Final Report

Washington State Provisional
Recreational Guidance for
Cylindrospermopsin and Saxitoxin

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Introduction

Numerous Washington lakes produce cyanobacteria (also known as blue-green algae) that can create toxins. Historically, many animals have become ill or have died after exposure to cyanotoxins in state lakes. For the past several years, Washington State Department of Health (DOH) has conducted passive surveillance of human and animal illnesses related to harmful algal bloom exposure (HABs). Reports of illnesses are entered into a national database at the Centers for Disease Control and Prevention (CDC) to track patterns of exposure and the extent of HAB public health impacts around the nation. Accordingly, cyanotoxins are a documented public health concern in Washington’s lakes.

The state legislature created and funded a Freshwater Algae Control Program in 2005, due in part to citizens’ concerns about health impacts from exposure to freshwater HABs. Washington Department of Ecology’s (Ecology) program provides funds to support laboratory costs for King County Environmental Laboratory (KCEL) to conduct toxicity tests on samples from state lakes with blooms. When algae grow to such an extent that they color the water, they are said to be blooming. A bloom that is not toxic one day may become toxic during the same growing season; the only way to know whether a cyanobacterial bloom is toxic is to test it for the presence of toxins.

Cyanobacteria are capable of producing a variety of different toxins. Originally, the laboratory tested for microcystins which are known liver toxins. Later, KCEL developed the capacity to test for anatoxin-a, a nerve toxin. During Ecology’s program development, stakeholders requested that state guidelines be developed to help with interpretation of toxicity results. In the absence of national recreational guidance for microcystins and anatoxin-a, DOH produced provisional guidance values for both cyanotoxins – Phase I (DOH 2008). State recreational guidance values will be updated when adequate toxicity data are published or when national recreational guidance is adopted, at which time the values will no longer be “provisional.” As part of the effort to provide assistance to local health jurisdictions and lake managers, DOH also developed a lake protocol that incorporated the guidance values as a reference for use by managers, agencies, and local health jurisdictions (LHJ). Ecology’s Freshwater Algae Control Program is ongoing at this time.

In 2009, DOH and partners began a five-year cooperative agreement with CDC to expand efforts to address HABs in Washington. Part of this project involved monitoring 30 Puget Sound lowland lakes for microcystins and anatoxin-a. By 2010, KCEL incorporated laboratory tests to monitor the 30 lakes for 2 additional cyanotoxins: cylindrospermopsin and saxitoxin. Cylindrospermopsin is a liver toxin and was first identified in the U.S. in Florida. Saxitoxin is the same neurotoxin that is produced by a marine water dinoflagellate and is known in the Pacific Northwest as Paralytic Shellfish Poison (PSP). Cylindrospermopsin and saxitoxin were each observed in two Puget Sound lakes during the 2009 and 2010 sampling seasons of the CDC project. The maximum concentration of cylindrospermopsin was 0.106 μg/L and the maximum concentration of saxitoxin was 193 μg/L.

No national freshwater recreational guidance has been developed for any of the four cyanotoxins even though recreational activities are the primary route of exposure to cyanotoxins.
At Ecology’s request, DOH has developed recreational guidelines for cylindrospermopsin and saxitoxin to direct LHJs in the second part of guidance implementation efforts - Phase II. This document offers provisional recreational guideline values for cylindrospermopsin and saxitoxin integrated into the three-tiered framework developed for microcystins and anatoxin-a. The tiered protocol is designed for lake managers and LHJs to follow in the event of a toxic occurrence.

Cyanobacteria of Concern

DOH has identified a list of cyanobacteria genera and species of concern for lakes in Washington. If the following genera are identified in a water sample from an algal bloom, the sample should be tested for toxicity:

- *Microcystis*
- *Anabaena*
- *Aphanizomenon*
- *Gloeotrichia*
- *Oscillatoria/Planktothrix*
- *Cylindrospermopsis*
- *Lyngbya*
- *Nostoc*

In a summary report for the 2008 legislature, Ecology identified the top three toxic cyanobacteria genera in Washington lakes as *Anabaena, Aphanizomenon*, and *Microcystis* (Ecology 2008). *Gloeotrichia* is included in this list because exposure to this genus has led to reports of human health impacts in Washington lakes. A recent study identified microcystin-LR production by *Gloeotrichia echinulata* (Carey et al. 2007).

Cyanobacterial Toxins and Symptoms

Cyanotoxins are a diverse group of natural toxins that fall into three broad chemical structure groups (Table 1). These are cyclic peptides (microcystins and nodularin), alkaloids (anatoxins, saxitoxins, cylindrospermopsin, alyssiatoxins, and lyngbyatoxin), and lipopolysaccharides (irritants). Anatoxin-a(s) is a naturally-occurring organophosphate. This guidance document addresses cylindrospermopsin and saxitoxin.
Table 1. World Health Organization (WHO) list of toxic cyanobacteria (modified from WHO 1999 and Graham et al. 2008).

<table>
<thead>
<tr>
<th>Toxin Group</th>
<th>Primary Target Organ in Mammals</th>
<th>Cyanobacterial Genera</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cyclic peptides</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microcystins</td>
<td>Liver</td>
<td><em>Microcystis, Anabaena, Planktothrix (Oscillatoria), Nostoc, Hapalosiphon, Anabaenopsis, Aphanizomenon, Synechococcus, Synechocystis, Aphanocapsa, Pseudanabaena</em></td>
</tr>
<tr>
<td>Nodularin</td>
<td>Liver</td>
<td><em>Nodularia</em></td>
</tr>
<tr>
<td><strong>Alkaloids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anatoxin-a</td>
<td>Nerve synapse</td>
<td><em>Anabaena, Planktothrix (Oscillatoria), Aphanizomenon, Raphidiopsis</em></td>
</tr>
<tr>
<td>Anatoxin-a(s)</td>
<td>Nerve synapse</td>
<td><em>Anabaena</em></td>
</tr>
<tr>
<td>Aplysiatoxins</td>
<td>Skin</td>
<td><em>Lyngbya, Schizothrix, Planktothrix (Oscillatoria)</em></td>
</tr>
<tr>
<td>Cylindrospermopsins</td>
<td>Liver</td>
<td><em>Cylindrospermopsis, Anabaena, Aphanizomenon, Umezakia, Raphidiopsis</em></td>
</tr>
<tr>
<td>Lyngbyatoxin-a</td>
<td>Skin, gastrointestinal tract</td>
<td><em>Lyngbya, Planktothrix (Oscillatoria)</em></td>
</tr>
<tr>
<td>Saxitoxins</td>
<td>Nerve axons</td>
<td><em>Anabaena, Aphanizomenon, Lyngbya, Cylindrospermopsis, Planktothrix (Oscillatoria)</em></td>
</tr>
<tr>
<td><strong>Amino Acid (Alkaloid precursor)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMAA</td>
<td>Neurotoxin</td>
<td><em>Cylindrospermopsis, Anabaena, Aphanizomenon, Planktothrix (Oscillatoria), Microcystis, Nodularia, Synechococcus, Synechocystis</em></td>
</tr>
<tr>
<td><strong>Lipopolysaccharides (LGS)</strong></td>
<td>Potential irritant; affects any exposed tissue</td>
<td><em>All</em></td>
</tr>
</tbody>
</table>

