no water or particles (including sodium sulfate) in the extract before proceeding.

6.8.1.5 If water or solid particles are present in the soil sample extract, remove as follows:

6.8.1.5.1 If there appears to be water in the sample extract flask, add a small amount of granular sodium sulfate to the flask and swirl the flask to allow the sodium sulfate to absorb excess water.

6.8.1.5.2 If there is a large amount of water in the extract, or both water and solid particles, place a small plug of silanized glass wool into the bottom of a clean glass powder funnel and pour 2-3 cm of granular sodium sulfate on top of it. Carefully pour the sample extract from the Erlenmeyer flask through the funnel and into the K&D flask. Rinse the Erlenmeyer flask three times with 5 mL or so of methylene chloride and add that to the funnel. Rinse the funnel with 5-10 mL of methylene chloride.

6.8.1.5.3 If the extract contains solid particles, but no water, proceed as in step 6.7.1.5.2, but omit the sodium sulfate. Filter the extract through a glass funnel containing silanized glass wool.

6.8.1.5.4 Make sure that there is no water or particles (including sodium sulfate) in the extract before proceeding.

6.8.1.6 If the extract was not transferred into a K&D flask in step 6.7.1.4-6.7.1.5, then pour the sample extract from the Erlenmeyer flask into a K&D flask. Rinse the Erlenmeyer flask three times with methylene chloride, adding the rinsate to the K&D flask.

6.8.1.7 Pre-wet a three-ball Snyder column by adding about 1-mL methylene chloride to the top. Attach the column to the K&D apparatus. Place the K&D apparatus on the S-Evap® with the hot water bath between 70 °C and 75 °C for water sample

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extracts and between 75 °C and 85 °C for soil samples. At the proper rate of distillation the balls of the column will actively chatter, but the chambers will not flood with condensed solvent. It should take 20-30 minutes to complete the distillation. **It is extremely important that the samples are not concentrated to dryness; if this occurs, a significant portion of the lighter molecular weight compounds of interest will be lost.**

6.8.1.8 Remove the K&D apparatus from the S-Evap® when there is only 3-5 mL left in the concentrator tube. **Do not let the tube boil dry.** Let the vapors in the condensing column cool and drain into the tube, then remove the Snyder column and rinse the inside of the K&D flask with a few mL of methylene chloride, allowing this to drain into the concentrator tube. If the sample would not concentrate or is very dark in color, contact the chemist in charge of the analysis for instructions before proceeding with that sample.

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

6.8.1.10 Proceed to section 6.8.2 for final extract concentration, or, if GPC cleanup is needed, dilute the sample extracts to 2 to 10 mL with methylene chloride and proceed to section 6.9.

6.8.2 N-Evap® Concentration

6.8.2.1 Turn on the N-Evap® water bath and set the dial to around 1.5 so that the water will be warm when needed; the temperature of the water bath must not exceed 32 °C.

6.8.2.2 Transfer the contents of the concentrator tube or Turbo-Vap® vessel into a labeled 15-mL screw-top centrifuge tube using a disposable Pasteur pipet. Rinse the inside of the concentrator tube or Turbo-Vap® vessel three times with 1 mL of methylene chloride, and add the rinsate to the centrifuge tube.

6.8.2.3 Before proceeding with the extraction, contact the chemist who will be analyzing the samples. The samples will need to be transferred to amber 2-mL auto sampler vials immediately upon completion, so the analyst will need to be ready to do so. If the analyst will not be ready to transfer the samples, blow down only to approximately 3 mL. Do not add internal standard. Cap the centrifuge tubes tightly and place them in the refrigerator until the analyst is ready.

6.8.2.4 Place the centrifuge tubes in the N-Evap®. Lower a needle into each tube, so that the tip of the needle is approximately 1 inch above the surface of the extract. Be sure that the valves at the top of the needles that are in use are open. Turn on the N_2 stream. Keep the gas flow at a gentle rate. The extract should not splash due to the nitrogen flow. Lower the centrifuge tubes into the water bath.

6.8.2.5 Concentrate the samples to a final volume of approximately 0.8 mL using nitrogen/N-Evap® apparatus (if possible -- see step 6.8.2.7); do not let the samples go dry! If a sample goes dry while on the N-Evap®, re-extract the entire sample.

