QUALITY ASSURANCE PROJECT PLAN

Addendum and Errata

Sheboygan AOC Pathway to Delisting Habitat BUI's—Survey and Assessment

Grant Number: GL00E00489-0

November 18, 2011

Errata

p.156 Qualifications for Wisconsin State Laboratory of Hygiene (WSLH): The laboratory does not hold NELAP accreditation for tissue analyses.

Amendments

The WSLH Quality Assurance Manual has been updated to revision 9.

Appendix C: Insert SOP for mudpuppy trapping from Gary Casper. The survey augments the Endangered Resources surveys, focusing on a species of greatest conservation need. As the SOP indicates, the survey is best conducted in late October or early November, when mudpuppies are most active.

Appendix F.5 Replace SOP ESS Org Method 1480 with revision 3 of the method.

Standard Operating Procedure for: Aquatic Funnel Trap Surveys for the Mudpuppy, *Necturus maculosus*

Version 1.0

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November 4, 2011

Mudpuppy, *Necturus maculosus* (Waukesha County, Wisconsin), photo © G. S. Casper

1.0 Introduction

The mudpuppy (*Necturus maculosus*) is a large aquatic salamander found throughout eastern North America, occupying benthic habitats of permanent lakes and streams, in both clear and silted water with or without aquatic plants (Lannoo, 2005). Mudpuppies forage nocturnally on a variety of organisms including aquatic invertebrates, crayfishes, small fishes and annelids, and are active year-round (Lannoo, 2005). Large populations may be present where retreats, such as flat rock slabs, logs, and planks are numerous. Adults prefer well-aerated water, often downstream or to the side of riffles (Bishop, 1941), and have been reported from depths to 17 m in Lake Erie (Pfingsten and White, 1989) and to 27 m in Green Bay, Lake Michigan, by (Reigle, 1967). In Wisconsin, large mating congregations form in late October or November in shallow (1-2 m), usually rocky, areas of lakes and streams (Vogt, 1981). Oviposition takes place in late spring, with nests excavated by females under rocks, logs, boards and other submerged cover, and eggs attached to the undersides of these objects (Shoop, 1965; Petranka, 1998; Vogt, 1981). Hatching occurs 1-2 months after laying, depending on water temperature (Petranka, 1998). Females apparently attend the eggs during development, protecting them from predation (Shoop 1965; Pfingsten and White 1989; Petranka 1998).

This Standard Operating Procedure describes a protocol for using baited aquatic traps to survey for mudpuppies. According to Pauley (no date), a variety of traps have been used to capture all life stages of mudpuppies and waterdogs. Funnel traps or commercial minnow traps are effective in capturing adults (Ashton and Braswell, 1979; Gendron, no date). These authors recommend them for larvae as well. Sever and Bart (1996) used modified Thiel-Turkey traps as described by Manning (1986) baited with earthworms. Pfingsten and White (1989) reported that commercial collectors use fyke nets baited with partially rotted fish and canned dog food. Hacker (1956) used traps but did not describe the type of trap. Detection probabilities and an understanding of how covariates such as temperature, time of year, bait type, or habitat type may affect trap success are unknown, and designing surveys to acquire and analyze data sufficient to calculate detection probabilities and understand covariate affects is strongly encouraged.

2.0 Instructions

Strict adherence to the rules is essential for sharing data with other programs and proper analysis of results. Please read all instructions prior to conducting surveys. Be sure to understand the purpose for which the various types of data will be collected and review the types of measurements and samples needed.

2.1 Requirements

Obtain all necessary permits (check with local authorities). It is desirable that surveyors be able to identify all species likely to be encountered. While the focus of the survey is on mudpuppies, various crayfishes, fishes and many aquatic insects will also be routinely captured. Numerous books and online resources are available (see Resources below). Surveys typically require working in cold water, so proper clothing is important, a partner is strongly recommended for safety, and a tolerance for cold hands is helpful.

2.2 Standard Operating Procedure

2.2.1 Seasonal Timing of Sampling

Surveys are recommended between mid-October and April, with late October through November probably being the best period in Wisconsin, as congregations are known to occur at this time (Vogt, 1981). Traps can be placed through the ice in winter. Seasonal variation in trapping success is currently unknown, but it is suspected to be low in mid-summer when water temperatures are higher.

