INTRODUCTION AND BACKGROUND

This document outlines a protocol for evaluating potential health concerns related to the presence of cyanobacteria (blue-green algae) in Massachusetts recreational freshwater bodies. Blooms can form when cyanobacteria, which are bacteria that grow in water, multiply quickly and form “scums” or “mats” on the surface of the water. Blooms can occur at any time but most often occur in late summer or early fall. The most common types of cyanobacteria that bloom are *Microcystis* and *Anabaena*. Certain strains of *Microcystis* and *Anabaena* manufacture toxins called microcystin and anatoxin, respectively, and these toxins can produce adverse health effects. Toxins are released from intact cyanobacteria cells when they die in the waterbody or when they are ingested by animals or humans. Once ingested, the digestive juices destroy their cell wall (lyse the cell) and the toxin is released into the gastrointestinal tract.

The scientific literature on health effects resulting from exposures to cyanobacteria-related toxins associated with blooms is developing, with the most widely cited guidance published by the World Health Organization (WHO) in 2003 (WHO 2003). This document reviews the WHO guidance as well as the current scientific literature for the purpose of updating current MDPH guidance with respect to responding to suspected or actual cyanobacteria blooms in Massachusetts recreational freshwater bodies.

REVIEW OF LITERATURE

Cyanobacteria, under the right conditions, can multiply quickly and pose a health risk to those coming into contact with the water. This ability to multiply quickly makes monitoring their numbers important. Because the health risk rises with the cell counts, the goal of any monitoring plan is to be able to take action before levels are reached that pose health risks.
This section reviews the current literature in order to make recommendations related to the presence of cyanobacteria in a recreational water body. There are three measures on which action can be taken:

1. Observation of visible scum or mat layer
2. Total cell count of cyanobacteria (units of total cells/mL water)
3. Concentration of cyanobacteria toxin (e.g., microcystin) (units of µg toxin/L of water)

These three measures will be evaluated based on a literature review of current studies on a) cell counts and health effects, b) cyanobacteria toxin levels and health effects, and c) correlations between cell counts and toxin levels.

**Literature on Cell Counts and Health Effects**

A prospective cohort study of 852 people was conducted in Australia in 1995 (Pilotto et al. 1997). Participants were interviewed at five freshwater bodies that had a history of cyanobacteria blooms. Information on their health and recreational water-related activities was collected. Follow-up interviews were held two and seven days later and noted any specific health symptoms, such as diarrhea, rashes, and eye or ear irritations. The responses from the interviews were compared with cyanobacteria counts from water samples collected at the freshwater bodies on the day the participants were first interviewed. No significant difference in reported health symptoms was found at two days following exposure. However, the authors reported that exposed individuals had an elevated odds ratio for symptoms seven days following exposure to the following:

- > 5,000 cyanobacteria cells/mL for over an hour
- Bathing in water with 5,000-20,000 cyanobacteria cells/mL
- > 80,000 cyanobacteria cells/mL

The odds ratio is based upon all symptoms reported. Thus, the cell counts or exposure period were not correlated with one specific symptom, but the odds of developing one of the seven symptoms the study examined.

This study forms the basis of the WHO guidance related to cell counts of 20,000/mL. At this level, the WHO recommends that there should be notification to inform individuals about possible health risks associated with contacting the water. WHO chose the level of 20,000 and not 5,000 because they noted that the reported effects at 5,000 were mild and not reported by a large number of people (WHO 2003).

More recent studies have used different methods to evaluate health effects from cyanobacteria, but the methods used cannot be translated to estimates of cell counts or toxin levels, hence are of limited use for purposes of developing guidelines. However, they do provide additional evidence that exposure to cyanobacteria can result in health effects, particularly dermal irritant effects.
A prospective cohort study found increased reporting of respiratory symptoms and of any symptom (respiratory, gastro-intestinal illness, ear, eye, dermal, or fever) at three days following exposure to cyanobacteria cell surface area > 12 mm\(^2\)/mL (Stewart et al. 2006b). The Stewart study chose to use cell surface area instead of cell counts, which prevents direct comparison of the thresholds found in the two studies.

