Extraction of Pesticides, Herbicides and other Chemicals from Water or Sediments/Waste

Note: LC-011 (A) is embedded in this SOP pertaining to Sediments/Waste Sample Preparation

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1 SCOPE AND APPLICATION

1.1 The SOP describes the extraction procedures for the isolation of chlorinated acid herbicides, Carbamates, urea herbicides and other chemicals from sediment/waste, ground water, or surface water matrices. If the water samples are not hydrolyzed with sodium hydroxide at basic pH of >12 for 1 to 2 hours, then this procedure isolates ONLY the acid forms of chlorinated acid herbicides, and it's not applicable for the extraction of the salt or ester forms of chlorinated acid herbicides.

1.2 Using this method, the analytes listed in Table 1 are reported in the Laboratory Information Management System (LIMS) for the tests. This method is in compliance with the requirements of EPA Method 8321B and USGS Method O-2060-01 for the parameters denoted as such in Table 5.1 of the laboratory's Quality Manual. USGS Method O-2060-01 is an approved test procedure of the National Pollutant Discharge Elimination System (NPDES) under the Clean Water Act (CWA) and Method 8321B is listed in the SW-846 publication under Subtitle C of the Resource Conservation and Recovery Act (RCRA). Furthermore, the NPDES and RCRA compliant tests employed by the laboratory using these methods are listed below.

Table 1: FDEP Laboratory Test IDs and equivalent methods reported by the SOP.

Laboratory Test Name	**Test Description**
W-AHERB-AA	USGS Method O-2060-01 analysis/EPA Method 3535A preparation, water sample
W-AHB-AA-R	EPA Method 8321B analysis/EPA Method 3535A preparation, water sample
S-AHERB-AA	EPA Method 8321B analysis/In House Method preparation, soil sample
SPLP-AH-AA	EPA Method 8321B analysis/EPA Method 3010/3535A preparation, SPLP water sample
TPLP-AH-AA	EPA Method 8321B analysis/EPA Method 3011/3535A preparation, TCLP water sample
W-CARB-AA	USGS Method O-2060-01 analysis/EPA Method 3535A preparation, water sample
W-CAB-AA-R	EPA Method 8321B analysis/EPA Method 3535A preparation, water sample
S-CARB-AA	EPA Method 8321B analysis/EPA Method 3570 preparation, soil sample
W-DALAPN-R	EPA Method 8321B analysis/EPA Method 3535A preparation, water sample
W-ENDO-AA	EPA Method 8321B analysis/Direct Injection preparation, water sample
W-GLYPH	EPA Method 8321B analysis/Direct Injection preparation, water sample
W-IND-AA-R	EPA Method 8321B analysis/Direct Injection preparation, water sample
S-INDZF-AA	EPA Method 8321B analysis/EPA Method 3570 preparation, soil sample
W-PCP-AA-R	EPA Method 8321B analysis/EPA Method 3535A preparation, water sample
W-PDQUAT	EPA Method 8321B analysis/Direct Injection preparation, water sample
W-SUC-AA-R	EPA Method 8321B analysis/EPA Method 3535A preparation, water sample
W-UHERB-AA	USGS Method O-2060-01 analysis/EPA Method 3535A preparation, water sample
W-UHB-AA-R	EPA Method 8321B analysis/EPA Method 3535A preparation, water sample
S-UHERB-AA	EPA Method 8321B analysis/EPA Method 3570 preparation, soil sample
SA-LCMSMS	EPA Method 8321B analysis, surface area wipe sample
O-LCMSMS	EPA Method 8321B analysis, unknown screening sample
W-MCYST-AA	EPA Method 8321B analysis, water sample
W-QUAT-NH4	EPA Method 8321B analysis, water sample
W-PYR-AA-R	EPA Method 8321B analysis, water sample
W-LCMS-DIJ	EPA Method 8321B analysis, water sample analysis by direct injection (Endothall, Glyphosate, Malathion, PDquat, Personal Care Products, Herbicides and Sucralose)

1.3 Filtered water samples are extracted by passing through solid phase column (Graphitized carbon-based solid-phase extraction column or Waters Oasis HLB columns or C18 SPE column) following EPA Method 3535A. Interferences are not removed by extraction of the base phase. Sucralose is sensitive to these acid/base extraction conditions and extraction at neutral pH 7 is used.

1.4 Following USGS method O-2060-01, 250 mL of filtered water sample is extracted by passing through a 0.5 gram graphitized carbon-based solid-phase extraction column (EPA Method 3535A) at neutral pH 7 for Chlorophenoxy acid herbicides, Carbamates, Personal Care Products (Acetaminophen, Carbamazepine,

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Fluridone, Indaziflam, Primidone and Sucralose) and Urea herbicides. Analyzed using high-performance liquid chromatography/ electrospray ionization–mass spectrometry to detect, identify, and quantify polar pesticides and pesticide metabolites in filtered water at concentrations as low as 2 nano grams per liter. This new method was designed to improve sensitivity and selectivity over the prior method, and to reduce known interferences from natural organic matter.

1.5 In sediment/waste extraction, samples are directly extracted with 0.3N sodium hydroxide followed by solid phase extraction. This simple procedure is most effective for the extraction of Chlorophenoxy acid herbicides. The base phase extraction also helps with the hydrolysis of the salts and esters to free acids.

1.6 EPA Method 3570 preparation method for the soil samples is used for the Carbamates, Urea Herbicides and Products Personal Care Products (Acetaminophen, Carbamazepine, Fluridone, Indaziflam, Primidone and Sucralose). Using this method, soil sample is extracted with 1:1 Acetonitrile – water and the filtered extract is analyzed by LC-MSMS.