2.2.2 Proper Conditions for Sampling

Avoid sampling after heavy rains when waters are flush with silt and streams experience strong currents.

2.2.3 Pre-survey Preparation

1. *Site selection and trap placement*. Obtain site maps and access instructions (do not trespass on private property without permission). If these are not provided, select sites based on habitat suitability (see field guides) – survey only sites with suitable habitat. This is generally permanent water, depths of 0.5 m or greater, and substrates with abundant cover such as large flat rocks or log jams. Visit the site(s) prior to beginning surveys to become familiar with the area. Traps should be placed in accessible areas and completely submerged, resting on the lake/stream bottom. They can be weighted by placing a rock inside, and should be tethered to a firm anchor (i.e. tree or large rock) with a strong waterproof line. Place traps where the salamanders are most likely to be, in areas with cover such as logs or large rocks.
2. *Survey effort*: Each trapping session should be 4-5 nights long. Check traps daily when they are set.
3. Make sure any vehicles have enough gas to complete the necessary travel.
4. *Safety*. Tell someone where you are going before heading out for the survey. Having a partner is strongly recommended for safety when working in the water.
5. Make certain you have all necessary equipment.

    Map of survey sites, directions to sites
    Traps (5 or 10 per trap site)
    Data sheets with survey instructions
    Clipboard
    Pencils
    Thermometer
    Waterproof rope and twine (for repairing traps and tethering to anchor points)
    Bait (canned dog food, raw giblets, fish heads, etc.)
    Field guides (see references)
    First aid kit
    Ziplock bags for collecting casualties
    Bait (or other) bucket for holding live specimens
    Proper clothing including hip or chest waders
    Digital camera for documenting finds
    Magnifying lense useful for identifying insects and small fishes (optional)
    Polarized sunglasses with light lenses, i.e. “fishing glasses” (optional)

2.2.4 Survey Procedure

1. Watch the weather forecast and plan a 5-day session to avoid significant rain events.
2. When setting traps record the location and GPS coordinates
3. Take the water temperature.
3. Check traps systematically, keeping each trap number consistent each time it is checked. Write the number on the trap if possible to avoid confusion.
4. Recording Data: Follow instructions on the data sheet. Record all species in traps to the best of your ability to identify, but *it is very important* that you record only animals actually *in* the traps. *Anything observed outside of the traps, including eggs, should be recorded under visual observations.* You may use tally marks as you go, but if so sum the marks when finished and circle the final total number. You may also use species groups where you cannot

determine the species, such as "unknown crayfish", or "unidentified minnow". Note anything you think might be affecting sampling, such as water level changes, holes in traps, or disturbance to traps.

5. A bucket may be used place the trap contents into for working, but you may also simply remove trap contents recording data as you go (this is where a partner really comes in handy as you are standing in the water with your hands full).
6. Handle animals gently. Some insects may bite or sting and crayfishes may pinch. Release all live animals into the water at the trap site (unless temporarily holding specimens for an expert identification).
7. Mortality is rare but sometimes occurs. Carcasses may be saved in labeled zip lock bags and frozen for donation to a museum. If in good condition, such carcasses are valuable voucher specimens for the study and should be deposited at a public museum with full data attached.
8. Take digital pictures of each species found at each trap site, plus any rare or unusual animals.
9. Record any other wildlife detected at the bottom of the data sheet, including eggs. Polarized sunglasses are useful to increase your ability to see into the water.

2.2.5 Post-survey

1. Consolidate all data sheets and place them in a secure location.
2. Store equipment.
3. Place all wet equipment in a place where it can dry.
4. If available, transcribe data from the data sheets into a spreadsheet or database. This can be started the next morning, especially if tired, to avoid errors.
5. Place paper data sheets in a safe dry place, and make copies or scans for your own records before submitting data to programs or otherwise sharing with others. It is important to have a backup of your data. Congratulate yourself on a job well done!

