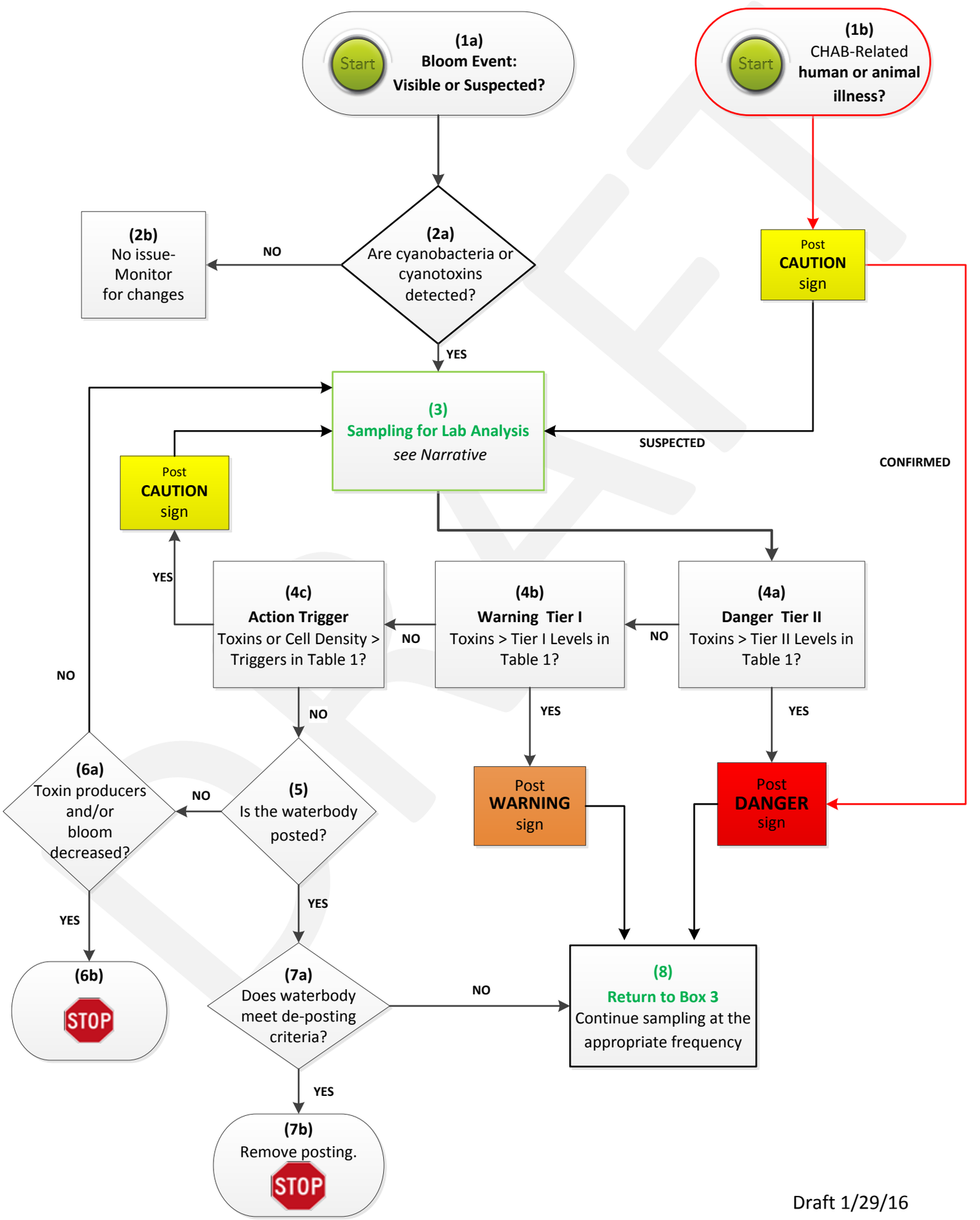
Figure 1. Decision Tree for Posting and De-posting Health Advisories for CyanoHABs

Proposed changes to consider for Voluntary CHAB Guidance (Working Draft)



Draft 1/29/16

Narrative for Figure 1 - Decision Tree for Posting and De-posting Health Advisories for CyanoHABs

Box 1a. Bloom Event: Visible or Suspected

- **If a bloom event is suspected proceed to Box 2a**

If there are visible signs of a harmful algal bloom, or a bloom may reasonably be suspected based on the indicators listed or past history, proceed to Box 2a.