1.7 Direct Injection Analysis: Water samples for Glyphosate, Glufosinate and AMPA, Diquat and Paraquat, Didecyldimethylammonium chloride, Endothall, Carbamazepine, Fluridone, Indaziflam, Malathion, Pyraclostrobin, Sucralose and other analytes with higher detection limits may be analyzed by direct injection.

2 SUMMARY OF THE METHOD

2.1 Chlorophenoxy acid herbicides, Carbamates, Urea Herbicides and Personal Care Products (Acetaminophen, Carbamazepine, Fluridone, Indaziflam and Primidone) are extracted from filtered 250 mL of water samples at neutral pH 7 with 250 mg of sodium chloride and 2.5 mg of EDTA using a 0.5 gram graphitized carbon-based solid-phase extraction column (EPA Method 3535A). After extraction, the adsorbed compounds are eluted from the SPE cartridge using two sequential elutions of 2 mL acetonitrile as fraction 1 and followed by 13 mL of an 80:20 ratio of methylene chloride – methanol containing 0.2% trifluoroacetic acid anhydride as fraction 2. The fraction 2 is reduced under nitrogen blowdown to near dryness and then combined with fraction 1. The final volume for the extract is 1 mL in 1:1 acetonitrile-MCAA water and the extract is filtered through 0.45 micron filter, and analyzed by LC-MSMS.

2.2 Urea Herbicides and Personal Care Products (Acetaminophen, Carbamazepine, Fluridone, Indaziflam, Primidone and Sucralose) are extracted from filtered 250 mL of water samples at neutral pH 7 with 250 mg of sodium chloride and 2.5 mg of EDTA using a 0.5 gram graphitized carbon-based solid-phase extraction column (EPA Method 3535A). After extraction, the adsorbed compounds are eluted from the SPE cartridge using two sequential elutions of 2 mL acetonitrile as fraction 1 and followed by 13 mL of an 80:20 ratio of methylene chloride – methanol as fraction 2. The fraction 2 is reduced under nitrogen to near dryness and then combined with fraction 1. The final volume for the extract is 1 mL in 10% acetonitrile - water and the extract is filtered through 0.45 micron filter, and analyzed by LC-MSMS.

2.3 Following EPA Method 3535A for Pyraclostrobin, filtered 250 mL of water sample at neutral pH 7 with 250 mg of sodium chloride is passed through C18 solid phase column and the column is eluted with 5 mL of acetonitrile. Acetonitrile eluent is concentrated to 0.1 mL by nitrogen blow down and re-constituted with 10% acetonitrile-water, and filtered through 0.45 micron filter, and analyzed by LC-MSMS.

2.4 Following EPA Method 3535A for Dalapon extraction, 500 mL of water sample (for waste water 100 mL) is pH adjusted to >12 with sodium hydroxide and left at room temperature for one hour to hydrolyze the salts and ester forms. Acidified and passed through Waters Oasis HLB solid phase column, and the column is eluted with 8 mL of methanol. Concentrated to 2 mL by nitrogen blow down and filtered through 0.45 micron filter, and analyzed by LC-MSMS.

2.5 One to thirty grams of sediment samples are extracted for acid herbicides by Sonication into an aqueous NaOH solution. The extract is then acidified and passed through C18 solid phase extraction column. Compounds of interest are eluted with 5 mL of acetonitrile. The 5 mL of acetonitrile eluate from above is then diluted to 10 mL with MCAA water and filtered through 0.45 micron filter, and analyzed by High Performance Liquid Chromatography with Turboionspray Mass Spectrometry (LC/MS MS).

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2.6 Ten grams of sediment sample is extracted for Carbamates, Urea Herbicides and Products Personal Care Products (Acetaminophen, Carbamazepine, Fluridone, Indaziflam, Primidone and Sucralose) by shaking in 10 mL of 1:1 acetonitrile-MCAA water for one hour following EPA Method 3570 Micro scale Solvent Extraction. Part of the extract is filtered through 0.45 micron filter and analyzed by LC-MSMS.

2.7 For Direct Injection Analysis: Water samples are filtered through 0.45 micron filter and analyzed by LC-MSMS for Glyphosate, Glufosinate and AMPA, Diquat and Paraquat, Didecyldimethylammonium chloride, Endothall, Carbamazepine, Fluridone, Indaziflam, Malathion, Pyraclostrobin, Sucralose and other analytes.

2.8 For Microcystins analysis, the whole water sample is autoclaved prior to analysis to release (lyse) the Microcystins from the algal and bacterial cells present. Therefore this method determines the "total" amount of Microcystins present in the sample. After autoclave, water samples are filtered through a 0.45 micron filter and an aliquot is injected onto a reverse-phase HPLC column and detected by triple quadrupole mass spectrometry using Turboionspray technique (LC-MSMS).