2.3 Literature Cited and Resources

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Shoop, C.R. 1965. Aspects of reproduction in Louisiana *Necturus* populations. The American Midland Naturalist. 74:357-367.
Vogt, R. C. 1981. Natural History of Amphibians and Reptiles of Wisconsin. Milwaukee Public Museum, Milwaukee, WI. 205 pp.
Wisconsin Herp Atlas: http://www4.uwm.edu/fieldstation/herpetology/

3.0 Forms and Attachments

- Survey Form

4.0 Equipment

- A variety of traps can be used, including 1/4 inch mesh galvanized steel or plastic or nylon mesh commercial minnow traps. These typically have 1-inch openings, which should be expanded to approximately 2 inches by cutting away the edges. Lobster or small turtles traps also should work, especially box style traps with flat bottoms and slit openings.

Aquatic Funnel Trap Survey Form **Observer Name(s):**_____ **Date:** ____/_____/_____

Start Time: _____ Air T: _____ Water T: _____ Wind: _____B Sky Code: _____ End Time: _____

Site Name and location (give coordinates):_____

list species below	Trap 1	Trap 2	Trap 3	Trap 4	Trap 5	Trap 6	Trap 7	Trap 8	Trap 9	Trap 10

Visual observations:

Calling frogs:

Instructions: Record all wildlife observed outside the traps as visual observations on the bottom of the form.

Data entry notes: Record start and end times. Record temperatures as C or F as appropriate. Use Beaufort wind scale and Sky Code below. Enter the site name.

Recording Data: Enter species in the first row as you remove them from the traps, and enter counts into the cell for each trap. The second species encountered becomes the second row, etc. So as you move from trap to trap add rows (species) and populate cells with counts of individuals. Record all species in traps to the best of your ability to identify, but ONLY critters actually IN the traps. *Anything observed outside of the traps, including eggs, should be recorded under visual observations.* You may use tally marks as you go, but if so sum the marks when finished and circle the final sum number. You may also use species groups where you cannot determine the species, such as "tadpole", or "crayfish". Note anything you think might be affecting sampling, such as water levels, trap damage, or disturbance to traps. Traps should be in suitable habitat (where the salamanders are expected to be), and can be moved with receding water levels if necessary.

Equipment Needs: A good attitude. Map and directions, traps, Data sheets, clipboard, pencils, thermometer, waterproof rope/twine, bait, field guides, first aid kit, ziplock bags, bucket, proper clothing (inc. waders), digital camera, magnifying lense, polarized sunglasses.

Sky Code	**Sky Condition**
0 -	Clear or only a few clouds
1 -	Partly cloudy or variable
2 -	Broken clouds or overcast
3 -	Fog
4 -	Drizzle or light rain (not affecting hearing)
5 -	Snow
6 -	Showers (is affecting hearing ability)

BEAUFORT WIND STRENGTH SCALE

B#	Wind Speed Mi/hr (Km/hr)	Description
0	<1 (<1.6)	**Calm:** Still: Smoke will rise vertically.
1	1-3(1.6-4.8)	**Light Air:** Rising smoke drifts, weather vane is inactive.
2	4-7(6.4-11.3)	**Light Breeze:** Leaves rustle, can feel wind on your face, weather vane is inactive.
3	8-12(12.9-19.3)	**Gentle Breeze:** Leaves and twigs move around. Light weight flags extend.
4	13-18 (20.9-29.0)	**Moderate Breeze:** Moves thin branches, raises dust and paper.
5	19-24 (30.6-38.6)	**Fresh Breeze:** Moves trees sway.
6	25-31(40.2-50.0)	**Strong Breeze:** Large tree branches move, open wires (such as telegraph wires) begin to "whistle", umbrellas are difficult to keep under control.
7	32-38 (51.5-61.2)	**Moderate Gale:** Large trees begin to sway, noticeably difficult to walk.
8	39-46(62.8-74.0)	**Fresh Gale:** Twigs and small branches are broken from trees, walking into the wing is very difficult.
9	47-54(75.6-86.9)	**Strong Gale:** Slight damage occurs to buildings, shingles are blown off of roofs.
10	55-63 (88.5-101.4)	**Whole Gale:** Large trees are uprooted, building damage is considerable.
11	64-72 (103.0-115.9)	**Storm:** Extensive widespread damage. These typically occur only at sea, and rarely inland.
12	>73 (>115.9)	**Hurricane:** Extreme destruction.

ESS ORG METHOD 1480

Analysis of Perfluorinated Compounds in Fish Tissue by HPLC-MS/MS

Matrix: Tissue

1. Scope and Application

1.1. This is a high performance liquid chromatographic triple quadrupole mass spectrometric (HPLC-MS/MS) method applicable to the determination of perfluorinated compounds in fish tissue.