The following are indicators of potential harmful algal blooms:

Visual suggestion -
Any of the following:

- water that is usually clear becomes bright green, opaque, or other discoloration
- water may appear soupy
- water may have planktonic (plankton-like) growth
- surface scum or algal mats on the water surface or on the shoreline
- benthic algal mats may accumulate on river/stream substrate
- foul odors

Suspected blooms may be compared with images shown in *Appendix X* or on the websites located at *(website)*;

OR

Measurable chemical factors –
An increase or change in pH or nutrient loading (increased levels of nitrogen and phosphorus compounds) may signal development of a bloom;

OR

Satellite imaging –
Positive detection of cyanobacteria pigments, via satellite imaging, will result in notifications that trigger field verification and possible sampling. A statewide program for notification will be available in the near future for waterbodies greater than 250 acres. For more information on satellite imaging visit (www.xxSWAMPsatelliteHABimagingxx);

OR

Historic evidence -
In some waterbodies with reoccurring blooms, cyanobacteria have been monitored for a sufficient time to identify trends. Blooms tend to occur under certain conditions that may be used as indicators to alert managers to potential blooms. These condition indicators are typically waterbody specific, therefore, they need to be

developed for each waterbody rather than developing a single set of indicators for statewide use.

Condition indicators may include:

- water reaching or exceeding a certain temperature
- water flow falling below a certain level
- decreasing depth of sunlight penetration/increased water opacity
- time of year associated with longer periods of sunlight (blooms may tend to begin and end around the same time each year in a particular waterbody).

Routine monitoring during the bloom season is essential to fully characterize the potential risks to public health and water quality. While full characterization of a bloom may not always be feasible, it is important to monitor during the bloom season, as the dominant cyanobacteria can turnover and a new bloom, involving different cyanobacteria, may develop. Historical information can also be used to help predict which toxins are likely to be produced during the bloom season; thus, reducing monitoring cost and event response time.

Box 1b. CHAB-Related Human or Animal Illness.

- **If suspected post CAUTION sign immediately and proceed to Box (3)**
- **If confirmed post DANGER sign immediately and proceed to Box (8)**

SUSPECTED:

Suspected acute human Illness *(1)*

Any of the following symptoms **AND** symptom onset within 48 hours of exposure to a waterbody with a suspected or confirmed algal bloom **AND** no other obvious cause of illness:

- sore throat or congestion
- coughing, wheezing, or difficulty breathing
- red, or itchy skin, or a rash
- skin blisters or hives
- earache or irritated eyes
- diarrhea or vomiting
- agitation
- headache
- abdominal pain

Human exposure to cyanotoxins most commonly occurs through ingestion or skin contact with contaminated water. Inhalation of spray or mist coming off water with high

toxin concentrations may also contribute to exposure during activities such as water-or jet-skiing. Children are considered more susceptible to effects from toxins than adults.

CAUTION signs should be posted immediately.

Collect water and algal mat samples immediately, including benthic mats in river systems. Samples should be collected and stored as appropriate for the lab identified for analysis. Contact with the lab prior to sampling is recommended.

NOTE: People with symptoms should contact their health care provider.

Suspected acute animal illness [(2)]

Any of the following symptoms **AND** symptom onset within 48 hours of exposure to a waterbody with a suspected or confirmed algal bloom **AND** no other obvious cause of illness:

- vomiting and/or diarrhea
- lethargy or general weakness
- abnormal liver function test results
- difficulty breathing
- foaming at the mouth
- muscle twitching that may lead to convulsions
- death

Pet and livestock exposure to cyanotoxins is typically through drinking water with sufficiently high concentrations of toxins, or ingestion of scum or drying mats along the shoreline. Dogs may ingest additional toxins or scum when they lick their fur after getting wet.