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6.8.2.6 If a sample extract is not dark in color or viscous, add 100 µL of 100 µg/mL the internal standard to the sample and bring the volume up to 1 mL with methylene chloride. Cap tightly, vortex and centrifuge. After adding internal standard, give to the analyst immediately. If the sample is dark in color or viscous, but will concentrate to the correct final volume, contact the analyst for specific instructions.

Note: For the analysis of drinking water, add 50 µL of 100 µg/mL the internal standard to the sample and bring the volume up to 1 mL with methylene chloride.

6.8.2.7 If an extract will not easily concentrate, blow down to whatever volume possible, be sure to note the final volume on the log sheet. Notify the analyst for special instructions, if any. Take an aliquot of exactly 1-mL of the extract and transfer to a clean, screw top centrifuge tube. Blow down to approximately 0.8 mL, then add appropriate internal standard and submit as in step 6.8.2.6.

6.8.2.8 If a sample extract will not concentrate to 5 mL, bring it to whatever volume is possible. Contact the analyst and follow any special instructions. Treat the extract as in step 6.8.2.7. However, if possible, re-extract the sample from the start, using an increased volume of spikes and surrogates proportional to the final volume of the sample (e.g., if a sample will reach a final volume of 10 mL instead of 1 mL, re-extract using 5 mL of surrogate instead of 500 µL.). Submit both extracts.

6.9 GPC CLEANUP PROCEDURE FOR PAHs

6.9.1 Calibration of the column: The column is calibrated using an HPLC-UV detector set at 254 nm. Load a 1.5-mL vial of the GPC Calibration Solution into the auto sampler. Attach the outlet flow line from the GPC system to the UV detector. Inject 1 mL of the calibration solution into the system. Monitor the detector recorder output chromatogram by collecting data by EZChrom. Note the retention times for each of the components. The analytes will elute in the following order: Corn oil, bis (2-ethylhexyl phthalate), methoxychlor, perylene and sulfur.

6.9.2 The peaks must exhibit the following characteristics:

- Peaks must be observed and should be symmetrical for all compounds in the calibration solution.
- The corn oil and phthalate peaks must exhibit > 85% resolution.
- The phthalate and methoxychlor peaks must exhibit > 85% resolution.
- The methoxychlor and perylene peaks must exhibit > 85% resolution.
- The perylene and sulfur peaks must not be saturated and must exhibit 100% resolution.

6.9.3 Using the information on the elution times, choose a time window for all the BNA components that will begin collection of effluent just before the phthalate peak elutes and will end collection just before the sulfur peak elutes. Dump time should allow corn oil and sulfur to be discarded, but not cut into the elution time of the other analytes.

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6.9.4 Using the information on the elution times, choose a time window for the PAHs components that will begin collection of effluent after 85% of the phthalate peak elutes and will end collection just before the sulfur peak elutes. Dump time should allow corn oil, phthalate and sulfur to be discarded, but not cut into the elution time of the other analytes. This collection window is same as pesticides.

6.9.5 Filter 1.5 mL of each sample extract through 0.45 μm syringe filter into an auto sampler vial. After inputting the correct collect and dump times into the fraction collector, load the aliquots of each extract onto the auto sampler. Program the auto sampler to inject 1 mL of each sample onto the system. If a sample is especially dirty, you may wish to split the 1 mL into multiple injections (2 injections of 500 μL, or 4 of 250 μL) to prevent breakthrough. After especially dirty samples, run a GPC blank (methylene chloride) to check for carry-over. Process the extracts using the dump, collect, and wash parameters determined from the calibration, and collect the cleaned extracts in 50 mL culture tubes that can be sealed with screw-caps. Cap the tubes and store them in the refrigerator if you will not be concentrating the extract right away. Concentrate the extracts using the N-Evap® (see Section 6.8.2). Transfer each extract to a centrifuge tube when it has blown down to 1-3 mL. As an alternative for samples that were injected more than once, you may combine the extracts from the all the vials for one sample, and concentrate by the K&D technique (section 6.8.1). If one-gram silica gel cleanup is required proceed to section 6.10.