1.2. The following compounds and reporting limits are listed below for this method:

Analyte	Report limit ng/g
Perfluoro-1-octanesulfonate	0.50
Perfluoro-1-butanesulfonate	0.50
Perflioro-1-hexanesulfonate	0.50
Perfluoro-1-heptanesulfonate	0.50
Perfluoro-1-decanesulfonate	0.50
Perfluoro-n-octanoic acid	0.50
Perfluoro-n-butanoic acid	2.0
Perfluoro-n-pentanoic acid	0.50
Perfluoro-n-hexanoic acid	0.50
Perfluoro-n-heptanoic acid	0.50
Perfluoro-n-nonanoic acid	0.50
Perfluoro-n-decanoic acid	0.50
Perfluoro-n-undodecanoic acid	0.50
Perfluoro-n-dodecanoic acid	0.50
Perfluoro-n-tridecanoic acid	0.50
Perfluoro-n-tetradecanoic acid	0.50
Perfluoro-1-octanesulfonamide	0.50

2. Summary of Method:

2.1. Perfluorinated compounds (PFCs) present in fish tissue samples are detected by an alkaline digestion/solid phase extraction , eluted with 0.1% NH_4OH in MeOH, and evaporated to dryness under nitrogen gas. The contents of the tube are reconstituted in 0.5 mL MeOH, and stored at -20 ^{o}F. Prior to analysis by HPLC-MS/MS, 0.5 mL 2mM NH_4Ac buffer is added, and the contents transferred to an autosampler vial via syringe filter (0.2 um). Separation of the analytes is achieved using gradient elution chromatography. After elution from the HPLC column, the analytes are analyzed using a turbo ion spray triple quadruple mass spectrometer in the negative ionization mode.

2.2. List Regulatory Deviations: This section is not applicable to this method.

3. Safety and Waste Management:

3.1. General safety practices for all laboratory operations are outlined in the Chemical Hygiene Plan for Environmental Sciences

3.2. All laboratory waste, excess reagents, and samples will be disposed of in a manner which is consistent with applicable rules and regulations. Waste disposal guidelines are described in the University of Wisconsin Chemical Safety and Disposal Guide.

4. Sampling Handling and Preservation: Samples must be iced or refrigerated at 4°C in transport and frozen as soon as possible,and shielded from light from the time of collection until analysis. Perfluorinated compounds have been shown to be stable for several months under these conditions.

5. Interferences: Matrix interference may be caused by contaminants that are present in the sample. The extent of matrix interference is unknown until further sample analysis is completed.

6. Reagents and Standards:

6.1. Reagents

6.1.1. Methanol, Reagent grade

6.1.2. Ammonium Acetate, Reagent grade

6.1.3. Sodium acetate, Reagent grade

6.1.4. Sodium Hydroxide (NaOH) pellets, Reagent grade

6.1.5. Ammonium hydroxide, Concentrated

6.1.6.

6.1.7. 18 Mohm water

6.2. Reagent Preparation

6.2.1. 2mM Ammonium Acetate in Water: Add 0.154 g of ammonium acetate to 1 liter of 18 Mohm water

6.2.2. 0.1% ammonium hydroxide in methanol: Add 1 mL ammonium hydroxide to 1 liter methanol.

6.2.3. 10 mM NaOH in methanol: Add 0.40 g NaOH to 1 L methanol.

6.2.4. 25 mM sodium acetate buffer (pH 4): Add 2.05 g sodium acetate to 1 liter of 18 Mohm water. Adjust to pH 4 using glacial acetic acid.

6.3. Standards

6.3.1. Prepare stock standard solutions by obtaining a known weight of pure material, dissolving the material in methanol and diluting to volume in a volumetric flask.

6.3.2. Stock standard solutions should be stored at 4 °F or below.

6.3.3. Working calibration standards are extracted matrix spikes.

6.3.4. Stock internal standard solution contains ^{13}C isotopically labeled compounds at a nominal concentration of 50 ng/ml each.