CAUTION signs should be posted immediately.

Collect water and algal mat samples immediately, including benthic mats in river systems. Samples should be collected and stored as recommended by the lab that will be conducting the analysis. Contact with the lab prior to sampling is recommended.

In the case of an animal death, ask the attending veterinarian to collect animal tissues (specifically the stomach contents, liver, and brain) for testing as soon as possible, prior to animal preservation or disposal. Stomach contents from an ill or dead animal are the most important samples for determining cyanotoxin exposure.

CONFIRMED:

Once a human illness, or animal illness or death, has been confirmed as being due to cyanotoxin exposure a **DANGER** sign should be posted immediately, and regular sampling and analysis of the water and scums, mats, or benthic algae should begin.

Confirmed acute human Illness

Meets criteria for *SUSPECTED CASE* **AND** there is laboratory documentation of at least one HAB toxin in the water **AND** professional judgment based on medical review by a health care provider.

DANGER signs should be posted immediately.

Confirmed acute animal illness or death

Meets criteria for *SUSPECTED CASE* **AND** there is laboratory documentation of HAB toxin(s) in stomach contents or in water and/or algal mats.

DANGER signs should be posted immediately.

NOTE: Dogs exposed to anatoxin-a may die within 20-30 minutes following onset of symptoms.

If humans or animals show symptoms of cyanotoxin exposure after contact with water, or with scums or mats of algae, they should receive immediate medical attention. The water and area where the contact has occurred should be sampled immediately to determine if cyanobacteria or cyanotoxins are present. Hospitals and veterinary clinics can be alerted to look for signs of cyanotoxin exposure in other patients, especially if these facilities are located near water where cyanobacteria may be present. Hospitals and veterinary clinics should be encouraged to report any suspected or confirmed cases of cyanotoxin exposure to the local health department and regional water board. (http://www.waterboards.ca.gov/waterboards_map.shtml).

Box 2a. Are cyanobacteria or cyanotoxins detected?

- **If No: Proceed to Box (2b), and continue routine monitoring for indicators**
- **If Yes: Proceed to Box (3)**

Box 2a provides an opportunity for initial screening using tools that do not require formal laboratory analysis. In some cases this is not a necessary step for making a management decision.

Are cyanobacteria detected?

There are a number of methods available for initial determination of cyanobacteria presence including:

- Visual screening for cyanobacteria using field or office-based microscopes, Smartphone tools (http://cellscope.berkeley.edu/), FlowCam (http://www.fluidimaging.com/)
- Field sensor or bench top fluorimeter measurements of phycocyanin pigments
- Stick test and jar tests (http://www.kdheks.gov/algae-illness/download/Jar_Test.pdf)

There are websites where photos of algal blooms and microscope images can be seen to compare to local conditions. These may help rule out non-harmful algal blooms and narrow down the type of bloom a waterbody is having. The USGS Field and Laboratory Guide can be helpful in some areas. https://pubs.er.usgs.gov/publication/ofr20151164

Are cyanotoxins detected?

The presence of some cyanotoxins may be confirmed quickly through the use of field test kits. Currently field test kits are available for measuring microcystins in water. Field test kits for measuring anatoxin-a and cylindrospermopsin will be available in 2016.

It is recommended that these kits be used for determining presence or absence of cyanotoxins. To detect intracellular toxins, a lysing step must be included (e.g. lysing chemical, freeze-thaw method) to release toxins from cells prior to testing with the kit.

NOTE: field test kits may not detect toxins from surface scum, algal mats, or benthic algal mats. These mats need to be sampled for identification and formal laboratory analysis.

If cyanobacteria or cyanotoxins are not detected, water managers should continue routine visual monitoring for indicators of cyanobacteria and cyanotoxins as described in Box 1a. If there is reason to believe there are toxins present that field methods did not detect, sampling for laboratory analysis should be considered.