Note that some genera, especially *Anabaena*, can produce both neuro- and hepatotoxins. If a toxic algal bloom contains both types of toxins, signs of neurotoxicity are usually observed first. Their effects occur sooner (minutes) than effects due to liver toxins (one to a few hours).
Cylindrospermopsin

Cylindrospermopsin is comprised of a tricyclic guanidine moiety combined with a hydroxymethyl uracil. Production of the toxin is strain-specific not species-specific (NIEHS 2000). Cylindrospermopsin exhibits a completely different mechanism of toxicity than the liver toxin microcystin (Hawkins et al. 1985, Griffiths and Saker 2003, Metcalf et al. 2004). Damage to cells is caused by blocking key protein and enzyme functions thereby inhibiting protein synthesis. Cylindrospermopsin targets the liver and kidneys but can also injure the lung, spleen, thymus, and heart as demonstrated in mouse studies (Terao et al. 1994, Falconer et al. 1999, WHO 1999). Animal toxicity studies also suggest that cylindrospermopsin may be carcinogenic (Falconer and Humpage 2001, Falconer 2005) and may produce genotoxicity in a human lymphoblastoid cell line (Humpage et al. 2000). Laboratory studies have shown that some of the compounds produced by Cylindrospermopsis may cause cancer and may be genotoxic (Humpage et al. 2000, NIEHS 2000, Shen et al. 2002, Humpage et al. 2005, USEPA 2006).

Cylindrospermopsis is a naturally-produced liver toxin found in certain strains of five genera: Cylindrospermopsis raciborskii (Australia, Hungary, and the U.S.), Umezakia natans (Japan), Anabaena bergii and Raphidiopsis curvata (Fastner et al. 2003), and Aphanizomenon ovalisporum (Australia, Israel) (Banker et al. 2000). It is most commonly observed in tropical and subtropical waters of Australia (NIEHS 2000). The first report of animal poisonings attributed to cylindrospermopsin was in drinking water in a farm pond in Queensland, Australia, where it was responsible for cattle deaths (Saker et al. 1999).

Cylindrospermopsis has been increasingly encountered in temperate regions and has caused blooms as far north as Vienna and northeastern Germany. Several studies have suggested that the temperate strains of this species are not capable of producing cylindrospermopsin (Fastner et al. 2003, Yilmaz et al. 2008, Xie et al. in press). Yilmaz et al. (2008) found that eight C. raciborskii isolates did not produce cylindrospermopsin, but a strain of Aphanizomenon ovalisporum from a fish pond in Jacksonville, Florida, was shown to produce this cyanotoxin. The detection of cylindrospermopsin in other water bodies in the U.S. [e.g., two lakes in western Washington (Ketchum and Sunday), Lake Valrico and Little Lake Wilson in West Central Florida (Yilmaz and Philips 2011)] indicates the presence of unidentified cylindrospermopsin producers. Cylindrospermopsis has been identified in North America (Canada and U.S.), Europe, Israel, Brazil, Southeast Asia, Japan, and Australia. (Banker et al. 1997, Chapman and Schelske 1997, Hamilton et al. 2005, Harada et al. 1994, Molica et al. 2002, Moore et al. 2004, Pham et al. 2011, Saker and Griffiths 2000, Stuken et al. 2006).

C. raciborskii appears to have an optimal growth temperature of 25°C or greater. One of the more alarming characteristics about Cylindrospermopsis is that some species do not form scums making them harder to detect and have highest cell concentrations well below the water surface (Falconer 2005).

Generally, toxins are retained in cyanobacterial cells when conditions are favorable; however studies have shown that it is not uncommon for 70%-98% of total cylindrospermopsin produced by cells to be dissolved in the water (Chiswell et al. 1999, NIEHS 2000). Cylindrospermopsin’s half-life is much longer (18 hours) than microcystin-LR (10 minutes) under shortwave UV light.
In two cases of human toxin poisoning in Australia, toxic symptoms occurred after application of copper sulfate to dense blooms. Interestingly, chlorine effectively degrades extracellular microcystins and cylindrospermopsin between pH 6.0 and 8.0, and saxitoxins at pH values at 9 and higher (Westrick 2008).

**Symptoms of Cylindrospermopsis Exposure**

Cylindrospermopsis causes extensive damage to the liver and kidney and is a potent inhibitor of protein synthesis (Terao et al. 1994; Falconer et al. 1999; Froscio et al. 2003). Symptoms of exposure to cylindrospermopsis include nausea, vomiting, diarrhea, abdominal tenderness, pain, and acute liver failure. Clinical symptoms after exposure to cylindrospermopsis may not show up immediately but may occur several days later. Thus it is often difficult to determine a cause–effect relationship between cylindrospermopsis exposure and symptoms.

The first report of animal poisonings attributed to cylindrospermopsis was by Saker et al. (1999) in drinking water in a farm pond in Queensland, Australia, where it was responsible for cattle deaths. However, *Cylindrospermopsis raciborskii* was implicated in one of the most significant cases of human poisoning from exposure to a cyanobacterial toxin in 1979. The local water supply on Palm Island, northern Queensland, Australia, had been dosed with copper sulfate to control a dense algal bloom (Byth 1980, Bourke et al. 1983). The copper sulfate caused cyanobacteria cells to break apart and resulted in the release of cyanotoxins (Hawkins et al. 1985). The outbreak was called “Palm Island Mystery Disease” before the cause was identified as cylindrospermopsis. One hundred and forty-eight people required hospitalization with symptoms that included vomiting, malaise, headache and constipation, later followed by bloody diarrhea. Blood and urine analysis revealed evidence of liver and kidney damage. There were no human fatalities associated with this outbreak.

The degree of the cyanotoxin impact for cylindrospermopsis and other cyanotoxins is influenced by animal size, species sensitivity, and individual sensitivity. According to the Merck Veterinary Manual, animals may need to ingest only a few ounces or up to several gallons to experience acute or lethal toxicity, depending on bloom densities and toxin content (Merck Veterinary Manual 2008: [http://www.merckveterinarymanual.com/mvm/index.jsp?cfile=htm/bc/210200.htm](http://www.merckveterinarymanual.com/mvm/index.jsp?cfile=htm/bc/210200.htm)). After removal from the contaminated water supply, affected animals should be placed in a protected area out of direct sunlight. The animal should have access to an unrestricted supply of clean water and good quality feed. Surviving animals have a good chance for recovery because both hepatotoxins and neurotoxins have a steep dose-response curve. Although no therapeutic antagonist has been found to be effective against cylindrospermopsis, activated charcoal oral slurry is likely to benefit exposed animals. The Merck web link states that an ion-exchange resin such as cholestyramine has proved useful to absorb the toxins from the gastrointestinal tract.

