Microcystins Sample Holding Time Study

I. Introduction

Sample holding times are established to ensure the analyte of interest is measured prior to significant degradation or loss. Sample holding times are typically defined as the time from sample collection until some action occurs in the laboratory to either stabilize the analyte against degradation or to measure the analyte. In some instances, there may be two holding times established – one from sample collection until the initiation of a preparatory step that temporarily stabilizes the analyte and another holding time between the sample preparation step and the analytical measurement. Although microcystins are thought to be heat stable, little is known about the degradation rate after sample collection or the effect of cell disruption techniques on the stabilization of microcystins. There is anecdotal evidence to suggest that the degradation rate may vary depending on the characteristics of the waterbody from which samples were collected, perhaps because of differences in the bacterial and/or algal populations. Autoclaving as a cell disruption technique may stabilize microcystins against further degradation (presumably by sterilizing the sample and destroying the microbe population). The goal of this experiment is to estimate the degradation rate of microcystins in natural water samples. These rates will be used to recommend standardized sample holding times for a Statewide Cyanobacteria HAB response plan.

II. Methods

The FDEP Central Laboratory in Tallahassee and the SFWMD Laboratory in West Palm Beach will participate in this pilot study. The laboratory contacts for this study will be Tim Fitzpatrick for FDEP and David Struve for SFWMD. When the HAB workgroup has chosen a bloom for sampling, the laboratory that is closest to it will be responsible for taking the sample, splitting it into aliquots, and distributing those aliquots to both laboratories. The aliquots will be analyzed for microcystins by each laboratory according to their standard SOP; however, both groups will use the SOP in Appendix I of this document for homogenizing and splitting samples into aliquots.

The holding time study will have two parts:

1. Examination of the degradation rate of microcystins in various natural waters, and;
2. Evaluation of autoclaving as a cell disruption technique to stabilize microcystins against further degradation.

Definitions

Primary Laboratory	The laboratory responsible for sampling, splitting, and distributing the samples from a particular algal bloom. This will be the lab that is physically the closest to a bloom selected for sampling.
Secondary Laboratory	The laboratory that receives and analyzes samples from the primary laboratory.

Sample Collection

1) Sufficient volume of sample will be collected to supply a total of forty-five 40 ml aliquots required for both parts of the study.

Sample Preparation

The **primary** laboratory will:

1) On the day of collection, split the collected water into 45 labeled 40ml VOA vials (amber glass) using the SOP in Appendix I (39 samples plus 3 extra samples for each laboratory = 45 samples).
2) Autoclave randomly selected (see the analysis schedule) 6 of the 45 bottles at 120°C for 30 minutes.
3) Chill all 45 bottles to 4°C.
4) Retain 3 autoclaved bottles and 21 randomly selected non-autoclaved bottles for analysis at the primary laboratory according to the schedule in the *Sample Analysis* section. Samples will be maintained at 4°C until the day of analysis.
5) Prepare a schedule with the date that each sample will be analyzed.
6) Ship (via overnight express delivery service) 3 autoclaved bottles, 18 randomly selected non-autoclaved bottles, and the analysis schedule to the secondary laboratory.
7) Analyze 3 of the retained, non-autoclaved bottles on the day of collection.

The **secondary** laboratory will:

1) Maintain the received samples at 4°C until the day of analysis.
2) The sample that has been autoclaved in the primary laboratory must be warmed up to room temperature before analysis.

Sample Analysis Schedule(summarized in Table 1.)

1) Day 0 (day of collection) - The primary laboratory will analyze 3 non-autoclaved bottles; the secondary lab will analyze nothing.
2) 48 Hours - Each (primary and secondary) laboratory will analyze 3 stored non-autoclaved bottles
3) Day 7 - Each laboratory will analyze 3 stored non-autoclaved bottles
4) Day 14 - Each laboratory will analyze 3 stored non-autoclaved bottles
5) Day 21 - Each laboratory will analyze 3 stored non-autoclaved bottles
6) Day 28 - Each laboratory will analyze 3 stored non-autoclaved bottles AND 3 stored autoclaved bottles

Table 1. Summary of number of laboratory analyses for each water body.