**Microcystins and World Health Organization Guidance**

The WHO recommended a drinking water guideline of 1 part per billion (ppb) microcystin, the toxin produced by certain strains of the cyanobacteria, *Microcystis*. The study forming the basis of the WHO drinking water guideline was a 13-week oral gavage mice study with microcystin (Fawell et al 1994). Based on liver histopathology and serum enzyme changes, a no-observed adverse effect level (NOAEL) of 40 µg/kg body weight/day was derived. WHO applied an uncertainty factor of 1,000 to derive a Tolerable Daily Intake (TDI) level of 0.04 ug/kg/day. [A TDI is the estimated amount of a substance that can be consumed daily over a lifetime without an appreciable health risk (WHO 2006).] WHO then applied standard exposure assumptions (e.g., a 70 kg adult drinks 2 liters of water a day) to derive a drinking water guideline of 1 ppb.

Although WHO discussed other animal studies, the above study was deemed to be the most conservative study on which to base a microcystin guideline. No other studies were available in the literature that would affect the use of the mice study as a basis for the microcystin guideline.

In order to assess health concerns related to microcystin (generally cell-bound) in recreational waters (as opposed to drinking water), WHO applied conservation exposure assumptions related to recreational water use. Specifically, WHO assumed an adult, weighing 60 kg, consumes 100 mL of water while swimming or wading, while a child, weighing 15 kg, may consume 250 mL of water during the same activities. If microcystin is present in the cyanobacteria and water (after lysing the cells) at a concentration of 1 ppb (or 1 µg/L), the total exposure to an adult would be nearly equal to the TDI while for a child, it would be about 10 times the TDI. Individuals with certain existing health conditions (i.e., liver ailments) could be at greater risk. Given the conservative assumptions used in deriving the TDI and exposure estimates for recreational water activities, WHO suggested that an appropriate guideline for microcystin in recreational waters could be 20 ppb.

**Other Health Effects Studies**

Two studies have examined the effects of individuals wearing skin patches containing cyanobacteria. One study involved placing dermal patches containing either whole or lysed cells at varying concentrations. This study found that approximately 20% of individuals had dermal reactions to the patches, whether they contained whole or lysed cells and independent of the cell count. The dermal reactions were reportedly all mild. The authors concluded that some percentage of the healthy population is susceptible to skin reactions from cyanobacteria (Pilotto et al. 2004). The second study involved placing dermal patches containing cyanobacteria and cyanobacteria toxins on volunteers. This study found that only one of 39 participants had a dermal reaction, and this reaction was to a non-toxin producing cyanobacteria (Stewart et al. 2006c).
Literature on Correlation Between Cell Counts and Toxin Levels

The available literature suggests there is some correlation between cyanobacteria cell counts and the toxin concentration in the water, but this correlation is uncertain. For example, the cells can begin to die, and as they die, they release the toxin. Thus, although the cell count may show a decreasing amount of cells, the toxin concentration in the water may actually increase for a period of time. In addition, it is difficult to select sampling locations as the cells and the toxins may not be equally distributed within a bloom.

Data available from Lake Champlain in Vermont show levels of microcystin greater than 20 ppb were generally found in waters with cell counts over 100,000 (Watzin et al. 2005). The WHO concluded that *Microcystis*-dominated algal blooms with 100,000 cells/mL may contain 20 ppb of toxin (WHO 2003). Thus, it is reasonable to assume, based on currently available data, that cell counts of 100,000 or more may have toxin levels of 20 ppb or more. The WHO recommended that at cell counts of 100,000 cyanobacteria cells/mL or greater, swimming should be discouraged and on-site advisory signs should be posted. This advisory also reflects concern that counts could rise rapidly, along with the associated toxin health risks. Based on the 1997 Pilotto et al. study, the WHO estimated that cell counts of approximately 20,000 could result in toxin concentrations in water ranging from about 2-4 ppb (WHO 2003).

MDPH RECOMMENDATIONS

The following paragraphs provide MDPH recommendations for cyanobacteria and toxin guidelines to prevent acute exposure to elevated levels of these substances in recreational waters. Dense blooms and scums can contain millions of cells/mL and toxin levels in the parts per million. They can form near embankments and in areas suitable for swimming and other forms of recreation. They can also move around in the water body and grow quickly, making management of them difficult (Watzin et al. 2005, WHO 1999, 2003). Exposure to high levels of cells and toxins is dangerous and the more serious published reports of acute health effects from cyanobacteria typically involves exposure to dense blooms and scums (Behm 2003, Hitzfeld et al. 2000, WHO 1999, 2003). The proposed guidelines are designed to allow preventive action to be taken prior to exposure, thereby mitigating possible health concerns.