6.10 ONE GRAM SILICA GEL CLEANUP FOR PAHs and PCP

6.10.1 Clean vacuum manifold valves and needles with methylene chloride.

6.10.2 For each sample place a properly labeled one-gram silica gel cartridge into the vacuum manifold.

6.10.3 Wash each cartridge with 6 mL of methylene chloride.

6.10.4 Apply and adjust vacuum if needed (vacuum is not usually needed), but do not let the cartridges go to dryness.

6.10.5 Place a rack containing labeled 15 mL centrifuge tubes inside the manifold, and replace the manifold top. Make sure that each cartridge position empties into the appropriate centrifuge tube.

6.10.6 Transfer crude extract (approximately 1 mL) or extract from GPC cleanup to the top frit of the appropriate silica gel cartridge.

6.10.7 Rinse each tube with 4 times with 3 mL of methylene chloride. Add rinsate to cartridge.

6.10.8 N-blow down and make up to a final volume with BNA internal standard in methylene chloride.

6.10.9 The extract from the ASE-350, make up to a final volume with BNA internal standard to 0.25 mL in methylene chloride before analysis.

6.11 FIVE GRAM SILICA GEL CLEANUP FOR PAHs

6.11.1 One-gram silica gel cleanup is a quick procedure for the relatively clean samples. Do five-gram silica gel column cleanup for bad matrices.

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6.11.2 For each extract, prepare one silica gel column from a 200 mL chromatographic column with a reservoir and a stopcock. With the stopcock closed, add a few mL of hexane to the column. Push a plug of silanized glass wool into the bottom of the column (a glass rod can be used for this) and tamp out all of the air bubbles. Pour approximately one-quarter to one-half inch of granular sodium sulfate on top of the glass wool plug. In a beaker, prepare a slurry of 5 g of silica gel in hexane. Using a funnel and a hexane filled wash bottle, transfer the slurry into the column on top of the sodium sulfate. Rinse the beaker and funnel well with hexane to ensure that no silica gel is left behind. The silica gel slurry will stick to the walls of the column. Rinse it down by opening the stopcock and washing the walls of the column with hexane (a Pasteur pipet can be used for this) until all of the slurry is packed into the column. Do not let the column go dry during this step. Pour another half inch of sodium sulfate on top of the silica gel. Before using the column, rinse with 20 mL of hexane, letting the hexane drip through. Again, do not allow the silica gel to go dry. Just before addition of the sample extract and fractionation surrogate, ensure that the hexane meniscus is resting on the sodium sulfate layer by opening the stopcock and adjusting the level accordingly.

6.11.3 Part of the sample extract should be put through the column. For most samples, this will be 0.5 mL out of 2 mL (Water) or 5 mL (Soil) dilute to 2 mL with hexane.

6.11.4 Transfer crude extract or extract from GPC cleanup to the top of the appropriate column. Open the stopcock for each column and collect in a centrifuge tube labeled for sample.

6.11.5 Rinse each tube with 4 times with 3 mL of 25:75 acetone-methylene chloride mixture. Add the rinsate to the column.

6.11.6 Collect up to 30 mL of 25:75 acetone-methylene chloride eluent.

6.11.7 N-blow down to 0.4 mL and add 50 µL of 100 µg/mL BNA internal standard and make up to 0.5 mL in methylene chloride. The final volume should result in a concentration equal to that of the extract initially taken for cleanup.

6.12 Sulfur Cleanup for Soil PAHs and Soil PAH Homologs with Copper Metal

6.12.1 Add copper metal prepared in section 4.15 to the sample extracts and vortex to mix. Add more copper metal if no shining surface left and cap. The extracts may be stored in the refrigerator in contact with copper metal for better sulfur removal. Filter through one gram or five gram silica gel column and makeup to required volume and proceed with GC/MS analysis.

7 SAMPLE ANALYSIS PROCEDURE

Reference analysis SOP **SV-006**.

8 QUALITY CONTROL

8.1 For every set of samples (not to exceed twenty samples), a reagent blank, two lab fortified blanks and duplicate matrix spikes must be prepared. If there is insufficient sample available to prepare duplicate sample matrix spikes, prepare one reagent blank, two laboratory fortified blanks, and one matrix spike. If there is insufficient sample available to prepare a single sample matrix spike, prepare one reagent blank, two laboratory fortified blanks, and no matrix spike.