If cyanobacteria or cyanotoxins are detected, sampling for laboratory analysis should begin immediately (proceed to Box 3).

Box 2b. No Detection - Monitor for changes.

If initial screening tools do not detect cyanobacteria or cyanotoxins, the bloom may be green algae or another nuisance. Green algae do not produce toxins, so the voluntary guidance will not cover these situations.

Continued routine visual monitoring for indicators of cyanobacteria and cyanotoxins is recommended as described in Box 1a.

Box 3. Sampling

If it is determined through field testing, bloom indicators, human illness, or animal illness or death that a harmful algal bloom is occurring in the waterbody, field sampling for formal laboratory analysis is strongly recommended.

Communication with the laboratory prior to sampling is strongly recommended to ensure appropriate sample collection, storage, shipping conditions, and other considerations.

NOTE: CAUTION signs should be posted while waiting for lab results.

Sample data received from the laboratory should be compared with the toxin and cell density triggers in Table 1 to determine if **ANY** toxin triggers (Primary Triggers) or Secondary Triggers have been exceeded. Primary Triggers are identified for toxin concentrations; Secondary Triggers are given for cell density of potentially toxin producing cells, and some site specific indicators of cyanobacteria, including visual monitoring. Exceedance of secondary triggers can result in an Action Trigger (see Box 4c).

Sampling Design Considerations

Monitoring should focus primarily on the protection of human health, and secondarily on the health of pets and livestock. Scums and mats often contain the highest levels of toxin; therefore these substances should be considered during sample collection. For human recreational safety, samples should be from areas of high use.

In rivers, and other flowing water, samples should be focused along shorelines where recreational users and animals are entering the water, backwater areas where algae and toxins may accumulate, and upstream and downstream of the area of concern.

Detailed guidance on sampling design is found in Appendix 1 of the 2010 Voluntary Guidance document.

Sampling frequency, location, and number of samples are vital elements to consider when designing a monitoring plan to characterize a bloom. It is recommended that sampling be conducted, at minimum, once a week. During favorable bloom conditions researchers have observed significant fluctuations of cyanobacteria and toxins over a 24 hour period; therefore, more frequent monitoring may be required to capture growth or changes. Although bloom characterization may be important, especially in waterbodies without a history of HABs, the cost of monitoring and turnaround time of laboratory testing should be taken into consideration.

NOTE: Sample frequency should increase if the bloom increases, visually changes, and/or scums/mats form, especially if initial toxin levels were at the Action Trigger or Tier I.

Primary Triggers

To obtain data needed for comparison to the primary triggers, monitoring should include the following sample types:

- *Cyanobacteria toxin analysis - to detect and quantify the concentration of cyanotoxins in water*
- **optional* cell identification - to identify the cyanobacteria present in water samples and if potential toxin producers are present*

The primary triggers are based on toxin concentrations measured in water, if **ANY** toxin exceeds the primary triggers, action is required. Measurements of water may include a combination of water, scum, and mats. The toxins addressed in this guidance, which are commonly detected statewide, include total microcystins, anatoxin-a, and cylindrospermopsin. Total microcystins[3] refers to the measurement of more than one variant of microcystin, at minimum the following microcystin (MC) variants should be analyzed: MC-LR, MC-LA, MC-RR, and MC-YR (can be analyzed by either ELISA or LC-MS). Cyanobacteria toxins predominantly collect within cells and the toxins are released upon cell death and damage. To measure the total toxin in water samples, a cell lysing step should be conducted by the lab prior to toxin analysis.

Characterization of a bloom involves identification of the dominant cyanobacteria to determine if there are potential toxin producers and if so, which types of toxins may be produced. Water samples can be examined by field or laboratory methods to identify the cells. This characterization can help direct laboratory testing for the most relevant toxins, thereby reducing the cost of analysis.