**Saxitoxin**

Saxitoxins are among the most potent natural toxins known (Arãoz et al. 2010). More than 30 different saxitoxin analogues have been identified, including pure saxitoxin (STX), neosaxitoxin (neoSTX), the gonyautoxins (GTX) and decarbamoylsaxitoxin (dc-STX) of which STX,
NeoSTX, GTX1 and dc-STX seem to be the most toxic. The term saxitoxin often refers to the entire suite of related neurotoxins produced by cyanobacteria.

This suite of closely related tetrahydropurines (saxitoxins-STX) is also described as a group of carbamate alkaloid toxins which are either nonsulfated (STXs), singly sulfated (gonyautoxins, GTSx), or doubly sulfated (C-toxins) (van Apeldoorn et al. 2007). Chemically, saxitoxin is stable and readily soluble in water, although it can be inactivated by treatment with a strong alkali. The half-lives for breakdown of a range of different saxitoxins in natural water have been shown to vary from 9 to 28 days, and gonyautoxins may persist in the environment for more than 3 months (Jones and Negri 1997). The toxicological database for STX-group toxins is limited and is comprised primarily of studies on acute toxicity following intraperitoneal (i.p.) administration. For monitoring purposes, toxicity equivalency factors (TEFs) have been applied to express the detected analogues (using HPLC) as STX equivalents (STX-eq). Until better information is available, the Scientific Panel on Contaminants in the Food Chain (EFSA 2009) proposes the following TEFs based on acute i.p. toxicity in mice: STX = 1, NeoSTX = 1, GTX1 = 1, GTX2 = 0.4, GTX3 = 0.6, GTX4 = 0.7, GTX5 = 0.1, GTX6 = 0.1, C2 = 0.1, C4 = 0.1, dc-STX = 1, dc-NeoSTX = 0.4, dc GTX2 = 0.2, GTX3 = 0.4, and 11-hydroxy-STX = 0.3. At present, monitoring freshwater systems using STX-eq/L gives us faster information to manage recreational areas in a timely manner, particularly since blooms are so dynamic.

Saxitoxins have been observed in numerous lakes around the world. Cyanobacteria genera that are documented as producing saxitoxin include Aphanizomenon sp. (U.S.); Aphanizomenon gracile, Aphanizomenon issatschenkoi, and Aphanizomenon flos-aqua (Europe); Anabaena circinalis (Australia); Anabaena lemmermannii (Denmark); Lyngbya wollei (U.S.); Cylindrospermopsis (Brazil); and Planktothrix (Italy) (Lagos et al. 1999, Pomati et al. 2000, Castro et al. 2004, van Apeldoorn et al. 2007). Wood et al. (2006) detected low levels of saxitoxins in 38 different New Zealand water bodies showing that saxitoxins may be more prevalent in New Zealand than previously assumed.

Low levels of saxitoxin were detected in water samples from a cyanobacterial bloom (predominantly Anabaena planktonica) in the Waikato River, New Zealand, in 2003. The bloom caused taste and odor problems in drinking water supplied to the city of Hamilton and other towns along the river (Kouzminov et al 2007). Clemente et al. (2010) studied cyanobacterial blooms at the Alagados Reservoir, Brazil, and found concentrations of 0.00515, 0.04384, and 0.05078 µg SXT-eq/L (see above) in water during the spring, summer, and autumn, respectively. Rapala et al. (2005) studied saxitoxin in bloom samples from freshwater sites in Finland in 2002 and 2003. Saxitoxin (STX) was the only analogue in the samples and was present in concentrations as high as 1,000 µg/L. The dominant species producing the toxin was Anabaena lemmermannii. In some instances, samples were collected from sites where swimmers had reported adverse health effects showing symptoms of fever, eye irritation, abdominal pains, and skin rash. Although no evidence of human intoxication from drinking water contaminated by saxitoxin has been reported, saxitoxin could represent a source of concern for acute effects due to their occurrence in freshwaters up to 2,700 µg/L (Batorèu et al. 2005).

Saxitoxins are produced by various cyanobacteria species but are more commonly produced by dinoflagellates in marine waters causing paralytic shellfish poisoning (PSP). Exposure to
Saxitoxin typically comes from eating shellfish contaminated by "red tides" or algal blooms of *Alexandrium catenella* (formerly *Gonyaulax catenella*) or *A. tamarense-excavatum* (formerly *G. tamarensis*). Detection of high concentrations of saxitoxin in shellfish such as mussels, clams and scallops frequently leads to closures of commercial and recreational shellfish harvesting, especially in California, Oregon, Washington, and New England. In freshwater, saxitoxins accumulate in *Daphnia magna*, freshwater bivalves including *Elliptio camoplanatus*, *Corbicula fluminea*, *Alathyria cygnea*, and the Australian mussel species *Alyathruia condola* (van Apeldoorn et al. 2007).

**Symptoms of Saxitoxin Exposure**

Saxitoxin is a potent nerve toxin that binds to the sodium channel of nerve and muscle tissues. This prevents propagation of action potentials in excitable cells, ultimately causing blockade of depolarization at the neuromuscular junction. Basically, saxitoxin prevents the passage of sodium ions through the cell membrane which blocks passage of the nerve impulse. Intoxication with saxitoxin can be a severe, life-threatening illness requiring immediate medical care (Kao 1993, Meyer 1953, Narahashi 1972, Rodrigue et al. 1990, WHO 1999, WHO 2003).

Saxitoxins are toxic by ingestion and by inhalation, with inhalation leading to rapid respiratory collapse and death. This group of toxins acts on the sodium channels of nerve cells, preventing normal cellular function and leading to paralysis. Age at exposure appears to influence susceptibility to saxitoxin in animal studies. Adult rats were about 10 times less sensitive to intraperitoneal exposure than young rats. In addition, impacts were lower for the route than the oral route of exposure (Wiberg and Stephenson 1960, Watts et al. 1966). Intoxication with saxitoxin can be a severe, life-threatening illness requiring immediate medical care.

Most information on saxitoxin symptoms comes from exposure through consumption of shellfish. Within minutes of eating toxic shellfish, a person would initially develop tingling of the lips and tongue. However, it can take up to an hour or two to develop tingling, depending on the dose and on the individual. Symptoms may progress to tingling of fingers and toes, followed by numbness and weakness with loss of control of arms and legs, developing into difficulty in breathing. Some people feel nauseous or experience a sense of floating after saxitoxin exposure. If a person consumes enough saxitoxin, muscles of the chest and abdomen become paralyzed, including muscles used for breathing, and the victim can suffocate. Terminal stages of saxitoxin poisoning can occur 2-12 hours after exposure, and death from PSP has occurred in less than 30 minutes (http://www.bt.cdc.gov/agent/saxitoxin/casedef.asp).