Guideline for Cyanobacteria Toxin (Microcystin) in Recreational Water

MDPH recommends adoption of the WHO TDI of 0.04 µg/kg/day of microcystin. In order to estimate a recreational water body concentration that would result in exposures at or below the TDI, the following assumptions were made:

**Adult**

| Weight: | 70 kg |
| Intake: | 0.05 L water/hour |
| Duration: | 1 hour/day |
Child

Weight: 35 kg
Intake: 0.1 L water/hour
Duration: 1 hour/day

These assumptions are taken from U.S. EPA guidance (1997; 1989). The average 10-year old child weighs approximately 35 kg and an average adult weighs approximately 70 kg. This average weight of a 10-year old child is also similar to the average weight of all children between the ages of 1-18 years old (EPA 1997). The intake rate is based on guidance from EPA on surface water ingestion while swimming (EPA 1989). For children, the intake rate was doubled to 100 ml, which is approximately seven tablespoons of water. According to EPA, non-competitive (recreational) swimmers consume more water than competitive swimmers (EPA 2003). Children playing in the water consume more water than those swimming for exercise. For exposure assessments of adults in swimming pools, EPA has created a model that assumes they consume either 0.0125 or 0.025 L/hr (EPA 2003). However, since this assessment is for cyanobacteria in freshwater bodies, and water from freshwater bodies is less distasteful to ingest than pool water, these lower intake rates for adults were not used. The duration of time spent in the water was estimated to be one hour per day, seven days a week during a 13-week season. The WHO TDI was based on a 13-week mice study. Thirteen weeks is approximately the length of the summer bathing season in Massachusetts.

To calculate a water concentration of microcystin that would result in a total dose of 0.04 µg microcystin/kg body weight/day (the TDI), the following equation is used:

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\text{Guideline Concentration} = \frac{(\text{weight}) \times (\text{TDI})}{(\text{intake}) \times (\text{duration})}
\]

Using the stated assumptions, the results indicate that a guideline based on adult exposure would be 56 µg microcystin per liter water, or 56 ppb. For a child, the guideline would be 14 ppb. Hence, to be most conservative, MDPH recommends the toxin guideline be 14 ppb.

**Guidelines for Cyanobacteria Cell Counts**

The available literature and the equation noted above suggest that at approximately 20,000 cells/mL, associated toxin levels may range between 2-4 ppb, while at 100,000 cells/mL, associated toxin levels may be approximately 20 ppb. If we assume a linear relationship between cyanobacteria cell counts and associated toxin levels (data are sparse in this area), a cell count of 70,000 cells/mL would correspond to a toxin level of approximately 14 ppb. This is also the concentration derived using the equation. Thus, to be protective and reduce potential exposures to levels at which there is a greater likelihood of health effects, MDPH recommends that at a cell count of 70,000 cells/mL, individuals should be advised to refrain from coming into contact with the affected water.
Recommendations for Monitoring or Advisory Posting

MDPH believes that the current literature supports the use of a cell count guideline of 70,000 cells/mL in order to prevent adverse health effects from exposure opportunities to cyanobacteria and related toxins during algal blooms. MDPH also recognizes that it is generally more feasible to monitor using cell count methods rather than toxin analytical methods. We do offer the following general guidance related to monitoring potential cyanobacteria problems with the stated goal of preventing health effects before cyanobacteria or toxins reach levels of concern or higher:

1. If a visible cyanobacteria scum or mat is evident, MDPH recommends an immediate posting by the local health department, state agency, or relevant authority to advise against contact with the water body.
2. If the cell count exceeds 50,000 cells/mL, toxin testing of lysed cells should be done to ensure that guideline of 14 ppb is not exceeded. The lysing should consist of three freeze and thaw cycles.
3. If either the cell count exceeds 70,000 cells/mL or the toxin level of lysed cells meets or exceeds 14 ppb, post an advisory against contact with the water. The lysing should consist of three freeze and thaw cycles.
4. Because cyanobacteria can multiply extremely rapidly, frequency of follow-up testing may depend in part on weather conditions, e.g., predicted hot, dry, and calm conditions, all of which promote rapid cyanobacteria generation, may suggest more frequent testing than weekly.
5. Since decreasing cell counts indicate cell die-off and lysing cells release toxins, algal toxin concentrations in the water may rise for a period of time after cell counts decrease. Many factors (e.g., wind, rain, temperature) can effect the progression of die-off, which supports a measured approach for lifting an advisory similar to that of Oregon and Australia: advisories may be lifted after two successive and representative sampling rounds one week apart demonstrate cell counts or toxin levels below those at which an advisory would be posted.