3 APPARATUS AND EQUIPMENT

3.1 Soil extraction: Metal spatulas, Aluminum weighing boats, Oven for dry weight determinations at 105 °C, Ultrasonic Bath (Fisher Scientific, Model FS220, 320 Watts power), Centrifuge bottles (250 mL and 500 mL), Centrifuge to fit bottles, Glass beakers (400 and 1000 mL), pH paper - 0.0 to 3.0 and 0.0 to 12.0, Top-Loading balance, accurate to + or - 0.1 g

3.2 Soil Micro-extraction: Vials – 40 mL with PTFE septa, Wrist Action Shaker, Vortex

3.3 Filtration - Vacuum filtration flasks, 500 and 1000 mL, Porcelain Büchner funnels, 9 cm and Rubber funnel supports. Whatman binder free glass microfiber filter, type: GF/F, 9 cm, 0.7 µm, Fisher catalog number 09-874-73 (1825-090) or TCLP Glass Fiber filter, Fisher catalog number 09-732-62 (FG75090MM)

3.4 Water extraction: Baker vacuum manifold or equivalent, capable of holding SPE cartridges with 75 mL polypropylene sample reservoirs, screw cap culture tubes 15 mL and Glass beakers 400 mL

3.5 Centrifuge tubes, graduated, with screw cap or ground glass stoppers – 15 mL. (Calibrate tubes to the required volume and record in the Extraction Log Book)

3.6 Gas-tight micro-syringes or volumetric pipets - 0.25 mL, 0.5 mL and 2 mL (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)

3.7 Eppendorf 10 µL to 100 µL and 100 µL to 1000 µL adjustable pipette (calibrated quarterly)

3.8 Pippet tips – 200 µL, 30 to 300 µL and 1000 µL

3.9 Volumetric flasks, 500 and 1000 mL

3.10 N-Evap®: Consists of heated water bath and apparatus to concentrate the extract using nitrogen gas

3.11 Graduated Polypropylene tube - 15 mL

3.12 Autoclave – Consolidated Stills and Sterilizers, Model # SSR-3A and Autoclave Temperature Indicator Tape.

3.13 Borex disposable culture glass tubes 16x125 mm) for Microcystin samples.

3.14 0.45 micron filtration disks, 2-mL crimp top vial and Polypropylene Autosampler vials – 0.3 mL

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4 REAGENTS AND CHEMICALS

4.1 C18 SPE Cartridge 500 mg (Restek, Resprep or equivalent), Waters Oasis HLB columns 200 mg/6cc, Supelco ENVIRO-Carb Graphitized Carbon Black 500 mg, 120/400 mesh, 6-mL, catalog number 57094 and UCT Enviro-Clean Graphitized Carbon Cartridge 500 mg, catalog number EC535156-P. Millipore 13 mm PTFE Syringe Filters 0.45 µ, pack of 1000 (catalog number - SLCR013NK).

4.2 Hydrochloric acid (HCl), reagent grade; Sulfuric acid (H_2SO_4), reagent grade; Anhydrous NaOH, reagent grade; Formic acid, Acros, Reagent ACS (96%), Trifluoroacetic acid anhydride (TFAA)—Pierce Chemical, Inc., reagent grade or equivalent and L–(+)–Ascorbic acid—J.T. Baker, reagent grade or equivalent.

4.3 Solvents, HPLC grade or equivalent (Fisher Scientific): Acetonitrile, acetone, methylene chloride, methanol and in house deionized water (HPLC water) tested for free of analytes.

4.4 1:9 HCl: Slowly add 100 mL of reagent grade HCl to 900 mL of HPLC water in a 1-liter volumetric flask. Mix well.

4.5 1:1 Sulfuric acid aqueous solution - Combine equal amounts of sulfuric acid and HPLC water. **REMEMBER: Add acid to water, NOT water to acid, otherwise a violent reactions may occur!**

4.6 Sulfuric acid, 0.1M: Slowly add 2.8 mL of concentrated sulfuric acid (18M) to approximately 450 mL of HPLC water in a 500 mL volumetric flask. Bring to a final volume of 500 mL and mix well. Transfer to re-pipet dispenser bottle.

4.7 Sulfuric acid, 0.1M with sodium chloride: add 250 grams of sodium chloride to 1000 mL of 0.1M H_2SO_4.

4.8 0.3N NaOH - Dissolve 6 g NaOH into 500 mL of HPLC grade water.

4.9 Sodium Chloride, reagent grade: Wash with methylene chloride and bake at 700 °C prior to use. EDTA, reagent grade.

4.10 Ottawa Sand for method blanks and lab-fortified blanks: and bake at 700 °C prior to use.

4.11 DEP well water: Ground water tested free of interferences and used for method blanks and lab fortified blanks (LFBs).

4.12 Estimate and prepare just enough conditioning and elution solutions for Graphitized Carbon Cartridges.

4.12.1 SPE cartridge conditioning solutions, Solution 1 - 80:20 methylene chloride - methanol. Mix 80 mL of methylene chloride with 20 mL of methanol. Store in a 250 mL stoppered Erlenmeyer flask. Replace solution at least weekly.

4.12.2 Solution 2 - Ascorbic acid solution. Dissolve 2 g of ascorbic acid (4.2) in 200 mL DI water and mix. Refrigerate at 4 °C and replace solution at least weekly.

4.12.3 SPE cartridge elution solution - 80:20 methylene chloride - methanol /0.2-percent trifluoroacetic acid anhydride. Mix 80 mL of methylene chloride, 20 mL methanol, and 0.2 mL of TFAA (4.2). Prepare solution daily and store in a 250 mL stoppered Erlenmeyer flask.

4.13 Positive Ion and Sucralose mix spike 1/4 µg/mL, Carbamates mix spike 1 µg/mL, Acid Herbicides mix spike 1 µg/mL and 8 surrogate spike mix 2/8 µg/mL (Sucralose-d6 at 8 µg/mL). Add 25 µL of each as required for a final volume of 1 mL extraction. Acid Herbicide Mix Spike and 2, 4-D-d3 surrogate at 10 µg/mL each for soil samples and add 100 µL to a final volume of 10 mL extraction. See SOP LC-039 for Monochloroacetic acid buffer solution (MCAA water) and all other spike standards preparation.