Secondary Triggers

Data may be obtained from the following parameters to compare to secondary triggers:

- *Cell density - to quantify the dominant cyanobacteria population*
- *Visual observation - to observe formation of blooms, scums, or mats*

There is research being done in several areas that may provide additional cost-effective secondary trigger parameters in the future. These include:

- *Satellite imagery - data or notification indicating formation of a bloom*
- *Cyanotoxin gene analysis - to detect cell genetic material capable of toxin production*

The secondary triggers are only developed for the “action trigger” level (box 4c). If data collected from these parameters meets the secondary trigger level, monitoring and posting of the waterbody is recommended.

NOTE: The Surface Water Ambient Monitoring Program[4] (SWAMP) has established a Statewide Freshwater HABs Program, including an Assessment and Support Strategy for fresh water HABs. An integral component of this program is the development of guidance documents for statewide event response. The guidance documents will include the following subjects:

- Standard operating procedures for sample collection

- Health and safety guidelines for sample collection
- Standard laboratory analysis methodology
- Decision frameworks for sampling and analysis
- Quality assurance objectives
- List of qualified labs and types of analyses they perform

These documents will be available on the SWAMP website under the highlights button labeled FHABs.

Box 4a. Danger Tier II. Have <u>ANY</u> Tier II toxin triggers been exceeded?

- **If No: Proceed to Box (4b)**
  - If Tier II triggers in Table 1 have **NOT** been exceeded, proceed to Box 4b and compare toxin levels with those in Table 1 for Tier I.
- **If Yes: Post DANGER signs immediately and proceed to Box (8)**
  - If Tier II triggers have been exceeded for **ANY** toxin, post the recommended **DANGER** signs in areas where the public is likely to recreate on the water or to encounter the signs and proceed to Box 8.

There is a heightened risk of adverse effects in people, pets, and livestock at these cyanotoxin concentrations. The public should be warned against contact with the water, including water spray from activities like jet skiing. Pets and livestock have very high risks of serious illness or death at these toxin concentrations because they consume more algal material compared to humans.

If conditions begin to change sampling should resume to determine when it is appropriate to move from Tier II to Tier I warning signs. Be aware that cyanotoxin levels may spike again, so even if cyanotoxin levels are decreasing, it is recommended that the decision tree be followed until de-posting criteria (Box 7a) are met in order to insure public safety and animal welfare.

Appendix A describes the basis of the Tier II Trigger levels for each toxin.

Box 4b. Warning Tier I. Have <u>ANY</u> Tier I toxin triggers been exceeded? (but not Tier II)

- **If No: Proceed to Box (4c)**
  - If Tier I triggers in Table 1 have **NOT** been exceeded, proceed to Box 4c and compare toxin levels with those in Table 1 for Action Trigger Levels.
- **If Yes: Post WARNING signs and proceed to Box (8)**
  - If Tier I triggers in Table 1 have been exceeded for **ANY** toxin, post the recommended **WARNING** signs where the public is likely to recreate on the water or to encounter the signs and proceed to Box 8.

There is cause to be concerned about exposure to cyanotoxin concentrations at this level. Prolonged contact with water can lead to an itching rash or other skin issues. Activities that lead to the ingestion of water, like swimming, can be dangerous to people and pets. Pets and livestock need to be kept out of the water and away from the shoreline; they tend to consume scum at the shoreline and drying algal mats that wash up on shore. These materials may have exceptionally high concentrations of cyanotoxins and can be lethal to pets and livestock.

Appendix A describes the basis of the Tier I Trigger level for each toxin.

Box 4c. Caution Action Trigger. Have ANY Primary or Secondary Action Triggers been exceeded? (but not Tier I)

- **If No: Proceed to Box (5)**
  - If the Action Triggers in Table 1 have **NOT** been exceeded, proceed to Box 5.
- **If Yes: Post CAUTION sign and proceed to Box (3)**
  - If Action Trigger for **ANY** toxin, or Secondary Trigger, has been exceeded, it is recommended that **CAUTION** signs be posted where the public is likely to recreate on the water or to encounter the signs and proceed to Box 3.