	This table is repeated for each of the 5 water bodies			
	Sample collected on Day 0 & aliquots not autoclaved until the day of analysis		**Autoclaved at Day 0 and stored until Day 28**	
	Primary Lab	***Secondary Lab***	***Primary Lab***	***Secondary Lab***
0 Hours	**3 reps**			
48 Hours	**3 reps**	**3 reps**		
Day 7	**3 reps**	**3 reps**		
Day 14	**3 reps**	**3 reps**		
Day 21	**3 reps**	**3 reps**		
Day 28	**3 reps**	**3 reps**	**3 reps**	**3 reps**
Total	**18 reps**	**15 reps**	**3 reps**	**3 reps**

Note to laboratories: For this study, it is important that both autoclave cell disruption technique preparation and all analyses (using ELISA) be conducted on the same day as prescribed. All sub-sample aliquots are to be kept at 4℃ until they are needed for analysis. The samples will be randomly selected and listed in the schedule for analysis in each scheduled date.

Data Reporting

Each laboratory will provide their results in an Excel worksheet to the other laboratory and to Lori Wolfe in the Biology Section at FDEP (Loretta.Wolfe@dep.state.fl.us). A electronic worksheet with a form for data entry will be provided to each laboratory.

Statistical Analysis

The analysis data file and statistical analyses will be done by Lori Wolfe at FDEP. The holding time data will be analyzed using a repeated measures analysis of variance (ANOVA) model with "laboratory" as a fixed factor.

The samples autoclaved right after collection, stored for 28 days, and then analyzed will be compared to the samples analyzed right after collection using a t-test. If the data for either experiment violate the assumptions of these parametric methods, nonparametric equivalents will be used.

Summary Report

A summary report will be produced from all the results by the FDEP Bureau of Laboratories. The draft report will be sent to each lab for comment and verification of results. Before the report is finalized, it will be distributed to the HAB Workgroup for discussion. A publication quality final report will be submitted to peer-reviewed journals if it is determined by the workgroup that the results will be of interest to the HAB scientific community.

Appendix I. SOP for homogenization and splitting of sampled bloom material.

1.0 Sample Collection

Aqueous Sampling for Cyanobacteria Blooms

1. INTRODUCTION: Use the procedures in this SOP to collect surface water samples for cyanobacteria toxin analyses and algal cell enumeration and identification. This SOP does not address sampling design issues which can be site or project specific. Care should be taken to ensure the sampling design will support the types of management decisions that the resulting data are intended to support.

1.1. Use the following DEP SOPs in conjunction with this SOP (available at: http://www.floridadep.org/labs/qa/sops.htm):

- FA 1000 Regulatory Scope and Administrative Procedures for Use of DEP SOPs
- FC 1000 Cleaning / Decontamination Procedures
- FD 1000 Documentation Procedures
- FM 1000 Field Planning and Mobilization
- FQ 1000 Field Quality Control Requirements
- FS 1000 General Sampling Procedures
- FS 2000 General Aqueous Sampling
- FS 2100 Surface Water Sampling
- FT 1000 General Field Testing and Measurement
- FS 7000 General Biological Community Sampling

1.1.1. Use additional SOPs as required for specific applications.

2. Equipment and Supplies: Sample Containers

2.1. Collect samples for algal toxin analysis in clean 250-ml wide mouth amber glass bottles with caps having a Teflon® liner.

2.2. Collect samples for algal cell enumeration and identification in clean 1-L brown plastic bottles.

2.3. Visually inspect bottles and caps for defects. Do not use if defects are present or containers do not appear clean.

2.4. Cyanobacteria bloom sampling requires specialized training, safety protocols and personal protective equipment (PPE). The degree and type of safety measures and PPE required depends on the unique characteristics of the bloom to be sampled. Typical risks include, but are not limited to, contact dermatitis and upper respiratory irritation.

2.4.1. MINIMUM SAFETY PLANNING AND PROCEDURES: It is beyond the scope of this field sampling SOP to describe safety protocols for every contingency. Follow the listed minimum safety procedures below, where applicable:

2.4.1.1. Produce a written sampling plan and review with all personnel.

2.4.1.2. Ensure that all personnel are appropriately trained.

2.4.1.3. Ensure that sampling personnel meet employer medical requirements.

2.4.1.4. Conduct site reconnaissance and identify hazards.

2.4.1.5. Provide appropriate PPE and sampling equipment to all samplers.

2.4.1.6. Establish site control (exclusion zones, access corridors, etc.).

2.4.1.7. Establish decontamination protocols for samplers.

2.4.1.8. Prepare for emergencies (backup personnel, spill containment, fire equipment, first aid, evacuations, etc.).

3. SAMPLE COLLECTION PROCEDURES

3.1. In order to provide consistency between sampling agencies and programs, it is recommended that all samples be collected as grab samples. Collect separate, discrete samples of the water column and of the scum layer. These samples should be analyzed and interpreted separately. Clearly mark the type of sample collected on the sample container.