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5 SAMPLE COLLECTION, PRESERVATION AND HANDLING

5.1 Water samples are collected in 1-Liter glass amber bottle for acid herbicides, Urea Herbicides and Personal Care Products (Acetaminophen, Carbamazepine, Fluridone, Indaziflam, Primidone, Pyraclostrobin and Sucralose). For Carbamates, samples are collected in pre-preserved 1-Liter bottles with Monochloroacetic acid to less than pH 5. For Endothall and Glyphosate samples are collected in 40 mL vials and for Diquat and Paraquat samples are collected in pre-preserved 250 mL brown plastic bottles with Heptafluorobutyric acid to less than pH 5. For Malathion, samples are collected in pre-preserved 40 mL vials with Monochloroacetic acid to less than pH 5. All the samples must be iced or refrigerated at 6 °C or less from the time of collection until extraction, and must be extracted within 7 days of collection and for Didecyldimethylammonium chloride and Sucralose 21 days of collection.

5.2 Sediment/waste samples are collected in glass jars, and must be iced or refrigerated at 6 °C or less from the time of collection until extraction. They must be extracted within 14 days of collection.

5.3 All sample extracts must be analyzed within 40 days of sample extraction and for Carbamates within 21 days of extraction.

6 SAMPLE PREPARATION PROCEDURE

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. Also record bottle number if more than one bottle available, lot numbers of reagents and solvents used, and bath temperature for extracts concentration, balance number used, and tubes calibrated to volume (if calibrated).

6.1 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.

6.2 All glassware used in these procedures must then be pre-rinsed with a 1:9 solution of HCl, and then thoroughly rinsed with deionized water followed by methanol. The analytes are strong organic acids that react readily with alkaline substances on glassware and may be lost during extraction, this is why the pre-rinse with acid is necessary, to remove all traces of possible alkaline residue on glassware.

6.3 Check out the water and sediment samples from the receiving room walk-in cooler.

6.4 Before proceeding, ensure that the spike and surrogate standards are at room temperature.

6.5 Acid Herbicides Sediment Sample Extraction (Method SOP-LC-011(A)) for the LIMS test S-AHERB-AA

6.5.1 Label all glassware with the appropriate sample number, using labeling tape or pre-printed sample bar code labels. There should be one method blank (Ottawa sand), two laboratory fortified blanks (Ottawa sand spikes) and two matrix spikes (duplicates) for each set of samples extracted, not to exceed 20 samples per set.

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

6.5.3 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.

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

6.5.4.1 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. 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 weight notebook.

6.5.4.2 A sample's percent solid 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.

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

6.5.5 Weigh approximately 30 g of each sample into a properly labeled 500-mL centrifuge bottle and record the weight to the nearest 0.1-g. Prepare method blanks and laboratory-fortified blanks from Ottawa Sand. Select one sample for duplicate matrix spikes. For waste analysis, weigh one gram instead of 30 grams.

6.5.6 Add 100 µL of 2, 4-D-d3 surrogate spike 10 µg/mL to all the samples in the set and 100 µL of Acid Herbicide Spike Mix 10 µg/mL to the laboratory- fortified blank and matrix spikes.

6.5.7 Add 200 mL of 0.3N NaOH solution to each sediment sample and mix with spatula. Sonicate all samples in an ultrasonic water bath for 5 minutes. Then, allow the samples to stand for 25 minutes, mix and sonicate again for 5 minutes, and allow it to stand for another 25 minutes period. Finally, mix and sonicate for a third time for 5 minutes. Centrifuge each for approximately 5 minutes at 50 rpm to settle suspended particulate matter. Decant the NaOH supernatant from each sample into a tarred, labeled centrifuge bottle and record the weight of the decanted extract.

6.5.8 Acidify each aqueous extract with approximately 6 mL of 1:1 sulfuric acid solution (or 1.5 mL concentrated sulfuric acid) and swirl to mix well. Check pH with narrow range pH paper (pH 0.0 to 3.0) to ensure it is less than or equal to 2.

6.5.9 After the extracts have been acidified, they may be stored overnight at 4 °C, if necessary. The sample integrity will be maintained only after pH has been adjusted to 2 or less with acid.

6.5.10 It is likely that precipitate will form during sample acidification. Centrifuge the cloudy extract before adding to SPE cartridge. After transferring the extract, rinse the bottle with 10 mL of 0.1M sulfuric acid, centrifuge again, and then decant the rinse into the reservoir.

6.5.11 Condition C18 cartridge and Label with the appropriate sample number, using a permanent marker. Firmly push an adapter into the top of each cartridge, and then attach a 75-mL reservoir to each adapter.

6.5.12 Condition each cartridge by aspirating 5-mL acetonitrile through the cartridge, followed by 10 mL of 0.1M sulfuric acid. Do not allow the cartridge to go dry during conditioning or sample introduction. When the level of liquid in the cartridge begins to approach the bottom, shut off the valve on the vacuum manifold for that cartridge. Do not let the cartridge go dry during conditioning.

6.5.13 Fill each reservoir with the appropriate sample extract. Open the valves on the vacuum manifold and allow the samples to begin passing through the cartridges. The vacuum gauge should not exceed 8-12 inches Hg which corresponds to a sampling rate of about 10mL/min. Do not let any cartridge go dry during sample addition. If a cartridge becomes clogged, and the sample will not pass through it, use a new cartridge.