Diagnosis of saxitoxin poisoning is confirmed by detection of toxin in the food, water, stomach contents, or environmental samples. Artificial respiration should be used to support breathing. When such support is applied within 12 hours of exposure, recovery usually is complete, with no lasting side effects (Fleming 2005, Halstead 1988, Kao 1993, Merck Veterinary Manual 2008). Stomach evacuation by the emergency department can be conducted if exposure is through ingestion. No antidote against saxitoxin exposure has been developed for human use.
Exposure Pathways

The most likely exposure pathways to cylindrospermopsin and saxitoxin in freshwater are through recreational contact and contaminated drinking water. Long-term chronic ingestion via drinking water and exposure through consumption of fish and shellfish are not considered in the following exposure scenario. This effort focuses on recreational exposure, which includes activities such as swimming, wind surfing, jet skiing, and water skiing. For this assessment, DOH assumes that a swimmer or other lake user ingests 0.05 liters of water per hour and that exposure lasts for two hours per day per year.

Cylindrospermopsin Risk Levels and Standards

Other Countries’ Guidelines

There are no published recreational guidance values for cylindrospermopsin. However, there are a few countries that have adopted interim drinking water guidance values for cylindrospermopsin as listed below (Table 2).

| Table 2. Cylindrospermopsin guidelines from other countries. |
|-----------------------------------------------|-----------------|---------------------------------|
| Country                        | Value           | Comment                          |
| Brazil                         | 15 μg/L         | Recommended Drinking Water Standard |
| Australia – Queensland         | 1.0 μg/L        | Interim Drinking Water Guidance Level |
| New Zealand                    | 1.0 μg/L        | Drinking Water Maximum Acceptable Value |

Brazil. Brazil currently has the most comprehensive federal legislation for various cyanotoxins. Legislation includes a “recommendation” drinking water standard of 15 μg/L for cylindrospermopsin (Burch 2008).

Australia. Australian Drinking Water Guidelines currently do not provide values for cylindrospermopsin due to the lack of adequate data (NH&MRC/ARMCANZ 1996). However, the Department of Natural Resources and Water (NRW) of Queensland recommends that a conservative approach be adopted using a value of 1.0 μg/L for cylindrospermopsin and saxitoxin as an interim drinking water guidance level until sufficient data have been collected to allow for the development of individual guidelines (http://www.derm.qld.gov.au/water/monitoring/pdf/monitoring_standard.pdf).

New Zealand. New Zealand applies maximum acceptable values (MAVs) for microorganisms and contaminants of health significance. A provisional drinking water MAV has been recommended for cylindrospermopsin using a Tolerable Daily Intake (TDI). MAVs are used in New Zealand compliance monitoring for drinking water (Kouzminov 2007). New Zealand has established a standard of 1.0 μg/L for cylindrospermopsin (Burch 2008).

USA. The U.S. uses Maximum Contaminant Levels (MCLs) as the highest level of a contaminant that is allowed in drinking water. Contaminants on the contaminant candidate list
(CCL) are a priority for the Environmental Protection Agency (EPA) to set MCLs (http://www.epa.gov/safewater/mcl.html). Cyanobacteria and their toxins are listed as microbiological contaminants on the CCL and are recognized as unregulated contaminants known to occur in public water systems. As such, they may require regulation under the Safe Drinking Water Act. No U.S. recreational guidance value is available for cyanotoxins in freshwater.

**Washington Recreational Guidance Values: Cylindrospermopsin**

For Washington, DOH recommends the use of a recreational guidance value for cylindrospermopsin (provisional) calculated as follows:

\[
\text{Guidance value (µg/L)} = \frac{\text{Subchronic RfD x BW}}{\text{IR}}
\]

where:

- Subchronic RfD = 0.03 µg /kg-day
- BW = 15 kg child
- IR = 0.05 L/h, assuming 2 h/d.

The resulting recreational guidance value using the subchronic RfD is **4.5 µg/L**. This limit accounts for chronic exposure to cylindrospermopsin, including daily swimming and incidental ingestion. The cylindrospermopsin guidance value is considered provisional and is recommended for use in DOH’s lake management protocol. This recommendation has been reviewed and accepted by DOH’s Scientific Advisory Committee.

The subchronic oral reference dose (RfD) used in the above equation was developed by EPA based on a Bench Mark Dose Lower confidence limit (BMDL) (statistical lower confidence limit on the benchmark dose) of 33.1 µg/kg-day for increased relative kidney weight in mice (EPA 2006). The BMDL was divided by a composite uncertainty factor (UF) of 1,000, resulting in a subchronic RfD for cylindrospermopsin of 0.03 µg/kg-day. The No Observed Adverse Effect Level (NOAEL) for increased kidney weight is 30 µg/kg-day (mouse study). Use of the NOAEL/Lowest Observed Adverse Effect Level (LOAEL) approach and an UF of 1,000 would also result in an RfD of 0.03 µg/kg-day since the NOAEL (30 µg/kg-day) and BMDL (33.1 µg/kg-day) for increased kidney weight are similar. An earlier review of cylindrospermopsin toxicity literature used results from a short-term oral toxicity study using a pure cylindrospermopsin compound to derive a subchronic tolerable daily intake (TDI) (NIEHS 2000). EPA (2006) concluded that the mouse and rat studies (14-day gavage and 21-day drinking water studies) were inadequately reported for use in deriving a short-term RfD.

DOH’s recommended recreational guidance value is provisional and may be updated as new information becomes available or if federal or international guidelines are developed. EPA (2006) concludes that at this time, no studies have been performed to assess the acute oral toxicity of pure cylindrospermopsin; short-term oral toxicity data using pure cylindrospermopsin
are inadequately reported; and no chronic, reproductive, developmental, or carcinogenicity studies of pure cylindrospermopsin have been conducted.

Some counties in Washington use cell densities to monitor lakes. DOH considered using cell counts in a state management approach but did not include cell densities to trigger an advisory because Ecology does not fund cell counts, only toxicity tests. Further, results to date from Washington’s monitoring efforts on 30 Puget Sound lowland lakes do not show a useful relationship between cell counts and toxin concentrations.

**Saxitoxin Risk Levels and Standards**

**Other Countries’ Guidelines**

As with cylindrospermopsin, no country has published a recreational guidance value for this cyanotoxin. Below is a list of the countries that have adopted interim drinking water guidance values for saxitoxin (Table 3).

<table>
<thead>
<tr>
<th>Country</th>
<th>Value</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brazil</td>
<td>3.0 μg/L</td>
<td>Recommended Drinking Water Standard</td>
</tr>
<tr>
<td>Australia</td>
<td>3.0 μg STX-eq/L</td>
<td>“Health Alert” for Acute Drinking Water Exposure</td>
</tr>
<tr>
<td>Australia - Queensland</td>
<td>1.0 μg/L</td>
<td>Interim Drinking Water Guidance Level</td>
</tr>
<tr>
<td>New Zealand</td>
<td>1.0 μg STX-eq/L</td>
<td>Drinking Water Maximum Acceptable Value</td>
</tr>
</tbody>
</table>

**Brazil.** Brazil currently has the most comprehensive federal legislation for drinking water guidance values for various cyanotoxins. Brazil’s legislation includes a “recommendation” standard of 3.0 μg/L for saxitoxin (Burch 2008, Costa et al. 2006).