3.1.1. If composite data are needed, collect individual grab samples over the specified time period or area.

3.1.2. Submit all samples for analysis.

3.1.3. Average the concentrations of the results to determine the average concentration over time or area.

3.2. Water Column Sample

3.2.1. Collect sample within the top 5-10 cm of the water column. If the sample is to be collected from a depth other than 5-10 cm, this should be noted on the sample container and sample submittal form. Do not collect the sample by skimming the surface.

3.2.2. Do not rinse the interior of the sample container with site water prior to collection.

3.2.3. Collect the sample directly into the container.

3.2.3.1. Slowly submerge the container, opening first, into the water. If an algal scum layer is present, attempt to avoid submerging the bottle through the algal scum layer as this may introduce algal scum into the sample and may not be representative of the water column.

3.2.3.2. Invert the bottle at the desired sampling depth so the opening is upright and pointing towards the direction of flow (if applicable). Allow water to enter the container until the bottle is almost full.

3.2.3.3. Return the filled container quickly to the surface.

3.2.3.4. Pour out a small volume of sample, if needed, to allow for homogenization back at the lab.

3.2.3.5. Quickly cap the container and tighten securely.

i. Surface Scum Sample

1. In order to collect an algal scum layer sample in a repeatable manner that would be representative of a potential recreational exposure, it is recommended that the scum layer be agitated to homogenize it with the underlying water such that it is dispersed in the upper 10 cm of the water column.

2. Homogenize the water and algal scum layer in a 0.5m diameter area by agitating the surface scum with a gloved hand or other device.

3. Quickly, collect a sample within the top 5-10 cm of the water column. Do not collect the sample by skimming the surface.

4. Do not rinse the interior of the sample container with site water prior to collection.

5. Collect the sample directly into the container.

a. Slowly submerge the container, opening first, into the water

b. Invert the bottle so the opening is upright and pointing towards the direction of flow (if applicable). Allow water to enter the container until the bottle is almost full.

c. Return the filled container quickly to the surface.

d. Pour out a small volume of sample, if needed, to allow for expansion of the sample and homogenization back at the lab.

e. Quickly cap the container and tighten securely.

4. TRANSPORT AND HANDLING OF SAMPLES

4.1. After the samples have been collected and containerized, clean the outside of the containers with water, paper towels or other absorbent materials to remove any spilled sample from the exterior of the container.

4.2. Protect glass containers from breakage ("bubble wrap" is recommended).

4.3. Samples need to be shipped to the laboratory in time to meet the prescribed holding time for the analyses being performed. Since there is limited data available to establish appropriate holding times for cyanobacteria toxin analyses, initial holding times may be quite conservative until additional studies are completed. Contact the analytical laboratory to which you are submitting samples for hold time information.

5. PRESERVATION

5.1. Samples for cyanobacteria toxin analysis should immediately be placed on wet ice and kept in the dark.

5.2. Samples for algal cell enumeration and identification should immediately be placed on wet ice and then preserved with 3 ml of Lugol's solution within 8 hours of collection time (See FS 7001, section 2). For long-term storage, add buffered formalin to achieve a minimum of 2.5% final concentration (approximately 25 mL (See FS 7001, section 1).

6. DOCUMENTATION

ii. Label each bottle with an appropriate field ID number.

iii. Complete field records.

iv. Make notes on the transmittal form and in field records about any relevant observations or problems.

2.0 Preparation of Samples

2.1 Split samples into the appropriate number of sample bottles including a number of extra bottles.

- 2.1.1 Homogenize sample by stirring for at least 30 min with a clean Teflon stir-bar on a magnetic stir plate.
- 2.1.2 Use a pump and Teflon tubing to fill the 40-ml VOA vials (collect at least the1st 300 ml in a waste beaker). Bottles should be filled in thirds; all bottles filled 1/3 the way full, then all bottles are filled 2/3 and finally full.
- 2.1.3 Cap vial, dry them and inspect for any irregularities. Vials with irregularities should be held aside. Randomly put vials into a box labeled with the site name.

2.2 Labeling Samples

- 2.2.1 Randomly select a vial from box and apply a label with laboratory name and sample number

3.0 Distribution and Laboratory Analyses of Samples

3.1 Once all samples are labeled, package them in a cooler with wet ice.

3.2 Samples are shipped to laboratories by overnight courier.

3.3 Laboratories should follow sample key provided for additional information regarding analysis schedule.