The presence of cyanotoxins above the primary action triggers indicates the need for concern for public health and animal welfare around the waterbody. The waterbody should be monitored for the possibility of increasing toxin concentrations. Cyanotoxin concentrations can change quickly during a bloom event.

Appendix A describes the basis of the Primary Action Trigger for each toxin.

NOTE: There is a heightened risk to dogs and livestock when the trigger level has been exceeded in a waterbody. Many pet and livestock deaths have been attributed to cyanotoxins in recreational waters in California. Deaths have occurred when toxin levels in water are below human thresholds for concern. This is likely due to increased exposure because animals are known to consume algal materials containing high toxin levels (e.g., dried mats, etc.). Dogs and livestock should be kept out of the water and away from the shoreline when action triggers are exceeded.

Secondary Action Triggers may lead to an affirmative response for Box 4c in the absence of toxin measurements. Secondary Action Triggers should not be used to rule out the presence of a harmful bloom because cyanotoxins may be present when cyanobacteria cell densities are low, or when visual cues of a bloom are absent. Use of the Secondary Action Triggers should be used at the discretion of local water or

public health managers and may be based on past experience with blooms in a particular waterbody.

Appendix B describes the basis for the Secondary Action Triggers. *[Appendix B has not been drafted yet.]*

Box 5. Is the waterbody posted?

- **If No: Proceed to Box (6a)**
  - If the factors indicating the need for sampling (Box 1a) are not still a concern, sampling may be discontinued. It is recommended that routine visual monitoring be continued.
- **If Yes: Proceed to Box (7a)**
  - If the factors indicating the need for sampling (Box 1a) are still a concern and the waterbody does not meet de-posting criteria, return to Box 3 and continue sampling**.**

It is recommended that toxin levels be below action trigger levels for a minimum of two weeks before considering de-posting, or posting at a lower level.

Box 6a. Toxin producing species, bloom, and favorable conditions decreased?

- **If No: Return to Box (3)**
  - If the bloom visually appears to be persisting or increasing, the concentration of potential toxin producing cells increases, or toxin levels are increasing, return to Box 3 and continue monitoring visually and sampling.
- **If Yes: Proceed to Box (6b)**
  - If the bloom appears to be decreasing and favorable conditions are decreasing, it is recommended to proceed to Box 6b.

A waterbody specific trend analysis may be helpful to determine which conditions signal that bloom events are ending.

Use caution when observing a bloom that appears to be decreasing; particularly for waterbodies undergoing their first recognized algal blooms and have limited historical data and observations to understand trends. When a bloom appears to be decreasing but conditions continue that are favorable for blooms (e.g. long days, low flows, high water temperatures, high nutrient concentrations), it is recommended that visual monitoring and sampling on a regular basis be continued until the favorable conditions decrease. During favorable conditions, a new bloom can quickly replace the previous bloom. Researchers have observed that cyanobacteria populations can fluctuate significantly within a 24 hour period so monitoring is needed to capture re-growth or growth of new blooms.

Box 6b. End of Decision Tree.

If a cyanobacteria bloom has significantly declined and conditions indicate that the bloom is at an end, managers may suspend sampling. Routine monitoring of the waterbody should continue, especially if conditions return that are favorable for blooms.

Box 7a. Does the Waterbody Meet the De-posting Criteria?

- **If Yes: Proceed to Box (7b)** and continue routine visual monitoring
- **If No: Proceed to Box (8)** and continue visual monitoring and sampling

De-posting criteria are intended to protect against the risk of exposure to toxins when water may appear suitable for recreational activities, but toxins may still be present and/or blooms may again increase.

Postings should remain in place until the following criteria are met:

- Quantitative samples confirm that all cyanotoxins are below Primary Action Triggers (see Table 1) **AND** declining bloom conditions (see Box 1a) continue for a minimum of two weeks.
- If posting was based on a Secondary Trigger, then either the secondary trigger OR primary triggers should no longer be exceeded (cell densities of toxin producers are below 4,000 cells/mL and no scums are visible).
- All evidence indicates the bloom is ending and favorable conditions are decreasing.