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8.2 REMEMBER: BNA analytes are very sensitive to many factors. It is easy to become confused during a long BNA extraction, particularly with a large batch of samples. DO NOT overextend yourself or rush the sample preparation! Sample holding times are satisfactorily met as long as the extraction into solvent is completed before the sample's expiration date; you do not have to rush to extract and K&D all in one day, unless you can ensure that the QC is not being compromised. Minimizing the number of people involved with the extraction and concentration steps can also keep things from becoming confused.

8.3 Take things slowly and carefully and write down any noted problems or deviations in the Extraction Notebook. Even if you believe the deviation will not affect the final result, you may not be aware of how dramatically some of these changes may affect surrogate and matrix spike recoveries. If you run into technical problems that cannot be answered by your supervisor, notify the BNA supervisor or the BNA senior chemist immediately for instructions.

8.4 See BNA SOP SV-010 for detailed information on quality control, definitions and interferences.

9 SAFETY/HAZARDOUS WASTE MANAGEMENT

9.1 See SOP OG-001, Safety and Waste Management Practices in the Organic Analysis Groups (Volatile, Semi-Volatile and Pesticide Laboratories) for specific details on safety and hazardous waste management.

10 REFERENCES

10.1 Test Methods for Evaluating Solid Wastes, Revision 6, SW-846, Method 8270E, Semi-volatile Organic Compounds By Gas Chromatography/Mass Spectrometry (GC/MS).

10.2 *Code of Federal Regulations, Title 40, Part 136, Vol. 49, No. 209*, Method 625.1: Base/Neutrals and Acids by GC/MS, 12/2016.

10.3 *Test Methods for Evaluating Solid Wastes, Third Edition, SW-846,* Method 3510C, *Separatory Funnel Liquid-liquid Extraction,* Revision 3 12/96

10.4 *Test Methods for Evaluating Solid Wastes, Third Edition, SW-846,* Method 3520C, *Continuous Liquid-liquid Extraction,* Revision 3 12/96

10.5 Test Methods for Evaluating Solid Wastes, Third Edition, SW-846, Method 3550C, Ultrasonic Extraction, Revision 3, 2/2007

10.6 Test Methods for Evaluating Solid Wastes, Third Edition, SW-846, Method 3580A, Waste Dilution, Revision 1, 07/92

10.7 ASTM method. D 5739-06. Standard Practice for Oil Spill Source Identification by Gas Chromatography and Positive Ion Electron Impact Low Resolution Mass Spectrometry.

10.8 DEP SOP SV-009. BNA Standard Preparation.

10.9 DEP SOP MT-004. The Toxicity Characteristic Leaching Procedure EPA Method 1311.

10.10 DEP SOP MT-023. The Synthetic Precipitation Leaching Procedure EPA Method 1312.

10.11 DEP SOP SV-006. Analysis of Base/Neutral/Acid Extractables by Gas Chromatography/Mass Spectrometry

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10.12 DEP SOP SV-010. Quality Assurance/Quality Control in the Semi-Volatile Organics Laboratory (BNA-GC/MS).

10.13 DEP SOP OG-001. Safety and Waste Management Practices in the Organic Analysis Groups (Volatile, Semi-Volatile and Pesticide Laboratories).

10.14 B. Richter; J. Ezzell; and D. Felix; "Single Laboratory Method Validation Report. Extraction of TCL/PPL (Target Compound List/Priority Pollutant List) BNAs and Pesticides Using Accelerated Solvent Extraction (ASE) with Analytical Validation by GC/MS and GC/ECD;" Document 116064.A; Dionex Corporation; June 16, 1994.

10.15 EPA Method 3545A, Pressurized Fluid Extraction for Semivolatile and Nonvolatile Organics. Revision 1, February 2007.