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6.5.14 After the entire 250-mL volume of a sample has been passed through a cartridge, rinse that sample's beaker with 5 mL of 0.1M sulfuric acid. Pour the rinsate into the sample reservoir and allow it to pass through. After this rinse has completely passed through the cartridge, allow the cartridge to go dry. Remove the reservoir, and let the cartridge vacuum dry for several minutes to remove all traces of water from it.

6.5.15 To elute the analytes from the cartridge. Attach a 5-mL syringe to the adapter on the cartridge. Put 5 mL of acetonitrile into the syringe. Push the acetonitrile through the cartridge using the syringe plunger. Collect elute in a labeled 15 mL graduated centrifuge tube. If more than one cartridge is used, then when eluting the sample, attach the second cartridge to the adapter on the first cartridge, and attach the syringe to the adapter on the second cartridge. Use only 5 mL of acetonitrile to elute and push it through both cartridges into a centrifuge tube.

6.5.16 Collect up to 5 mL of acetonitrile eluate from the SPE cartridge, and dilute with MCAA water to 10 mL. Vortex to mix and filter through 0.45 micron filtration disk into 2-mL crimp top vial. Proceed with LC-MSMS analysis. Store extracts at 4 °C if not using immediately.

6.6 Acid herbicides Extraction in SPLP and TCLP Extracts for the Lims tests SPLP/TCLP-AH-AA

6.6.1 For SPLP and TCLP extraction, 250 mL of SPLP/TCLP extracts are spiked as in step 6.5.6. The acidified samples extracts are passed through C18 cartridge as in steps 6.5.11 to 6.5.16 to complete the preparation for the tests SPLP/TCLP-AHB-AA.

6.7 Water Samples Preparation for Dalapon by Waters Oasis HLB Solid Phase Extraction (EPA Method 3535A) for the LIMS test W-DALAPN-R

6.7.1 Follow the steps in section 6.5.11 to 6.5.16 used above for soil acid herbicides extraction with the following changes when Dalapon is extracted from water samples. Extract 500 mL of water sample (100 mL of sample for waste water), add 50 µL of 10 µg/mL 2, 4-D-d3 surrogate Spike to all the samples in the set and 50 µL of 10 µg/mL Acid Herb Mix Spike to the laboratory-fortified blank and matrix spikes. After base hydrolysis for one hour, the acidified water samples are passed through Waters Oasis HLB Solid Phase Extraction columns and the columns are eluted with 8 mL of methanol. Nitrogen blow down to 2 mL. Vortex to mix and filter through 0.45 micron filtration disk into 2-mL crimp top vial. Proceed with LC-MSMS analysis. Store extracts at 4 °C if not using immediately.

6.8 Water Samples Preparation for Pyraclostrobin by C18 Solid Phase Extraction (EPA Method 3535A) for the LIMS test W-PYR-AA-R.

6.8.1 Pre-filter all the water samples in the set before extraction using a 0.7-µm pore size glass-fiber filter. Measure or weigh 250 mL of water sample and check pH of the sample and record. Add 250 mg of sodium chloride and mix well.

6.8.2 For every 20 samples in the set, prepare one extraction blank and duplicate Lab-fortified blanks using DI water. Prepare duplicate matrix sample spikes if enough sample is collected.

6.8.3 For all the samples in the set add 25 µL of 2/8 µg/mL surrogate mix. For QC samples add 25 µL of 1/4 µg/mL Positive Ion and sucralose mix spike. Each sample stir to mix well with glass rod. Record all the sample weights, volume of spikes and surrogates with Lims serial number in the extraction notebook.

6.8.4 Pass the water samples through 500 mg C18 Solid Phase Extraction columns and elute the columns with 5 mL of acetonitrile. Nitrogen blow down to 0.1 mL and re-constitute to 0.5 mL with 10% acetonitrile-water. Filter through 0.45 micron PTFE filter for LC-MSMS analysis and store extracts

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at 4 °C if not using immediately.

6.9 Acid Herbicides, Carbamates, Urea Herbicides and Personal Care Products Water Samples Preparation by Graphitized Carbon Cartridge Solid Phase Extraction (EPA Method 3535A & USGS O-2060-01) for the LIMS tests W-AHERB-AA, W-AHB-AA-R, W-CARB-AA, W-CAB-AA-R, W-UHERB-AA and W-UHB-AA-R

6.9.1 Filter all the water samples in the set using a 0.7-µm pore size glass-fiber filter. Measure or weigh 250 mL of filtered water sample. Check pH of the sample and record.

6.9.2 For every 20 samples in the set, prepare one extraction blank and duplicate Lab-fortified blanks using DEP well water. Prepare duplicate matrix sample spikes and sample duplicate. One sample duplicate per batch may be prepared to confirm low level positives present.

6.9.3 For each sample add 250 mg of sodium chloride and 2.5 mg of EDTA, and shake well to mix. For all the samples in the set add 25 µL of 2/8 µg/mL surrogate mix. For QC samples add 25 µL of 1 µg/mL Negative Ion mix spike, 40 µL of 25 µg/mL Benzoic acid spike, 25 µL of 1 µg/mL Carbamates mix spike and 25 µL of 1/4 µg/mL Positive Ion and sucralose mix spike. Record all the sample weights, volume of spikes and surrogates with Lims serial number in the extraction notebook.