**Australia.** Australian Drinking Water Guidelines currently do not have a recommended guideline for saxitoxin (NH&MRC/ARMCANZ 1996). However, Australia issues a “Health Alert” for acute drinking water exposure of 3.0 μg STX-eq/L. Further, the NRW of Queensland recommends that a conservative approach be adopted until sufficient data have been collected to allow for the development of individual guidelines ([http://www.derm.qld.gov.au/water/monitoring/pdf/monitoring_standard.pdf](http://www.derm.qld.gov.au/water/monitoring/pdf/monitoring_standard.pdf)). Queensland uses a value of 1.0 μg/L for cylindrospermopsin and saxitoxin as interim drinking water guidance levels.

**New Zealand.** New Zealand applies MAVs for microorganisms of health significance. A provisional drinking water MAV has been recommended for saxitoxin using a (TDI). MAVs are used in New Zealand compliance monitoring for drinking water (Kouzminov 2007). New Zealand has established a recommended provisional saxitoxin MAV (as STX-eq) of 3.0 μg/L (Burch 2008).
**Shellfish Saxitoxin Standards.** Other regulatory action for saxitoxin involves setting a maximum tolerable value in shellfish tissue. In the U.S. and Canada, the maximum tolerable value of saxitoxin is 0.8 mg/kg of mollusk meat. The European regulatory limit is 800 μg/STX eq/kg shellfish meat, the same action level as in the U.S. and Canada. The regulatory limit for saxitoxin was established in the 1930s and is based on bioassays measuring toxic activity in mice. ([http://www.thefreelibrary.com/The+origin+of+the+regulatory+limits+for+PSP+and+ASP+toxins+in...-a0130777682](http://www.thefreelibrary.com/The+origin+of+the+regulatory+limits+for+PSP+and+ASP+toxins+in...-a0130777682)).

**Washington Recreational Guidance Values: Saxitoxin**

DOH considered several options on which to base a recreational guidance value (provisional) for saxitoxin in Washington. Options 1 and 2 differ in the assumed acute reference dose used in each calculation. Option 3 is based on direct human exposure data, and Option 4 is calculated using Europe’s and U.S.’s shellfish tissue regulatory maximum tolerable value for saxitoxin. DOH recommends adoption of Option 1, as described below.

The first option uses an acute reference dose (acute RfD) developed by the European Food Safety Association (EFSA) based on acute toxicity of STX-equivalent intoxications in humans (>500 individuals) (EFSA 2009). The resulting guidance value is calculated as follows:

\[
\text{Guidance value (μg/L)} = \frac{\text{Acute RfD x BW,}}{\text{IR}}
\]

where:

- Acute RfD = 0.5 μg STX-eq/kg-day
- BW = 15 kg child
- IR = 0.05 L/h, assuming 2 h/d.

The (provisional) recreational guidance value using EFSA’s acute RfD is 75 μg/L. This freshwater guidance value accounts for daily swimming and incidental ingestion. For comparison purposes with Australia’s drinking water guidance value (3 μg STX-eq/L), a child’s drinking water guidance value using the above assumed weight and acute RfD but assuming an IR of 1 L (instead of the recreational assumption of 0.05 L/h for 2 h/d) would be 7.5 μg/L and an adult drinking water guidance value would be 3.7 μg/L (assuming IR = 2 L).

The acute reference dose used in the above equation was developed by a Panel on Contaminants in the Food Chain (CONTAM Panel) to answer questions about marine biotoxins in shellfish in Europe (EFSA 2009). No data on the chronic effects of STX-group toxins in animals or humans were available, so the CONTAM Panel could not establish a tolerable daily intake (TDI). In view of the acute toxicity of STX-group toxins, the CONTAM Panel decided to establish an acute reference dose (ARfD). The Panel determined that a lowest observable adverse effect level (LOAEL) in the region of 1.5 μg STX equivalents/kg BW could be established; however, many individuals in the data set (>500 individuals) did not suffer adverse reactions at higher intakes. Thus, the CONTAM Panel concluded that the LOAEL is close to the threshold for effects in sensitive individuals and determined that a factor of 3 was sufficient to estimate a NOAEL of 0.5
μg STX equivalents/kg BW. The Panel did not use an additional factor for variation among humans since the data included a large number of affected consumers, including sensitive individuals. This limit was developed to account for acute exposure to saxitoxin through consumption of shellfish meat.

A similar process was used by a Joint FAO/IOC/WHO ad hoc Expert Consultation on Biotoxins in Bivalve Molluscs to determine a provisional LOAEL of 2.0 μg/kg BW STX equivalents (Report of the Joint FAO/IOC/WHO 2004). The Expert Consultation established a provisional acute reference dose of 0.7 μg STX equivalents/kg BW, based on the LOAEL of 2 μg STX equivalents/kg BW (humans) and a safety factor of 3. The Expert Consultation used a safety factor of 3 “because documentation of human cases includes a wide spectrum of people (occupation, age, and sex) and mild illness is readily reversible.”

A guidance value based on the ad hoc Expert Consultation’s acute reference dose is calculated as follows:

\[
\text{Guidance value (μg/L)} = \frac{\text{Acute RfD} \times \text{BW}}{\text{IR}}
\]

where:

- Acute RfD = 0.7 μg STX-eq/kg-day
- BW = 15 kg child
- IR = 0.05 L/h, assuming 2 h/d.

The resulting (provisional) recreational guidance value (protective of a child) using the Expert Consultation’s recommended acute RfD would be 105 μg/L, Option 2. For comparison with drinking water guidance from other countries, a drinking water guidance value calculated using an acute RfD of 0.7 μg STX-eq/kg-day for a 15 kg child who ingests 1 L would be 10.5 μg/L.

A different process was used by South Australian authorities to calculate a drinking water guidance value (Option 3). They recognized that guidance for freshwater cyanobacteria related to drinking water needs to consider acute health effects and, in the case of saxitoxin, used food data to determine a guideline value for the drinking water pathway. Based on human exposure data, Fitzgerald et al. (1999) chose a dose of 124 μg saxitoxin as the LOAEL. For an average daily water consumption of 2 L for an adult and assuming 60 kg for the case weight and 70 kg for the average Australian adult body weight (with the proportion ascribed to water = 0.5 % and using an uncertainty factor of 10):

\[
\text{Health alert} = \frac{(124 \, \mu g/60 \, kg \times 70 \, kg \times 0.5)}{(10 \times 2L) \, \mu g/L} = 3.6 \, \mu g/L, \text{rounded down to 3 } \mu g/L.
\]

The South Australian health alert value of 3 μg STX-eq/L of drinking water was calculated for acute exposure associated with occurrence of intermittent blooms of cyanobacteria (Fitzgerald et al. 1999). The health alert value of 3 μg STX-eq/L of drinking water would require cell densities exceeding 20,000 cells/mL (Anabaena circinalis) based on Australian monitoring data. Water
associated with cell densities of this magnitude would normally smell and taste bad, with the threshold for off tastes in water being 1,000–2,000 cells/mL.