Signage should be posted at (all) water body entry points and should include the following: date of the posting, contact information for the posting authority, language (to be provided or reviewed by MDPH) advising against contact with the water, and a recommendation that pets accidentally entering the water be rinsed.

This proposed protocol does not pertain to the toxin anatoxin, which is produced by several species of cyanobacteria. There is no guidance in the literature for responding to detections of anatoxin. Thus, if anatoxin is detected, MDPH will evaluate such situations episodically, using supplemental information such as cyanobacteria counts, exposure scenarios (popular swimming site, for instance), and upcoming weather forecasts. The cyanobacteria *Anabaena*, which produces anatoxin, would be included in any cell counts of cyanobacteria. Therefore, there is some mechanism for managing the risk it poses.
References


Appendix: Guidelines from Other Health Organizations

California State Water Resources Control Board
At cell counts greater than 40,000 cell/mL of *Microcystis* and *Planktothrix* or at cell counts greater than 100,000 cells/mL of potentially toxic cyanobacteria (e.g., *Anabaena* and *Microcystis*), the Board’s draft guidelines recommend that a beach be closed. The 40,000 cells/mL value was derived using a risk assessment approach based on child’s recreational exposure to the toxin (CSWRCB 2006). This approach is not described further in the Board’s draft guidance document. The 100,000 cells/mL value appears to be taken from the WHO guidance.

California Department of Health Services
At cell counts greater than 20,000 cells/mL, the Department’s draft guidance recommends that a beach be closed. No supporting information is given. However, it is likely that this number is taken from the WHO guidance, which advises notifying bathers of the presence of cyanobacteria at this cell count.

Vermont
At cell counts greater than 4,000 cells/mL, Vermont recommends that the water be tested for toxins. This threshold is based upon the results from 6 years of research in Lake Champlain and other waterbodies in the state. They have found that the toxin levels do not approach their guideline of 6 ppb of toxin until the cell counts are higher than 4,000 cells/mL (Watzin et al. 2003, 2005, and Stone and Bress 2007). This low threshold enables them to monitor developing situations and minimize potential exposure to elevated levels of toxin.

The Vermont guidance level of 6 ppb of toxin is based upon the same study that the WHO used to generate their provisional guideline for drinking water consumption. The study was conducted in 1994, and involved administering the cyanobacteria toxin microcystin orally to mice. Based upon liver histopathology and serum enzyme level changes, and adding an uncertainty factor of 1,000, the WHO generated a TDI (Tolerable Daily Intake) of 0.04 ug/kg/day. This TDI is a level of the toxin that should be safe to consume daily over a lifetime. Assuming that an adult weight 60 kg and drinks 2 liters of water per day, using this TDI, the WHO derived a drinking water guideline of 1 ppb of microcystin in water.

Vermont took the TDI that the WHO had generated, and using different assumptions about body weight and water consumption, generated a guideline for recreational exposure to the cyanobacteria toxin. They assumed an exposure scenario where a child, weighing 15 kg, ingests 100 mL of beach water per day (EPA guidance). Based on this scenario, Vermont calculated a recreational water guideline of 6 ppb.

The World Health Organization
The WHO does not recommend a cell count at which to test for the toxin. The lowest WHO cell count guideline is 20,000 cells/mL, and that is due to health concerns based on irritative or allergenic effects of cyanobacteria described in a study by Pilotto et al (1997 cited in WHO 2003). At this level, the WHO recommends that officials “post on-site risk advisory signs” and “inform relevant authorities”. The Pilotto study is one of the two studies upon which Australia bases its 5,000 cells/mL guidance described above.
Australia
At toxin levels greater than 10 ppb, Australia recommends that a beach be closed. This concentration is based on a LOAEL derived from a pig study by Falconer et al (1994 cited WHO 1999; also discussed in Kuiper-Goodman et al. 1999 as cited Australian Guideline 2005). In this study, pigs consumed drinking water that contained microcystin. Based upon general liver damage (observed from histopathology and serum enzyme level changes), a LOAEL of 100 ug/kg/day was derived. Australia then added an uncertainty factor of 5,000 and assuming a child weighing 15 kg consume 100 mL of water for 2 weeks, generated a recreational water guideline of 10 ppb.
References