6.9.4 Condition 0.5 gram Supelcoclean or UCT graphitized carbon cartridge sequentially eluting with two 5-mL aliquots of 80:20 methylene chloride - methanol (v/v) without vacuum. This step is followed by elution with 5 mL of methanol through each cartridge. Elute three 5-mL aliquots of the ascorbic acid solution to complete the conditioning. Do all these steps under gravity flow without vacuum. The ascorbic acid conditioning step should take place slowly to ensure activation of binding sites in the cartridge bed. Keep the flow rate of ascorbic acid solution at no more than 3 mL/min. Cover conditioned cartridges with foil and set aside until ready for use. Cartridges can be used up to 8 hours after conditioning. Cartridges that have not been used within 8 hours need to be reconditioned. Collect the conditioning solvents in the vacuum manifold; place these solvents in a chlorinated waste container for proper disposal. NOTE: To avoid deactivation of the SPE sorbent surface, cartridges should never be allowed to dry after conditioning.

6.9.5 Label each conditioned, 6- mL graphitized carbon-based SPE cartridge with appropriate sample number. Mount 75 mL reservoir with adopter and load sample. Adjust water sample flow rate through the conditioned cartridges at 20 mL/min. Upon completion of extraction, air dry the SPE cartridge for few minutes and make sure no drops of water sticking in the tube. Each cartridge is eluted under gravity as in steps 6.9.6 and 6.9.7 without vacuum.

6.9.6 After sample loading rinse the cartridge 3 times with 0.5 mL of DI water to remove any salts left on the column and air dry, cartridges retain a small amount (around 0.1 mL) of residual water. Chemically dry the cartridge by eluting the analytes with two times 1 mL of acetonitrile and label as fraction 1. Some compounds are eluted in this fraction. This 2 mL fraction is collected separately and labeled with the appropriate sample identifications. The last drops of acetonitrile in the cartridge bed is removed by applying positive pressure with a bulb on top of the cartridge.

6.9.7 Most of the analytes are collected in the second elution fraction of the SPE cartridge. Place 15 mL centrifuge tube in the elution rack to collect the fraction 2. Label these tubes with the appropriate sample identifications. Make sure the tubes are arranged in the correct order so that they will receive the eluent from appropriate SPE cartridges. Elute each SPE cartridge with three aliquots of 80:20 methylene chloride - methanol (v/v) with 0.2 percent trifluoroacetic acid anhydride (TFAA) solution. The three aliquot volumes are, in sequence, 5, 5, and 3 mL. Eluting with three aliquots improves analyte recoveries compared to continuous elution with a single 13-mL aliquot. Start the

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elution and do not let the cartridge beds go dry. When the elutions of the three aliquots for each sample are completed, remove the tube and go to nitrogen blow down step.

6.9.8 Nitrogen blow down slowly at 34 °C bath temperature. Rinse the top of the tube with 0.5 mL of methanol at 5 mL and 0.5 mL and blow down to 0.2 mL. Transfer fraction 1 with acetonitrile into fraction 2 and Nitrogen-blow down at 42 °C bath temperature to 0.5 mL of acetonitrile and makeup to 1 mL with MCAA water. There may be residual methylene chloride left in the fraction 2. If this occurs, remove methylene chloride by adding 1 mL of methanol and blowing down to 0.2 mL. Repeat this step until no more methylene chloride left. Typically, methylene chloride is present as an immiscible layer underneath the aqueous phase or as small bubbles at the bottom of the test tube, or as a cloudy or "milky" appearance. As this description indicates, the presence of methylene chloride in a sample may be barely perceptible, so each sample must be carefully examined for the presence of methylene chloride.

6.9.9 Vortex to mix and filter through 0.45 micron PTFE filter for LC-MSMS analysis. Store extracts at 4 °C if not using immediately.

6.10 Urea Herbicides and Personal Care Products (Acetaminophen, Carbamazepine, Fluridone, Indaziflam, Primidone and Sucralose) Water Samples Preparation by Graphitized Carbon Cartridge Solid Phase Extraction (EPA Method 3535A & USGS O-2060-01) for the LIMS tests W-PCP-AA-R, W-SUC-AA-R, W-UHERB-AA and W-UHB-AA-R

6.10.1 Following steps in 6.9, prepare Urea Herbicides and Personal Care Products Water Samples Preparation by Graphitized Carbon Cartridge Solid Phase Extraction with few modifications. Measure or weigh 250 mL of filtered water sample with 250 mg of sodium chloride and 25 mg of EDTA. For all the samples in the set add 25 µL of 2/8 µg/mL surrogate mix. For QC samples add 25 µL of 1/4 µg/mL Positive Ion and sucralose mix spike. After sample loading rinse the cartridge with 3 times 0.5 mL of DI water to remove any salts left on the column and air dry, collect 2 times 1 mL of acetonitrile as fraction 1. Collect fraction 2 with three aliquots in sequence of, 5, 5, and 3 mL of 80:20 methylene chloride - methanol (v/v). Do not use trifluoroacetic acid anhydride (TFAA). Nitrogen blow down slowly at 34 °C bath temperature to 0.2 mL as in step 6.9.8 and transfer fraction 1 with acetonitrile into fraction 2 and Nitrogen-blow down at 42 °C bath temperature to 0.1 mL of acetonitrile and makeup to 1 mL with 10% acetonitrile – water. Vortex to mix and filter through 0.45 micron PTFE filter for LC-MSMS analysis. Store extracts at 4 °C if not using immediately.