South Australia’s resulting health alert value for drinking water (3 µg STX-eq/L) is much lower than the recreational values calculated above due to the higher amount of water ingested for the drinking water exposure assumption. When a higher amount of ingested water is assumed along with other assumptions for the first two options, values in the same range result. (Option 1 using an acute RfD of 0.5 µg STX-eq/kg-day = 7.5 µg/L – child ingesting 1 L and 3.7 µg/L – adult drinking 2 L) (Option 2 using an acute RfD = 0.7 µg STX-eq/kg-day = 10.5 µg/L – child drinking 1 L and 5.3 µg/L – adult drinking 2 L).

A fourth way to determine a freshwater recreational guidance value for saxitoxin is to use equivalents based on the maximum tolerable value in shellfish tissue. The U.S. regulatory limit for saxitoxin equivalents is based on mouse bioassays conducted in the 1930s, resulting in 80 µg STX-eq/100 g shellfish meat which is equivalent to 0.8 ppm (800 µg STX-eq/kg in Europe, or 0.8 mg/kg = 0.8 mg/L, or 800 µg STX-eq/L). This value is much higher than the three other potential guidance values above. While 80 µg STX-eq/100g shellfish meat has proven to be protective via the ingestion route, it is not known whether the equivalent concentration of 800 µg/L for recreational ingestion would be protective of human health.

Table 4. Four options considered for use as saxitoxin recreational guidance value.

<table>
<thead>
<tr>
<th>Option</th>
<th>Basis</th>
<th>Resulting Saxitoxin Guidance Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acute RfD, based on STX-eq intoxications in &gt;500 humans</td>
<td>75 µg/L</td>
<td>EFSA 2009</td>
</tr>
<tr>
<td>2</td>
<td>Acute RfD of STX-eq, based on LOAEL of 2 ug STX eq/kg BW and safety factor of 3</td>
<td>105 µg/L</td>
<td>Report of the Joint FAO/IOC/WHO 2004</td>
</tr>
<tr>
<td>3</td>
<td>South Australian drinking water guidance value, based on LOAEL of 124 ug STX-eq, calculated for acute exposure</td>
<td>3 µg/L</td>
<td>Fitzgerald et al. 1999</td>
</tr>
<tr>
<td>4</td>
<td>Equivalents based on Maximum Tolerable Value in shellfish</td>
<td>800 µg/L</td>
<td><a href="http://www.thefreelibrary.com/The+origin+of+the+regulatory+limits+for+PSP+and+ASP+toxins+in+-a0130777682">http://www.thefreelibrary.com/The+origin+of+the+regulatory+limits+for+PSP+and+ASP+toxins+in+-a0130777682</a></td>
</tr>
</tbody>
</table>

DOH recommends Option 1 as a recreational guidance value primarily because it is the most protective (lower) of the four options that is calculated for the recreational scenario (not calculated for drinking water as in Option 3) (Table 4). Options 1 and 2 use similar calculations but different acute RFDs based on human intoxication data. While Option 3 resulted in a lower recreational guidance value, it was calculated for a drinking water exposure scenario and is therefore not recommended for our use. Option 4 is not recommended since it is unknown whether the resulting guidance value is protective of those exposed through aquatic recreational activities.
Washington Lakes: Three-Tiered Approach to Managing Lakes with Cyanobacterial Blooms

The following framework for managing toxic or potentially toxic cyanobacterial blooms uses a three-tiered approach. The framework applies the recreational guidance values derived above (6 µg/L microcystins, 1 µg/L anatoxin-a, 4.5 µg/L cylindrospermopsin, and 75 µg/L saxitoxin) and is recommended for managing Washington lakes (Figure 1).

A unique feature of the Washington approach to cyanobacterial blooms in lakes is that bloom samples from all water bodies are eligible for toxicity testing. DOH and Ecology have incorporated outreach and educational efforts to encourage local health jurisdictions (LHJs), other agencies, lake residents, and the general public to notify Ecology or LHJ when a potential bloom is observed. The reported incidence of blooms may be associated more with the circumstance of observation than any other single factor.

Observers should look for developing blooms and surface accumulations which can occur in any nutrient-rich water such as lakes, ponds, or river embayments. Although conditions needed for a bloom to form or produce toxin are complex and uncertain, elevated nutrients such as nitrogen and phosphorous, pH 6-9, and favorable temperatures support cyanobacteria growth. Weather conditions can influence the area of biomass accumulation. For example, intense rain or heavy wind conditions mix water so that surface accumulations may not be evident. Also, a steady, light wind may push cyanobacteria toward a given shore and cause biomass accumulations. Because scum formations and blooms are extremely transitory in nature and highly influenced by wind patterns, it is challenging to quantify toxin levels from one or two samples unless a scum covers the entire lake.

Upon notification of a potential bloom, the LHJ or other agency staff (or lake resident) will obtain a sample number from Ecology, sample the water body experiencing the bloom, then send the sample to the laboratory for toxicity tests. Sampling and shipping directions are available from Ecology’s Freshwater Algae Control Program: http://www.ecy.wa.gov/programs/wq/plants/algae/monitoring/index.html.

At present the King County Environmental Laboratory (KCEL) is under contract with Ecology to test for microcystins, anatoxin-a, cylindrospermopsin, and saxitoxin. Results of toxicity analyses will be incorporated into Ecology’s database and accessible via Ecology’s website.
Figure 1. Three-tiered approach to managing Washington water bodies with cyanobacterial blooms.

Bloom forming? Bloom or scum visible?

No

No Action

Yes

TIER I:
Local Health posts CAUTION sign
Samples taken and sent for toxicity tests
Weekly sampling until bloom dissipates

Bloom dissipates
Remove Sign

Microcystin Level ≥ 6 μg/L
and/or Anatoxin-a ≥ 1 μg/L
and/or Cylindrospermopsin ≥ 4.5 μg/L
and/or Saxitoxin > 75 μg/L

No

Yes

TIER II:
Local Health posts WARNING sign
Local Health takes additional site-specific steps
Minimum weekly sampling

- History of high toxicity, or
- Reports of illness, pet death

Return to
TIER II at LHJ discretion

Microcystin < 6 μg/L
and/or Anatoxin-a < 1 μg/L
and/or Cylindrospermopsin < 4.5 μg/L
and/or Saxitoxin < 75 μg/L
for at least one week (e.g., two consecutive weekly samples)

TIER III:
Local Health Posts DANGER sign
Lake Closed
Tier I

A sample of a visible cyanobacteria bloom or scum is sent for phytoplankton examination and toxicity testing. Results are sent to the appropriate LHJ, to the agency that sent in the sample, and posted to Ecology’s Freshwater Algae Program list serve. If the sample is dominated by potentially toxic cyanobacteria, the LHJ should post a CAUTION sign (Figures 1, A1). A CAUTION advisory is intended to provide the public with information that a public health hazard might exist. In Washington, local jurisdictions have the authority to post advisories on water bodies within their districts (RCW 70.05.070).