6.3.4.1. Perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid

6.3.4.2. Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid

6.3.4.3. Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid

6.3.4.4. Perfluoro-n-[1,2,3,4,5-$^{13}C_5$]nonanoic acid

6.3.4.5. Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid

6.3.4.6. Perfluoro-n-[1,2-$^{13}C_2$]undecanoic acid

6.3.4.7. Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid

6.3.4.8. Sodium perfluoro-1-hexane[$^{18}O_2$]sulfonate

6.3.4.9. Sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate

7. Apparatus:

7.1. Sample bottles: avoid use of Teflon septa/cap liners

7.2. Analytical Balance capable of accurately weighing to the nearest 0.0001 g.

7.3. 15-ml conical screw capped polypropylene centrifuge tubes, graduated

7.4. Vortex mixer

7.5. Disposable polypropylene luer tip syringes

7.6. 13-mm syringe filters, 0.2 micron nylon

7.7. 2-ml auto-injector vials with aluminum crimp caps (polypropylene septa)

8. HPLC/MS/MS Instrument Conditions

8.1. The HPLC-MS/MS method is performed on an Applied Biosystems/SCIEX API 4000 triple quadrupole mass spectrometer (Foster City, CA), which is interfaced to an Agilent 1100 HPLC equipped with a degasser, autosampler and column heating compartment.

8.2. General Method Parameters

8.2.1. Synchronization Mode: LC Sync

8.2.2. Auto-Equilibration: Off

8.2.3. Acquisition Duration: 17 minutes 0 seconds

8.2.4. Number of Scans: 937

8.2.5. Period In File: 1

8.2.6. Acquisition Module: Acquisition Method

8.2.7. Software version: Analyst 1.5

8.3. Source height setting-5, Source L/R setting-5

8.4. Agilent 1100 LC Pump Method

8.4.1. Pump Model: Agilent 1100 LC Binary pump

8.4.2. Column: Zorbax Rapid Resolution, 3.5 µm, 30 mm long x 2.1 mm I.D. (Part # 873700-902).

8.4.3. Agilent 1100 LC Pump Method Properties

Minimum Pressure (psi)	0.0
Maximum Pressure (psi)	5801.0
Dead Volume (µl)	40.0
Maximum Flow Ramp (ml/min²)	100.0

Maximum Pressure Ramp (psi/sec)	290.0
Left Compressibility	50.0
Right Compressibility	115.0
Left Dead Volume (µl)	40.0
Right Dead Volume (µl)	40.0
Left Stroke Volume (µl)	-1.0
Right Stroke Volume (µl)	-1.0
Left Solvent	A2
Right Solvent	B2

8.4.4. Step Table:

Step	Total Time (min)	Flow Rate (µl/min)	A (%)	B (%)
0	0.00	250	70	30
1	0.50	250	20	80
2	9.00	250	20	80
3	9.01	500	10	90
4	11.00	500	10	90
5	11.01	500	70	30
6	14.00	500	70	30
7	14.01	250	70	30
8	17.00	250	70	30

8.5. Agilent 1100 Autosampler Properties

Autosampler Model	Agilent 1100 Autosampler
Syringe Size (µl)	100
Injection Volume (µl)	5.00
Draw Speed (µl/min)	200.0
Eject Speed (µl/min)	200.0
Needle Level (mm)	1.00
Temperature Control	Not used
Wash Vial Number	100
Wash Rack Number	1
Use Custom Injector Program	No

8.6. Agilent 1100 Column Oven Properties

Left Temperature (°C)	30.00
Right Temperature (°C)	30.00
Temperature Tolerance $^{\pm}$(°C):	1.00
Start Acquisition Tolerance $^{\pm}$(°C)	0.50
Time Table	(Not Used)
Column Switching Valve	Installed
Position for first sample in the batch	Left
Use same position for all samples in the batch	

8.7. MS/MS Method Properties:

8.7.1. Period 1:

8.7.1.1. Scans in Period: 937

8.7.1.2. Relative Start Time: 0.00 msec

8.7.1.3. Experiments in Period: 1

8.7.2. Period 1 Experiment 1:

Scan Type:	MRM (MRM)
Scheduled MRM	No
Polarity:	Negative
Scan Mode:	N/A
Ion Source:	Turbo Spray
Resolution Q1:	Unit
Resolution Q3:	Unit
Intensity Thres.:	0.00 cps
Settling Time:	0.0000 msec
MR Pause:	5.0000 msec
MCA:	No
Step Size:	0.00 Da