Table 1: BNA Analytes

Base/Neutral Extractables	Diethylphthalate	Dinoseb
Nitrobenzene-d5 Surrogate	Dimethylphthalate	Diphenylamine (CCC)
Acetophenone	2,4-Dinitrotoluene	Di-n-butyl phthalate
Aniline	2,6-Dinitrotoluene	Di-n-octyl phthalate (CCC)
Benzyl alcohol	2,6-Dimethylnaphthalene	
Benzaldehyde	Fluorene	
2-Butoxyethanol	Hexachlorocyclopentadiene (SPCC)	
1-Butoxy-2-propanol	Triethyl-2-acetylcitrate	
Alpha-Terpineol	1-Methylnaphthalene	
Bis(2-chloroethoxy)methane	2-Methylnaphthalene	
Bis(2-chloroethyl)ether	1-Naphthylamine	
Bis(2-chloroisopropyl)ether	2-Naphthylamine	
1,3-Dinitrobenzene	2-Nitroaniline	Fluoranthene (CCC)
Dipropylene glycol monobutyl ether	5-Nitro-o-toluidine	
Ethyl Methanesulfonate	2,3,5-Trimethylnaphthalene	
Hexachlorobutadiene (CCC)	Pentachlorobenzene	3-Methylcholanthrene
Hexachloroethane		1-Methylphenanthrene
Hexachloropropene		
Isophorone	**Terphenyl-d14 Surrogate**	4-Nitroquinoline-1-oxide
Isosafrole	2-Acetylaminofluorene	N-Nitrosodiphenylamine/ Diphenylamine
Naphthalene	4-Aminobiphenyl	Phenacetin
Nitrobenzene	Anthracene	Phenanthrene
N-Nitrosodi-n-butylamine	Azobenzene/1,2-Diphenylhydrazine	Pentachloronitrobenzene
N-Nitrosodiethylamine	Benzidine	Pyrene
N-Nitrosodimethylamine	Benzo[a]anthracene	1,3,5-Trinitrobenzene
N-Nitrosodi-n-propylamine (SPCC)	Benzo[b]fluoranthene	
N-Nitrosomethylethylamine	Benzo[k]fluoranthene	***Acid Extractables***
N-Nitrosomorpholine	Benzo[a]pyrene (CCC)	**Phenol-d5 / 2-Fluorophenol Surrogates**
N-Nitrosopiperidine	Benzo[g,h,i]perylene	4-Chloro-3-methylphenol (CCC)
N-Nitrosopyrrolidine	Bis(2-ethylhexyl)adipate	2-Chlorophenol
Pentachloroethane	Bis(2-ethylhexyl)phthalate	2,4-Dichlorophenol (CCC)
2-Picoline	4-Bromophenylphenyl ether	2,6-Dichlorophenol
Pyridine	Butyl benzyl phthalate	2,4-Dimethylphenol
Safrole	Caffeine	2-Nitrophenol (CCC)
1,2,4,5-Tetrachlorobenzene	Carbazole	Phenol (CCC)
1,2,4-Trichlorobenzene	Chrysene	o-Cresol
o-Toluidine	Coprostanol	m, p-cresol

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2-Fluorobiphenyl Surrogate	Dibenzo[a,h]anthracene	Benzoic acid
Acenaphthene (CCC)	Dibenzothiophene	**2,4,6-Tribromophenol Surrogate**
Acenaphthylene	3,3'-Dichlorobenzidine	2,4-Dinitrophenol (SPCC)
Biphenyl		2-Methyl-4,6-dinitrophenol
2-Chloronaphthalene	p-(Dimethylamino)azobenzene	4-Nitrophenol (SPCC)
4-Chlorophenylphenyl ether	7,12-Dimethylbenz(a)anthracene	Pentachlorophenol (CCC)
Dibenzofuran		2,3,4,6-Tetrachlorophenol
Diethylphthalate	Triethyl-2-acetylcitrate	2,4,5-Trichlorophenol
Dimethylphthalate	Hexachlorobenzene	2,4,6-Trichlorophenol (CCC)
Dibenzofuran	Indeno[1,2,3-cd]pyrene	

Appendix of Changes

04/26/2010 Minor revisions to the Table 1, added 5 new analytes.

05/11/2011 Added new analyte Dipropylene glycol monobutyl ether, Soil PAH Homolog analysis test and Petroleum Hydrocarbon Pattern Identification.

06/01/2012 Minor changes to PAHs/Homologues and Petroleum Profile analyses.

03/31/2014 Added EPA method 3545A, ASE sediment extraction.

07/29/2015 Added few analytes in table and some other minor changes.

06/07/2016 Removed preparations not in use.

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