If the bloom dissipates, the LHJ should remove the sign. If the bloom remains but microcystin levels are below 6 µg/L, anatoxin-a levels are below 1 µg/L, cylindrospermopsin levels are below 4.5 µg/L, and/or saxitoxin levels are below 75 µg/L, the CAUTION sign should remain posted. If the sample contains microcystin values equal to or greater than 6 µg/L, anatoxin-a values equal to or greater than 1 µg/L, cylindrospermopsin levels equal to or greater than 4.5 µg/L, and/or saxitoxin levels equal to or greater than 75 µg/L, move to Tier II.

Given the tremendous spatial and temporal variability in toxin concentrations, LHJs are encouraged to factor in the spatial extent of the bloom when deciding if a warning level or closed level advisory is warranted. As long as people comply with the CAUTION advisory and avoid areas with a moderate or heavier density of cyanobacteria, they should not be subject to any ill effects from cyanotoxins.

Tier II

If microcystin levels are 6 µg/L or higher, anatoxin-a levels are 1 µg/L or higher, cylindrospermopsin levels are 4.5 µg/L or higher, and/or saxitoxin levels are 75 µg/L or higher, the LHJ should post a WARNING sign (Figures 1, A2). The lake should be sampled weekly, at a minimum, with the WARNING sign posted as long as microcystin concentrations are 6 µg/L or higher, anatoxin-a levels are 1 µg/L or higher, cylindrospermopsin levels are 4.5 µg/L or higher, and/or saxitoxin levels are 75 µg/L or higher. DOH recommends that LHJs wait one week once microcystin levels fall below 6 µg/L, anatoxin-a levels fall below 1 µg/L, cylindrospermopsin levels fall below 4.5 µg/L, and/or saxitoxin levels fall below 75 µg/L before retracting an advisory.

Toxin levels may be at their highest during bloom die-offs even though the water looks “normal.” Another consideration is that toxin levels may be significantly lower due to temporary changes in weather conditions rather than changes in the cyanobacteria population. Heavy wind and/or intense rainfall can redistribute cyanobacteria throughout the lake and throughout the water column with little change in the total number of cyanobacteria cells. This makes it difficult to assess whether a bloom is declining or not. Therefore, DOH recommends that LHJs do not lift advisories unless they check the lake under weather conditions that are conducive with biomass accumulation (relatively calm or a light steady wind and little or no rainfall).
At this point, the LHJ might want to take additional steps in communicating risk, depending on severity of the bloom and historical use of the lake (i.e., a highly used access point such as a dog park might warrant greater outreach efforts as compared with a lake not known for any recreational activity). Time of year is another factor to consider since there is usually much less human recreational activity in Washington lakes in the winter due to lower temperatures. One possible risk communication tool is a press release by the LHJ. Another would be to notify local veterinarians and fish and wildlife officials so that they may issue information regarding the bloom. In certain situations, some LHJs have mailed notifications to local lakefront residents after confirmation of cyanobacterial toxicity. Other possible measures used to reach lakefront residents include radio messages or the internet via a list serve or “blast” email. DOH anticipates that successful LHJ outreach efforts may be duplicated in other counties as results of successful efforts become known.

If a lake has a history of high toxicity, if toxin concentrations are extremely high, or if pet illnesses/death or human symptoms are reported, move to Tier III. Implementation of Tier III is based on judgment of the LHJ and local knowledge of the water body.

**Tier III**

Under certain circumstances, a LHJ may wish to close a lake with unusually high microcystin, anatoxin-a, cylindrospermopsin, or saxitoxin concentrations. At the discretion of the LHJ, a water body can be posted as DANGER – Closed (Figures 1, A3). Examples include:

- Very dense blooms covering an entire lake
- Confirmed pet illnesses or death
- Reported human illness

The LHJ should post a press release to notify the general public of a lake closure. Also, LHJs should follow whatever additional methods of outreach, including those listed under Tier II, that will best inform public beach users and lake front residents of the risks from cyanotoxins and how to avoid these risks.

Retraction of a lake closure is also at the discretion of the LHJ. DOH recommends posting a WARNING sign and following Tier II recommendations after retracting a lake closure until microcystin levels are less than 6 µg/L, anatoxin-a levels are less than 1 µg/L, cylindrospermopsin levels are less than 4.5 µg/L, and/or saxitoxin levels are less than 75 µg/L (Figure 1).

Ecology’s Freshwater Algae Control Program will add microcystin, anatoxin-a, cylindrospermopsin, and saxitoxin results to their website database as results are received from the laboratory. For Tier II and Tier III, actions taken by the LHJ such as posting or closing a lake will be published on the website and posted on Ecology’s list serve.
Risk Perspective

Recognizing that local health jurisdictions may not have sufficient funds to enable the level of effort described in these guidelines, DOH would like to provide some perspective on the relative public health importance of cyanobacteria monitoring activities though it is beyond the scope of this document to compare or rank a relative risk of environmental contaminant exposures such as cyanotoxins, PCBs, lead, or mercury. We know that exposure to cyanobacterial toxins may cause symptoms ranging from skin irritation to gastrointestinal upset to neurological problems to death. In the recent past, toxic blooms in Washington lakes have led to the death of small and large animals. DOH is concerned about potential impacts on humans and pets after short-term exposure to the nerve toxins and on impacts to humans and pets from long-term exposure to the liver toxins.

While potential impacts to public health from cyanotoxins are high, associated costs for sampling and toxicity tests are low. Ecology has provided financial assistance to cover the expense of microcystin, anatoxin-a, cylindrospermopsin, and saxitoxin toxicity testing through the Freshwater Algae Control Program. In summary, LHJ costs would include staff time to sample a potential toxic bloom, cost of sample jars for non-microcystin sampling, and cost of mailing sample kits to the laboratory.

Summary

DOH offers freshwater recreational guideline values for microcystin, anatoxin-a, cylindrospermopsin, and saxitoxin (Table 5). This document provides background information, toxicity reviews, and guideline development for cylindrospermopsin and saxitoxin.

| Table 5. Washington State provisional recreational guidance for freshwater cyanotoxins. |
|-----------------------------------------------|-----------------------------------------------|
| Toxin                                         | Recreational Guidance Value                   |
| Microcystins                                  | 6 µg/L                                        |
| Anatoxin-a                                    | 1 µg/L                                        |
| Cylindrospermopsin                            | 4.5 µg/L                                      |
| Saxitoxin                                     | 75 µg/L                                       |

DOH recommends a (provisional) recreational guidance value of 4.5 µg/L cylindrospermopsin, based on a subchronic RfD. This recommended recreational guidance value is used in a three-tiered framework for LHJs or the appropriate local agency to follow in the event of a toxic bloom.