8.7.3. Parameters:

Analyte	Q1 Mass (Da)	Q3 Mass (Da)	Dwell (msec)	DP	CE	CXP
PFOS-1	498.90	80.00	25.00	-55.00	-72.00	-5.00
PFOS-2	498.90	99.00	25.00	-55.00	-64.00	-17.00
PFOS-3	498.90	180.00	25.00	-55.00	-50.00	-9.00
PFOA-1	413.10	368.80	25.00	-25.00	-14.00	-1.00
PFOA-2	413.10	168.90	25.00	-25.00	-26.00	-9.00
PFOS IS-1	502.92	80.27	25.00	-100.00	-70.00	-3.00
PFOS IS-2	502.92	98.96	25.00	-100.00	-62.00	-7.00
PFOA IS-1	416.78	372.10	25.00	-35.00	-14.00	-9.00
PFOA IS-2	416.78	169.00	25.00	-35.00	-26.00	-7.00
PFBS	299.00	80.00	25.00	-70.00	-58.00	-5.00
PFHxS	399.05	80.20	25.00	-80.00	-68.00	-1.00
PFHpS	449.00	80.20	25.00	-80.00	-74.00	-5.00
PFDS	599.03	80.10	25.00	-70.00	-86.00	-5.00
PFBA	213.00	169.18	25.00	-25.00	-12.00	-13.00
PFPA	263.15	219.16	25.00	-25.00	-12.00	-11.00
PFHxA	313.05	269.20	25.00	-25.00	-15.00	-15.00
PFHpA	363.10	319.05	25.00	-30.00	-12.00	-7.00
PFNA	463.06	418.82	25.00	-30.00	-14.00	-11.00
PFDA	513.09	468.99	25.00	-35.00	-16.00	-11.00
PFUdA	563.09	519.17	25.00	-40.00	-16.00	-9.00

PFDoA	613.12	569.20	25.00	-35.00	-18.00	-9.00
PFTrDA	663.04	619.23	25.00	-35.00	-16.00	-11.00
PFTeDA	713.09	669.14	25.00	-40.00	-18.00	-11.00
PFBA IS	216.97	171.93	25.00	-40.00	-14.00	-9.00
PFHxA IS	314.98	270.03	25.00	-45.00	-14.00	-7.00
PFHxS IS	403.02	103.18	25.00	-100.00	-52.00	-5.00
PFNA IS	468.00	423.00	25.00	-60.00	-16.00	-5.00
PFDA IS	515.06	469.93	25.00	-60.00	-18.00	-7.00
PFUdA IS	565.05	520.04	25.00	-55.00	-18.00	-15.00
PFDoA IS	615.09	570.19	25.00	-70.00	-18.00	-9.00
FOSA	498.02	78.14	25.00	-80.00	-60.00	-5.00
FOSA IS	506.08	78.15	25.00	-85.00	-60.00	-5.00

8.8. Parameter Table (Period 1 Experiment 1):

CUR:	35.00
GS1:	40.00
GS2:	40.00
IS:	-4500.00
TEM:	400.00
ihe:	ON
CAD:	10.00
EP	-10.00

8.9. Electron Multiplier Settings

Detector Paramenters (Negative):	
CEM	2200.0
DF	200.0

9. Quality Control

9.1. For general quality control, procedures see the Quality Assurance Manual. For specific quality control acceptance limits that apply to laboratory control samples, surrogates, calibration check standards, matrix spikes, and duplicates for this analytical procedure please consult the laboratory's LIMS system. For details, see the standard operating procedure "ESS ORG QA0001 QAWRKSHT" located at O:\SOP\EHD\ESS\Enviro Organic\Organic and Air Chem\Final\Quality Assurance (QA)\ESS ORG QA 0001_QAWRKSHT.doc.

9.2. Before any analysis is done, the MS/MS detector should pass a polypropylene glycol (PPG) tune check. A standard containing 300 µM of SCIEX Mixed PPG solution is run. The spectra should meet the recommended SCIEX operating criteria before samples are

run. A tune check should be performed periodically (eg. Quarterly and after PM or other service) See example SCIEX tuning criteria in 9.3 below:

9.3. SCIEX tuning criteria for PPGs in negative Turbo Ion Spray mode (NOTE: cps = counts per second and FWHM = field width at half mass.

