Washington State Recreational Guidance for Microcystins, Anatoxin-a, Cylindrospermopsin and Saxitoxin

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Washington State Department of Health

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For more information or additional copies of this report:

Division of Environmental Public Health

Office of Environmental Public Health Sciences

P.O. Box 47825

Olympia, Washington 98504-7825

360.236.3385

360.236.2261 (fax)

Rad.Cunningham@doh.wa.gov

Report Author

Joan Hardy, Ph.D.

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Tracie Barry, Rad Cunningham, Christopher Johnson, and Kaitlyn Kelly
Washington Department of Health

Debra Bouchard, Daniel Nidzgorski, and Rachael Gravon
King County Department of Natural Resources

Marissa Burghdoff
Snohomish County Surface Water Management

Lindsay Tuttle
Tacoma-Pierce County Health Department

Alyssa Payne
Clark County Public Health

Ian Rork
Kitsap Public Health District

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Preface

In January 2021, DOH received six-month's funding to address several topics associated with Washington's Freshwater Algae Control Program at the request of local health jurisdictions and lake managers. Objectives of the project were to:

1) Determine local health jurisdictions' and lake managers' preference regarding a Two-Tier Lake Management Protocol vs. the original Three-Tier approach.
2) Update signs to reflect a Two-Tier approach, if chosen, and determine additional languages for key message translations.
3) Develop an Informational Sign for posting by LHJs and lake managers at lakes with a history of blooms.
4) Create materials and communicate methods for reporting animal and human illnesses associated with freshwater cyanobacteria.

We surveyed interested parties on several cyanobacteria topics to inform decisions on the above objectives. Survey results were presented in a statewide freshwater cyanobacteria workshop where stakeholders provided additional preferences regarding protocol and sign development.

Two previous recreational guidance value reports (Microcystins and Anatoxin-a, Cylindrospermopsin and Saxitoxin) are merged into this updated report that includes changes produced during the project. EPA's updated national recreational guidance values for Microcystins and Cylindrospermopsin have been incorporated into the state recreational guidance protocols. The project's scope did not allow for an updated literature review.

To report animal or human health incidents associated with cyanobacteria exposure, contact your local health jurisdiction, or waterborne-illness@doh.wa.gov, or call 206-418-5500. For instructions on sampling a waterbody with a suspected cyanobacteria bloom, visit https://www.nwtoxicalgae.org/. To request signs for posting, call DOH EPH at (360) 236-3385.

Introduction

Cyanobacteria, also known as blue-green algae, are found in numerous Washington lakes, rivers and ponds. This group of photosynthetic bacteria is capable of producing a variety of different cyanotoxins, including microcystins and cylindrospermopsin, which are liver toxins, and anatoxin-a and saxitoxin, which are nerve toxins. Historically, many animals have become ill or have died after exposure to cyanotoxins in state lakes.

The Washington state legislature funded a Freshwater Algae Control Program in 2005, due in part to citizens' concerns about health impacts from exposure to freshwater cyanobacterial harmful algal blooms (cyanoHABs). Since cyanotoxins were determined to be a documented public health concern in Washington's lakes, Washington State Departments of Health (DOH) and Ecology (ECY) in collaboration with local health jurisdictions and other stakeholders developed a passive surveillance program to monitor cyanobacteria blooms. Ecology's 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 cyanobacteria 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.

During ECY's program development, stakeholders requested that state guidelines be developed to help with interpretation of toxicity results. Originally, this program only tested for microcystins, but KCEL later developed the capacity to test for anatoxin-a, cylindrospermopsin, and saxitoxin. In the absence of national or international recreational guidance values for microcystins and anatoxin-a, DOH produced "provisional" guidance values for both cyanotoxins (DOH 2008). Later, DOH developed "provisional" recreational guidance for saxitoxin and cylindrospermopsin (DOH 2011). As part of the effort to provide assistance to local health jurisdictions and lake managers, DOH also developed a three-tier lake management protocol that incorporated guidance values as a reference for use by managers, agencies, and local health jurisdictions (LHJs). ECY's Freshwater Algae Control Program is ongoing at this time and includes a small grants program for algal or nutrient management projects.

History of Washington's Freshwater Algae Control Program

1995 -2004: Citizen action in response to animal deaths in Pierce County lakes.

2005: State legislature funded the Freshwater Algae Control Program, under ECY.

2006: DOH and ECY held workshops around the state for stakeholder feedback on program development.

2007: ECY incorporated a Freshwater Algae Grant Program.

2008: DOH produced recreational guidance values for microcystins (MCs) and anatoxin-a (ATX) and Three-Tier Lake Management Protocol.

2008: King County Environmental Lab (KCEL) incorporated methods to test for MCs and ATX.