6.11 Carbamates, Urea Herbicides and Personal Care Products (Acetaminophen, Carbamazepine, Fluridone, Indaziflam and Primidone) Sediment Sample Preparation (EPA Method 3570) for the LIMs tests S-CARB-AA, S-INDZF-AA and S-UHERB-AA

6.11.1 Follow the steps in section 6.5.1 to 6.5.5 for soil extraction of acid herbicides. Weigh 10 grams of well-mixed sediment sample into 40-mL vial for each sample. Also prepare one sample duplicate matrix spikes and Ottawa sand laboratory blank and laboratory fortified blanks (LFB).

6.11.2 Add 125 µL of 2/8 µg/mL surrogate mix (or 25 µL of 10 µg/mL each of individual surrogates Acetaminophen-d4/Carbamazepine-d10/Carbaryl-d7/Diuron-d6/Indaziflam-N15/Primidone-d5) to all samples in the set. Add 250 µL of 1/4 µg/mL Positive Ion and Sucralose Mix Spike, and/or 250 µL of 1 µg/mL Carbamates Spike to the laboratory-fortified blank and matrix spikes. Note the amount added and the concentration of analytes in the extraction log book.

6.11.3 Add 10 mL of 1:1 acetonitrile – water (for Carbamates use 10 mL of 1:1 acetonitrile – MCAA water) to each sample and cap it tightly and shake for 1hour using wrist action shaker.

6.11.4 After 1hour, centrifuge the samples and filter the extract through 0.45 µm PTFE filtration disk into a

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2–mL crimp top vial. Proceed with LC-MSMS analysis. Store extracts at 4 °C if not using immediately.

NOTE: If pH of sample is greater than 3, recoveries of some Carbamates, particularly Oxamyl, Methomyl and Methiocarb may be low. These Carbamates will degrade in neutral or alkaline aqueous solutions. If soil is alkaline, it is unlikely that these Carbamates will be found.

6.12 Water Samples Preparation for Direct Injection Analysis of Didecyldimethylammonium chloride (Lims test W-QUAT-NH4)

6.12.1 Water Samples for the analysis of Didecyldimethylammonium chloride are collected in 40 mL VOC vials with only 1/3 filled. For each vial, add equivalent volume of methanol and record the volume. Shake and let it stand for 30 minutes. Take 2 mL of this 1:1 methanol-water sample for each sample and for duplicate matrix spikes. Also, prepare method blanks and laboratory fortified blanks from 1:1 methanol-reagent water.

6.12.2 Add 20 µL of 0.020 µg/mL Carbamazepine-d10 surrogate to all the samples in the set for 2 mL of water sample. Add 20 µL of 1 µg/mL of Didecyldimethylammonium chloride to 2 mL of QC samples. This results in a 10 ppb spike level. Vortex to mix and filter all the samples in the set through 0.45 µm filter for LC-MSMS analysis or store at 5 °C for further analysis.

6.13 Water Samples Preparation for Direct Injection Analysis of Endothall (Lims test W-ENDO-AA)

6.13.1 Prepare 5 mL each of the well shaken sample and duplicate matrix spikes, and 5 mL each of method blanks and laboratory fortified blanks using reagent water. Add 125 µL of 1 µg/mL Endothall-d6 surrogate to all the samples in the set for 5 mL of water sample. Add 125 µL of 1 µg/mL of ICAL and Spike mix containing Endothall and Endothall-d6 to 5 mL of QC samples. This results in a 25 ppb spike level. Vortex to mix.

6.13.2 Filter all the samples in the set through 0.45 µm filter for the LC-MSMS analysis of store at 5 °C for further analysis.

6.14 Water Samples Preparation for Direct Injection Analysis of Diquat/Paraquat (Lims test W-PDQUAT)

6.14.1 Prepare 5 mL each of the well shaken sample and duplicate matrix spikes, and 5 mL each of method blanks and laboratory fortified blanks using reagent water. Add 50 µL of 10 µg/mL Diquat-d4 surrogate to all the samples in the set for 10 mL of water sample. Add 25 µL of 10 µg/mL of Diquat/Paraquat spike mix to 10 mL of QC samples. Vortex to mix.

6.14.2 Filter all the samples in the set through 0.45 µm filter for the LC-MSMS analysis of store at 5 °C for further analysis.

6.15 Water Samples Preparation for Direct Injection Analysis of Glyphosate/Glufosinate/AMPA (Lims test W-GLYPH)

6.15.1 Prepare 1 mL each of the well shaken sample and duplicate matrix spikes, and 1 mL each of method blanks and laboratory fortified blanks using reagent water. Add 50 µL of 10 µg/mL Glyphosate 13C 15N surrogate to all the samples in the set for 1mL of water sample. Add 50 µL of 1 µg/mL of Glyphosate/AMPA spike mix to 1 mL of QC samples. Vortex to mix.

6.15.2 Filter all the samples in the set through 0.45 µm filter for the LC-MSMS analysis of store at 5 °C for further analysis.

6.16 Water Samples Preparation for Direct Injection Analysis of Personal Care Products and Sucralose (Lims test W-LCMS-DIJ)

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6.16.1 Prepare 9 mL each of the well shaken water sample and one sample in duplicate for matrix spikes, and 9 mL each of reagent water for method blanks and laboratory fortified blanks. Add 1 mL each of acetonitrile and mix well, this results a 10% acetonitrile is water. Add 25 µL of 0.1 µg/mL surrogate mix to all the samples in the set for 10 mL of water sample. Add 25 µL of 0.1 µg/mL of Spike mix containing Personal Care Products and Sucralose to 10 mL of QC samples. This results in a 2 ppb spike level. Vortex to mix.

6.16.2 Filter all the samples in the set through 0.45 µm filter for the LC-MSMS analysis of store at 5 °C for further analysis.