Mass	Q1 cps	Mass criteria	Q3 cps	FWHM
934	>/= 2.0e7	0.6 to 0.8 amu	>/= 2.0e7	0.6 to 0.8 amu
2036	>/= 3.0e6	0.6 to 0.8 amu	N/A	N/A

9.4. Minimum quality control (QC) requirements may include initial demonstration of laboratory capability, analysis of laboratory reagent blanks, laboratory fortified samples, laboratory fortified blanks and QC samples.

9.5. Laboratory reagent blanks - The analyst must demonstrate that all glassware and reagent interferences are under control. Before a new set of samples is extracted, a laboratory reagent blank (LRB) must be analyzed. If within the retention time window of any analyte of interest the LRB produces a peak that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples.

9.6. Assessing laboratory performance with laboratory fortified blanks (LFB) – Laboratory fortified blanks spiked over the working range are the calibration standards for this method.

9.7. Assessing analyte recovery with laboratory fortified sample matrix – If sufficient sample is available, the laboratory will add a known concentration to a minimum of 5% of the routine samples or one sample per set, whichever is greater. The spiking concentration should not be less than 2-5 times the background concentration of the sample selected for fortification. Calculate the percent recovery, P, of the concentration for each analyte, after correcting the analytical result, X, from the fortified sample for the background concentration, b, measured in the unfortified sample, i.e.:

$$P = 100 (X - b) / \text{fortifying concentration}$$

9.8. Until the laboratory acquires the appropriate number of LFB data points (20 - 30) for in house statistical limits, the limits specified in the EPA Solid Waste methods manual will be used as guidance which is 70-130%.

9.9. Assessing precision with duplicates: If sufficient sample is available, with each batch of samples, the laboratory should analyze one serum in duplicate. Based upon the analyst's experience, a sample which is expected to contain the analytes above the limit of quantitation should be chosen to duplicate, Until the laboratory acquires the appropriate number of data points (20-30) for in house statistical limits, the limits specified in the EPA Solid Waste methods manual, which are 70-130%, will be used as guidance.

9.10. Identification of Analytes - The retention time window for all compounds is monitored. In addition, each compound that is detected is confirmed by a confirmation ion pair.

10. Method Calibration

10.1. Working Standard Preparation Procedure

10.1.1. Due to matrix effects, volumetric dilutions of stock methanolic standard solutions cannot be used as working calibration standards. Instead, all quantitation is based upon matrix matched extracted standard curves, using the same extraction protocol that is employed for the samples (Section 11). The matched matrix, ocean perch tissue, is spiked at a minimum of 5 standard curve levels - suggested levels follow: 0.10, 0.50, 1.0, 5.0, and, 10.0ng/g.Inject each extracted calibration standard. The Applied Biosystems Analyst data system is used to prepare an internal standard linear calibration curve for each analyte. An R value of greater than or equal to 0.995 will be used as guidance to verify the acceptability of the curve.

10.2. The working calibration curve must be verified on each working day by the injection of one or more calibration standards at the beginning and end of each analytical run, and after the analysis of 10 samples if 10 or more samples are analyzed in an analysis day. In addition, or minus 20% will be used as guidance for acceptability of the calibration check standard. If the check standard varies by more than 20 percent, then a new calibration curve may need to be generated.

10.3. One calibration check standard, which is a LFB, is extracted with each batch of samples.

11. Sample Preparation Procedure

11.1. A frozen aliquot of about 0.5g of tissue is transferred into a 50 ml polypropylene centrifuge tube. 0.5 mL of 18 Mohm water is added, and the sample is blended to visual homogeneity (up to 60 sec.) using a Tissue Terror probe-style hand-held blender.

11.2. 9 mL of 10 mM NaOH in methanol is added via serological pipette (10 mL) in a manner which simultaneously washes the tip of the hand-held homogenizer.

11.3. Spike the sample with mass labeled internal standard solution at the same concentration as the standards (ISTD target concentration is nominally 0.5 ng/g).

11.4. Securely cap the 50 mL polyprop. tube(s) and place on shaker (orbital or rocker) for at least 16 hours at room temperature.