2010: KCEL incorporated methods to test for saxitoxin (STX) and cylindrospermopsin (CYL).

2011: DOH produced recreational guidance values for CYL and STX.

2013: DOH developed veterinary outreach posters and diagnostic cards.

2019: EPA finalized national recreational guidance values for MCs and CYL.

2020: WA adopted EPA's national recreational guidance values for MCs and CYL.

2021: DOH incorporated EPA's MC and CYL guidance values into an updated Two-Tier Lake Management Protocol. DOH added an Informational Sign for posting during bloom season or throughout the year.

The following describes Washington's freshwater toxins, updated recreational guidelines, and a two-tier lake management protocol designed for lake managers and LHJs to follow in the event of a toxic occurrence. Recreational guidance values for anatoxin-a and saxitoxin will be updated when adequate toxicity data are published or when EPA adopts national recreational guidance values.

Summary of Freshwater Toxins Affecting Public Health in Washington

DOH 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, we recommend that the sample be tested for toxicity:

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

In a summary report for the 2008-2009 legislature, ECY identified the top three toxic cyanobacteria genera in Washington lakes as *Dolichospermum*, *Aphanizomenon*, and *Microcystis* (Ecology 2009).

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, aplysiatoxins, and lyngbyatoxin), and lipopolysaccharides (irritants). Guanotoxin (anatoxin-a(s)) is a naturally occurring organophosphate.

Table 1: World Health Organization (WHO) list of toxic cyanobacteria (modified from WHO 1999 and Graham et al. 2008).

Toxin Group	Primary Target Organ in Mammals	Cyanobacterial Genera
Cyclic Peptides		
Microcystins	Liver	*Microcystis, Dolichospermum (Anabaena), Planktothrix (Oscillatoria), Nostoc, Hapalosiphon, Anabaenopsis, Aphanizomenon, Synechococcus, Synechocystis, Aphanocapsa, Pseudanabaena*
Nodularin	Liver	*Nodularia*
Alkaloids		
Anatoxin-a	Nerve synapse	*Dolichospermum (Anabaena), Planktothrix (Oscillatoria), Aphanizomenon, Raphidiopsis*
Guanotoxin [anatoxin-a(s)]	Nerve synapse	*Anabaena*
Aplysiatoxins	Skin	*Lyngbya, Schizothrix, Planktothrix (Oscillatoria)*
Cylindrospermopsins	Liver	*Cylindrospermopsis, Dolichospermum (Anabaena), Aphanizomemon, Umezakia, Raphidiopsis*
Lyngbyatoxin-a	Skin, gastrointestinal tract	*Lyngbya, Planktothrix (Oscillatoria)*
Saxitoxins	Nerve axons	*Dolichospermum (Anabaena), Aphanizomenon, Lyngbya, Cylindrospermopsis, Planktothrix (Oscillatoria)*
Amino Acid (Alkaloid precursor)		

BMAA	Neurotoxin	*Cylindrospermopsis, Dolichospermum (Anabaena), Aphanizomemon, Planktothrix (Oscillatoria), Microcystis, Nodularia, Synechococcus, Synechocystis*
Lipopolysaccharides (LGS)	Potential irritant; affects any exposed tissue	All

Note that some genera, especially *Dolichospermum*, 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).

Microcystins

General Information. Microcystins are the most thoroughly investigated cyanobacterial toxins (Falconer 2004). Over 250 structural variants have been identified, and microcystin-LR is the variant most commonly found in cyanobacteria (Huisman et al. 2005, Botana 2007, Welker and von Doehren 2006, Bouaïcha et al. 2019). Microcystins have been identified in *Dolichospermum, Microcystis*, *Oscillatoria* (*Planktothrix*), *Nostoc* and *Anabaenopsis* species and from the terrestrial genus *Hapalosiphon* (WHO 1999). More than one microcystin may be found in a particular cyanobacteria strain. Microcystins are cyclic heptapeptides that primarily affect the liver in animals. A lethal dose of microcystins in vertebrates causes death by liver necrosis within hours or up to a few days. Microcystins block protein phosphatases 1 and 2A (important molecular switches in all eukaryotic cells) with an irreversible covalent bond (Bagu et al. 1997). Liver injury is likely to go unnoticed and results in (external) noticeable symptoms only when it is severe (WHO 2003). Other studies have shown that microcystin toxicity is cumulative (Fitzgeorge et al. 1994). Researchers suspect microcystins are liver carcinogens, which could increase cancer risk to humans following continuous, low level exposure.