6.17 Water Samples Preparation for Direct Injection Analysis of Malathion (Lims test W-MALATHN)

6.17.1 Prepare 1 mL each of one blank and duplicate laboratory fortified blanks using MCAA water. Filter all the samples pre-preserved with Monochloroacetic acid buffer in the set through 0.45 µm filter and collet 5 mL each. Filter one of the sample for duplicate matrix spikes in the set (not to exceed 20 samples).

6.17.2 Add 25 µL of 1 µg/mL Malathion-d6 surrogate to all the samples in the set for 1mL of water sample. Add 20 µL of 0.5 µg/mL of Malathion spike to 1 mL of QC samples. Vortex to mix. Store at 5 °C or proceed directly to UPLC analysis.

6.18 Water Samples Preparation for Direct Injection Analysis of Microcystins by Autoclave (Lims test W-MCYST-AA)

6.18.1 Tighten the sample bottle cap and thoroughly shake the bottle and its contents to ensure good mixing prior to sub-sampling. Weigh 10 grams or measure 10 mL of each sample and quality control sample for autoclave in a pre-labeled 16x125 mm borosilicate culture tube. The extraction batch consists of up to 20 samples including one method blank (reagent water), one or two lab fortified blanks (i.e. reagent water spike) and duplicate sample matrix spikes. Method blank, lab fortified blanks and sample matrix spikes are quality control samples.

6.18.2 Prepare quality control samples (lab fortified blanks and sample matrix spikes) by adding 20 µL of Microcystin Mix Spike standard solution (1000 µg/L) to 16x125 mm borosilicate culture tubes and diluting to 2 mL with reagent water and sample respectively. This results in a 10 µg/L spike level. Add to blank and all the samples in the set, 20 µL of Carbamazepine-d10 Spike standard solution (100 µg/L). Vortex to mix.

6.18.3 Cap the tubes loose or cover with aluminum foil and mark the sample level with marker before placing the tray with the tubes in the autoclave. Place Autoclave Temperature Indicator tape on the tray. Follow these steps for the Autoclave.

6.18.4 Turn ON Generator Control button (located at the bottom) and wait to reach the pressure to 20 psi (bottom dial) and it takes about 30 minutes.

6.18.5 Open the chamber and place the sample tray. Close the door and hand tight. Make sure all the doors are closed (check door open signal). If the door open signal is ON, check in the back side (from back room).

6.18.6 Fluid cycle button should be pressed down (normally it is pressed down, unless somebody used and changed it). Push to start the Autoclave by pressing ON button. The pressure dial will go down and builds back to 20 psi. Once the needle reached to 120 °C (248 °F) (takes about 5-7 minutes), then timer will start.

6.18.7 Check after 35 minutes, if it is done (timer will show zero). Then press the generator control button

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to turn OFF the Autoclave. Wait for the chamber pressure to drop to zero on the top panel.

6.18.8 Open the chamber and carefully remove the sample tray and allow it to come to room temperature.

6.18.9 When the autoclave is complete, check the chart to make sure everything is normal, and make sure that the Indicator paper has turned black. Record in the note book and paste the Indicator tape in the extraction log book.

CAUTION: DO NOT OPEN THE AUTOCLAVE UNTIL PRESSURE GAUGE READS ZERO!!
Autoclave contains steam under pressure, which will vent and could cause injury if opened too soon.

6.18.10 Allow the tubes to reach room temperature, check the level of the sample (if sample is not up to the mark, then the sample need to be re-autoclaved with fresh sample) and cap them tightly. Vortex to mix and centrifuge at 2000 RPM for 10 minutes, filter through 0.45 micron filtration disk and proceed with the analysis by ELISA or LC-MSMS.

7 SAMPLE ANALYSIS PROCEDURE

7.1 See SOP LC-001, Analysis of Pesticides, Herbicides and other Chemicals in Water and Sediment/Waste Sample Extracts by High Performance Liquid Chromatography with Turboionspray Mass Spectrometry (HPLC/MS/MS).

8 QUALITY CONTROL

8.1 See SOP LC-010, “Quality Assurance/Quality Control in the HPLC Pesticides Laboratory” for specific details 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 EPA Method 3535A. Solid-Phase Extraction (SPE) Revision 1, February 2007

10.2 USGS O-2060-01, “Determination of Pesticides in Water by Graphited Carbon-Based Solid-Phase Extraction and High-Performance Liquid Chromatography/Mass Spectrometry”. U.S. Geological Survey, Denver, CO 80225, 2001.

10.3 EPA Method 3570. Microscale Solvent Extraction (MSE) Revision 0, November 2002

10.4 DEP SOP LC-039. HPLC Standard Preparation.

10.5 DEP SOP LC-001. Analysis of Pesticides, Herbicides and other Chemicals in Water and Sediment/Waste Sample Extracts by High Performance Liquid Chromatography with Turboionspray Mass Spectrometry (HPLC/MS MS)

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

Appendix of Changes

08/22/2011 Edited preparation for Sucralose in water

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08/15/2012	Edited preparation for Sucralose in water
02/19/2013	Edited Graphitized Carbon Black SPE preparation for low level Chlorophenoxy acid herbicides, Carbamates and Urea herbicides in water
01/10/2014	Merged SOPs LC-005-1, LC-006-2, LC-011-2 and LC-021-2 into LC-011-2
01/14/2015	Merged SOPs LC-038-1 and LC-011-2 into LC-011-2
01/15/2016	Added new analytes
01/26/2016	Added Benzoic acid
08/03/2016	Added Malathion

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