DOH recommends a provisional recreational guidance value 75 µg/L saxitoxin. This recommendation uses an acute reference dose (acuteRfD) developed by the European Food Safety Association (EFSA 2009) based on acute toxicity of STX-equivalent intoxications in humans (>500 individuals). The (provisional) recreational guidance value of 75 µg/L saxitoxin is also incorporated into a three-tiered lake management framework.
When a cyanobacterial bloom is developing or a bloom or scum is observed, LHJs may post CAUTION signs while samples are being tested for toxicity (Tier I). The CAUTION signs remain posted until the bloom dissipates (if microcystin levels remain below 6 µg/L, anatoxin-a levels remain below 1 µg/L, cylindrospermopsin levels remain below 4.5 µg/L, and/or saxitoxin levels remain below 75 µg/L).

If cyanotoxin levels are above those shown in Table 5, the LHJ posts WARNING signs until levels fall below guidance values for at least one week (Tier II). LHJs may conduct additional outreach efforts as needed. Under rare circumstances, a LHJ may need to post DANGER signs (Lake Closed), which revert to WARNING signs at the LHJ’s discretion.

Ecology and DOH personnel are available to discuss results of lake testing and consult with LHJs during their decision-making process. Ecology will update and maintain lake toxicity data on its Freshwater Algae Control Program website and list serve. Finally, each agency has additional information on freshwater algae and toxicity issues available on its website as a public education and risk communication resource.
References


Appendix A

Caution, Warning, and Danger Signs: A Three-Tiered Approach
Figure A1. Caution sign for use in Tier 1.

CAUTION
TOXIC ALGAE MAY BE PRESENT
Lake may be unsafe for people and pets

Until further notice:

• Do not swim or water ski in areas of scum.
  No nade o practique el esquí acuático en áreas con espuma o verdín.

• Do not drink lake water.
  No tome el agua del lago.

• Keep pets and livestock away.
  Mantenga alejados las mascotas y el ganado.

• Clean fish well and discard guts.
  Limpie bien el pescado y deseche las tripas.

• Avoid areas of scum when boating.
  Evite las áreas con espuma o verdín cuando ande en lancha.

Call your doctor or veterinarian if you or your animals have sudden or unexplained sickness or signs of poisoning.

Report new algae blooms to Department of Ecology:
360-407-6000

Call your local health department:

For more information: www.deh.wa.gov/ehp/algae/

Health
May 2005
Figure A2. Warning sign for use in Tier II.

**WARNING**

**TOXIC ALGAE PRESENT**

Lake unsafe for people and pets

Until further notice:

- Do not swim or water ski.  
  No nade o practique el esquí acuático.
- Do not drink lake water.  
  No tome el agua del lago.
- Keep pets and livestock away.  
  Mantenga alejados las mascotas y el ganado.
- Clean fish well and discard guts.  
  Limpie bien el pescado y deseche las tripas.
- Avoid areas of scum when boating.  
  Evite las áreas con espuma o verdín cuando ande en lancha.

Call your doctor or veterinarian if you or your animals have sudden or unexplained sickness or signs of poisoning.

<table>
<thead>
<tr>
<th>Report new algae blooms to Department of Ecology:</th>
<th>Call your local health department:</th>
</tr>
</thead>
<tbody>
<tr>
<td>360-407-6000</td>
<td></td>
</tr>
</tbody>
</table>

For more information:  
www.doh.wa.gov/ehp/algae/  
Figure A3 - Danger sign for use in Tier III.
Appendix B

Algal Bloom Monitoring:
Washington Department of Ecology
Questions and Answers
Appendix B1 - How to Sample a Lake

How to sample a lake

How do I have a lake water sample tested for algae?
If you think that your lake has an algae bloom and you want to have the algae identified, please contact Tricia Shoblom at the Washington Department of Ecology at:

- Telephone: 425-649-7288
- Email address: tsho461@ecy.wa.gov

Ms. Shoblom will ask you questions about the bloom and help you decide what to do. If you have a digital camera, you may be asked to email her a photograph of the bloom. The algal identification and toxicity testing service started April, 2007 and is ongoing.

To report an algae bloom after hours or on weekends, go to:
http://www.ecy.wa.gov/reportenviroproblem.html

Others may have reported the same bloom.
You may not be the only person who lives on your lake who has reported the bloom. Ecology may already be working with county health district staff or other lake residents about the same bloom that you are reporting. If so, Ecology may already have people collecting samples from that water body. In some locations, county health district or lakes program staff have requested that they be notified when a potential algal bloom is identified. In some situations, county staff may prefer to collect the samples themselves. If requested, Ecology staff will keep you informed about the algae identification and toxicity testing results from your lake.

How to participate in the algal bloom identification program

What happens if I am asked to send in an algae sample?
If Ecology staff asks you to send in an algae sample, they will provide specific directions about how to collect the sample and how to send or deliver it to the laboratory. It is very important for you to carefully follow the directions. Once at the laboratory, specialists will identify the algae species. If the sample contains an alga known to produce toxins, the laboratory will run a toxin analysis on the sample. If the sample contains a known toxin-producing alga, you may be asked to send in more samples. Be aware that Ecology cannot reimburse postage or delivery costs.

How do I collect a sample?
If you are approved to collect a sample, Ecology staff will provide you with specific directions about sample collection, preservation, and shipping information. Please work through the Washington Department of Ecology. The laboratory will not accept outside samples unless they have been pre-approved by Ecology. The lake or water body must be located in Washington State to be eligible for this service.
Where do I send the sample?
If asked to collect a sample, Ecology staff will provide you with mailing or delivery addresses. Be aware that Ecology cannot reimburse postage or delivery costs.

How do I get the results?
Ecology or county staff will contact you with information on algae identification and toxicity. Ecology staff will also enter this information into Ecology's web-based algae database. However, it may take a few weeks before Ecology can upload the most recent information.

Can I see the results from other lakes?
Ecology has an on-line algae database. You can enter a lake name and if there is any algae information about this lake in the database, the information will be displayed. You will also be able to search for algae by genus or common names to see the locations in Washington where specific species have been reported.

Because this is a new database, Ecology entered historical information on algae blooms for some Washington lakes. As Ecology collects new identification and toxicity information, staff will update the database. You should not assume that because there is no information in the algae database about a lake, that there is no information. Ecology is not able to enter all historical information.

Here is the link to the algae database: https://fortress.wa.gov/ecy/toxicalgae

Join Ecology's Freshwater Algae Program Listserv
Ecology has set up a listserv for freshwater algae. The Freshwater Algae Program Listserv will act as an information exchange and discussion forum about freshwater algae in Washington. A listserv is a program that allows people to send an email to one address, which automatically resends that message to all of the other subscribers on the list. See Ecology's website to subscribe to the list: http://www.ecy.wa.gov/programs/wq/plants/algae/monitoring/index.html