Symptoms. Symptoms of microcystin poisoning may take 30 minutes to 24 hours to appear, depending upon the size of the animal affected and the amount of toxic bloom consumed. Gross and histopathologic lesions caused by microcystins are quite similar among species, although species sensitivity and signs of poisoning can vary depending on the type of exposure. One of the earliest effects (15-30 min) of microcystin poisoning is increased serum concentrations of bile acids, alkaline phosphatase, y-glutamyltransferase, and AST. Microcystin symptoms in mammals and other animals may include jaundice, shock, abdominal pain/distention, weakness, nausea/vomiting, severe thirst, rapid/weak pulse, and death. It is likely that the number of incidents with low-level symptoms such as nausea, vomiting and diarrhea associated with recreational exposure to cyanobacterial toxins are under reported. Death may

occur following exposure to very high concentrations within a few hours (usually within 4-24 hr) or up to a few days. Death is due to intrahepatic hemorrhage and hypovolemic shock. In animals that live more than a few hours, hyperkalemia or hypoglycemia, or both, may lead to death from liver failure within a few days to a few weeks (Merck Veterinary Manual - https://www.merckvetmanual.com/toxicology/algal-poisoning/overview-of-algal-poisoning?query=microcystins

According to the Merck Veterinary Manual, surviving animals have a good chance for recovery because the toxins have a steep dose-response curve. Activated charcoal oral slurry is likely to benefit exposed animals, even though therapies for cyanobacterial poisonings have not been investigated in detail. The link states that an ion-exchange resin such as cholestyramine has proved useful to absorb the toxins from the GI tract and that certain bile acid transport blockers such as cyclosporin A, rifampin, and silymarin injected before dosing of microcystin have been effective in preventing hepatotoxicity in laboratory studies.

Anatoxin-a

General information. Anatoxin-a is one of three neurotoxic alkaloids that have been isolated from cyanobacteria (Falconer 2005). It is produced by various species of cyanobacteria including *Dolichospermum*, *Planktothrix* (*Oscillatoria*), *Aphanizomenon*, *Cylindrospermum* and *Microcystis* spp. Anatoxin-a was first detected in Canada in the 1960s (Gorham 1964). Between 1961 and 1975, cattle and dog poisonings associated with *Dolichospermum* (*Anabaena*) *flos-aquae* blooms occurred in six locations in Canada. Since then reports of anatoxin-a poisoning have been documented many times around the world (Testai 2021). Most anatoxin-a detections have been in Europe. Second to Europe, most reports of anatoxin-a have been in North America (Botana 2007). Anatoxin-a also has the potential of high concentrations in floating benthic mats or macrophytes (WHO 2021, Bouma-Gregson et al. 2018, Foss et al. 2018).

Anatoxin-a is a bicyclic secondary amine. It binds to the nicotinic acetylcholine receptor at the axon terminal at the neuromuscular interface (Botana 2007, Huisman et al. 2005). Binding of anatoxin-a is irreversible; the sodium channel is locked open, becomes overstimulated, fatigued, and eventually paralyzed. In the respiratory system, anatoxin-a exposure results in a lack of oxygen to the brain, subsequent convulsions and death by suffocation. Anatoxin-a is about 20 times more potent a nicotinic agonist than acetylcholine (Botana 2007). A methylene analogue of anatoxin-a called homoanatoxin-a was isolated from *Planktothrix formosa* in Norway. Symptoms of homoanatoxin-a are similar to those observed with anatoxin-a. This analogue has been found recently in Ireland and Japan (Botana 2007).

Alkaloid toxins are more likely to be present in free water than the cyclic peptide toxins microcystins and nodularin (WHO 2003). While microcystins appear to be more common than neurotoxins, neurotoxins have caused severe animal poisonings in North America, Europe and Australia (WHO 2003). Anatoxin degrades readily to nontoxic

degradation products in sunlight and at a high PH (Botana 2007). In natural blooms in eutrophic lakes, anatoxin-a half-life is typically less than 24 hours, while the half-life in the laboratory was about five days (WHO 1999). The rapid degradation of anatoxin-a presents problems with determining toxin levels after exposure. According to Botana (2007), samples should be protected from light and acidified prior to storage at -20^{o}C in order to limit anatoxin-a degradation.

Symptoms. Neurotoxins are notoriously rapid acting poisons; anatoxin-a was originally called very fast death factor (VFDF) due to its potency (Botana 2007). Illness and death to an animal may occur within a few minutes to a few hours after exposure, depending on the size of the animal and amount of toxic bloom consumed. An animal with anatoxin-a toxicosis may exhibit staggering, paralysis, muscle twitching, gasping, convulsions, backward arching of neck in birds, and death. Livestock that drink large amounts of contaminated water and pets that collect scum on their fur then ingest it by licking are at highest risk from anatoxin-a exposure. While anatoxin-a is largely retained within cells when conditions for growth are favorable, toxins will be liberated in the gastrointestinal tract if water containing toxic cells is consumed (WHO 1999, Botana 2007). However, ingestion of a sublethal dose of these neurotoxins leaves no chronic effects and recovery appears to be complete with no ongoing injury (WHO 2003). Exposure leaves no sign of organ damage and residual toxin is rapidly degraded (Botana 2007).