NOTE: In some instances, blooms may still be active when seasonal changes begin and continued sampling is no longer safe, such as sustained near-freezing temperatures, heavy rains, or snowfall. Professional judgment should be used to inform decisions to remove postings under these circumstances, considering potential for exposure by children, pets, or livestock, and potential for returning warmer weather and bloom resurgence.

Box 7b. Remove Posting

When the waterbody meets the de-posting criteria per Box 7a, all **CAUTION**, **WARNING**, and **DANGER** signs should be removed. Signs left up when cyanotoxins are no longer an immediate concern may result in the public disregarding signs when an immediate danger is present.

Box 8. Return to Box 3

If toxin trigger levels in Table 1 have been exceeded for **ANY** toxin during monitoring, post the appropriate **CAUTION**, **WARNING**, or **DANGER** signs immediately. Return to Box 3 to continue sampling at an appropriate frequency (see Appendix 1) to monitor for changes in cyanotoxin levels necessary to protect public health.

After the waterbody is posted with appropriate signs, return to Box 3 and follow a sampling plan designed for monitoring a toxic bloom. It may not be necessary to continue sampling for toxins as long as conditions do not change, based on observation, and the signs remain in place to ensure that people are sufficiently warned to stay out of the water.

SIGNS

Signs, such as those shown in Appendix [X], should be posted in prominent water access points to alert the public to the dangers of exposure, especially for children, pets, and livestock.

Signs should be highly visible, and made of sufficiently durable materials to remain highly visible throughout the duration of the bloom. Laminated signs can be put up rapidly, but are not durable to weather and vandalism; if used, they should be checked daily and replaced as needed. More permanent signage (e.g., metal) is recommended. In areas where cyanotoxins are annually present in the water, it may be appropriate to install permanent signs that can be easily modified to reflect current conditions (blank, caution, warning, or danger).

Additionally, signage alerting the public to the ongoing issue and providing additional information about cyanobacteria and risks related to cyanotoxins may be warranted; informational signs should be significantly different from **CAUTION**, **WARNING**, and **DANGER** signs so that they are not confused.

Footnotes & References

(1) Human case definitions in this document use criteria slightly modified* from the Ohio Department of Health's *Blue-Green Algae/Cyanobacteria Harmful Algal Bloom (HABs) Physician Reference*, cited on 10-15-2015, available at: http://www.odh.ohio.gov/~/media/ODH/ASSETS/Files/eh/HABs/habproviderreference.ashx. The Ohio document references the Center for Disease Control and Prevention (CDC) *Proposed Case Definition for Algal Toxin-related Diseases* as the source of their case definitions.

* Ohio/CDC lists three categories of cases: suspect, probable and confirmed; while this guidance document captures illnesses in two categories: suspect and confirmed.

(2) Animal case definitions in this document use criteria developed for CDC's Harmful Algal Bloom Illness Surveillance System (HABISS). For further details see Backer LC et al *Canine Cyanotoxin Poisonings in the United States (1920's-2012: Review of Suspected and Confirmed Cases from Three Data Sources; Toxicon, 2013, Full text available online at* stacks.cdc.gov/view/cdc/21319/cdc_21319_DS1.pdf

(3) Welker, M. and von Dohren, H. Cyanobacterial Peptides—Nature's Own Combinatorial Biosynthesis. FEMS Microbiology Reviews, 2006, 30, 530-563.

*Microcystins are a class of toxins synthesized by some cyanobacteria. Approximately 90 variants of the microcystin compound have been discovered. Laboratory analytical capabilities are limited to testing a fraction of the discovered variants. Communication with the laboratory is recommended to learn about their available testing methods.

(4) The Surface Water Ambient Monitoring Program (SWAMP) is conducted by the State Water Resources Control Board and Regional Water Quality Control Boards.