11.5. Following $\geq$ 16 hour alkaline digestion, centrifuge samples at 2000 rpm for 5 minutes.

11.6. Transfer 1.0 mL of the clear, upper layer to a 15 mL polyprop. conical tube, and add 9 mL 18 Mohm water.

11.7. Prepare a 12 port manifold by carefully cleaning all surfaces with methanol, and affix SPE cartridges (OASIS WAX, 3cc, 60mg).

11.8. Condition cartridges with the following sequence of reagents, 4 mL per reagent, flow at approximately 1 mL/minute;

11.8.1. 0.1% NH4OH in methanol

11.8.2. Methanol

11.8.3. 18 Mohm water

11.9. Load samples onto SPE cartridges and adjust flow to about 1 mL per minute.

11.10. After entire sample has passed, wash cartridges with the following sequence of reagents, 4 mL per reagent, flow approx. 1 mL/minute (note that this is a wash process and eluents are not be collected);

11.10.1. Sodium acetate (25 mM) buffer (pH 4)

11.10.2. Methanol

11.11. Allow vacuum air to pass through emptied SPE cartridges for up to 5 minutes as a drying step.

11.12. Elute SPE cartridges into properly-labeled collection tubes (15 mL polyprop.) using 4 mL of 0.1% NH_4OH in methanol. Adjust flow at about 1 mL/minute.

11.13. Evaporate eluent just to dryness under nitrogen gas (5 – 15 psi) using a TurboVap at 40 ^oC.

11.14. Reconstitute the evaporate in 0.5 mL methanol, cap tightly, and store at -20 ^oF until time of analysis.

11.15. At time of analysis, add 0.5 mL of 2 mM NH_4Ac buffer and transfer to 2 mL amber autosampler vial via filter syringe (0.2 um) if necessary.

11.16. Cap atuosampler vials using caps that are absent of teflon.

12. Calculations: Calculations are performed using the Applied Biosystems Analyst software, performing a multilevel calibration, and using a linear fit.

13. Data Management: Data is collected and calculations are made on a PC-based system running SCIEX Analyst Software by the analyst. Perfluorinated analyte data is transcribed onto the sample worksheet, reviewed, and transferred to the Laboratory's LIMS system manually by the analyst (or designee). It is then reviewed by peers or the section supervisor before being released.

14. Definitions: General definitions of other terms that may be used in this method are found in Section 19 of the SLH Quality Assurance Manual.

15. Method Performance: Where applicable the laboratory's initial accuracy and precision data (MDLs and IDCs) were generated in compliance with the reference method and the Departments standard operating procedure "ESS ORG QA0012 LOD and LOQ Determinations". All data is stored with sample date in the year it was run. QC personnel keep a spreadsheet of the MDLs data.

16. References:

16.1. Ye, X., Strynar, M., Nakayama, S., Varns, J., Helfant, L., Lazorchak, J., Lindstrom, A. “Perfluorinated Compounds in Whole Fish Homogenates from the Ohio, Missouri, and Upper Mississippi Rivers, USA”, *Environmental Pollution*, 156:3, 1227-1232.

16.2. “Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry”. 3M Environmental Laboratory Method Number ETS-8-4.1 (03/01/99).

16.3. Kurunthachalam, K., Franson, J.C., Bowerman, W.W., Hansen, K.J., Jones, P.D., and Giesy, J.P. “Perfluoooctane Sulfonate in Fish-Eating Water Birds Including Bald Eagles and Albatrosses”, *Environ. Sci. Technol.* 2001, 35, 3065-3070.

16.4. Hansen, K.J., Clemen, L.A., Ellefson, M.E., Johnson, H.O. “Compound-Specific, Quantitative Characterization of Organic Fluorochemicals in Biological Matrices”, *Environ. Sci. Technol.* 2001, 35, 766-770.

16.5. “Test Methods For Evaluating Solid Waste Physical/Chemical Methods (SW-846) Third Edition,”1996.

16.6. "Methods for the Determination of Organic Compounds in Drinking Water," US EPA/600/4-88/039, 1995.

16.7. "Quality Assurance Procedures and Policies", The ESS QA Manual.

16.8. "Constitution, Bylaws, and Standards", National Environmental Laboratory Accreditation Conference, (July 1999)

17. Tables, figures, diagrams, charts, checklists, appendices: None

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