Cylindrospermopsin

General Information. 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, Froscio et al. 2003). 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, EPA 2006a).

Cylindrospermopsin 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). Further, *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 cylindrospermopsin. One hundred and forty-eight people required hospitalization with symptoms that included vomiting, malaise, headache and constipation, later followed by bloody diarrhea.

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. 2011. 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. Cylindrospermopsin 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^{o} 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. Symptoms of exposure to cylindrospermopsin include nausea, vomiting, diarrhea, abdominal tenderness, pain, and acute liver failure. Clinical symptoms after exposure to cylindrospermopsin may not show up immediately but may

occur several days later. Thus it is often difficult to determine a cause–effect relationship between cylindrospermopsin exposure and symptoms.

The degree of the cyanotoxin impact for cylindrospermopsin 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 2013: https://www.merckvetmanual.com/toxicology/algal-poisoning/overview-of-algal-poisoning

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 cylindrospermopsin, 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

General Information. Saxitoxins are among the most potent natural toxins known (Aràoz 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); *Dolichospermum* (*Anabaena) circinalis* (Australia); *Dolichospermum* (*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 *Dolichospermum (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 *Dolichospermum* (*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. 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 this 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 (https://emergency.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.

Cyanotoxin Risk Levels and Standards

DOH offers freshwater recreational guideline values for microcystin, anatoxin-a, cylindrospermopsin, and saxitoxin (Table 5). This document provides reference links to EPA's literature review and recreational guidance development for microcystins and cylindrospermopsins. Washington has adopted EPA's recommended recreational guidance values for microcystins and cylindrospermopsin. For anatoxin-a and saxitoxins, background information, toxicity reviews, and guideline development for provisional state recreational guidance values are provided, below. When national recreational

guidance values are developed for anatoxin-a and saxitoxins, DOH recommends their adoption in place of current provisional guidance.

Microcystins and Cylindrospermopsin

- EPA. Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin. EPA 822-R-19-001. May 2019.
- https://www.epa.gov/sites/production/files/2019-05/documents/hh-rec-criteria-habs-document-2019.pdf

EPA published recommended values for microcystins and cylindrospermopsin under the Clean Water Act (CWA) section 304(a) for states to consider as the basis for swimming advisories for notification purposes to protect public health in recreational waters. For microcystins, the recommended recreational value is 8 µg/L and for cylindrospermopsin, the recommended recreational value is 15 µg/L.

These values are based on the exposure experienced by recreating children. EPA recommends that these values not be exceeded on any single day when used as a swimming advisory to protect swimmers at a beach. DOH has incorporated the two recreational criteria values into Washington's Lake Management Protocol (Figure 1).

Anatoxin-a

- (Originally published in July 2008: https://www.doh.wa.gov/Portals/1/Documents/4400/334-177-recguide.pdf)
- EPA Toxicological Review – Anatoxin-a: https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=160546

Anatoxin-a is a potent acute neurotoxin. Available data indicate that health concerns based on chronic toxicity (vs. acute toxicity) of anatoxin-a would not be significant (WHO 1999). In 1999, WHO concluded that the toxicity database is insufficient for derivation of a TDI for anatoxin-a. Metcalf and Codd (2004) report that further toxicity data via oral and lifetime exposure are needed to derive guidance values for remaining known cyanobacterial toxins, including anatoxin-a. Oral toxicity and lifetime-exposure studies are ideal to minimize uncertainties and have been determined only for microcystin-LR and recently for cylindrospermopsin. Because acute effects are the primary endpoint of concern for recreational exposure to anatoxin-a and data are not available for derivation of an acute RfD, DOH reviewed other options for use in recreational guidance and has recommended the most protective approach until an acute RfD is available to calculate an anatoxin-a guidance value.

A 2006 EPA DRAFT toxicological review of anatoxin-a concluded that available oral toxicity information is inadequate to support derivation of oral RfD values for acute and

chronic exposure durations to anatoxin-a. The anatoxin-a database is limited in number and quality of studies on effects following oral exposure to sublethal levels. However, neurotoxicity and death were observed in acute, short-term, and subchronic oral animal studies (EPA 2006b). The authors conclude in their draft report that available data are insufficient to derive acute and chronic oral RfDs.

Based on the above data, EPA (2006b) was able to derive a DRAFT short-term RfD of 3 X 10-3mg/kg-day by dividing the no observed adverse effect level (NOAEL) by an uncertainty factor of 1000 (10 for interspecies extrapolation, 10 for interindividual variability, and 10 for database deficiencies). The NOAEL of 2.5 mg/kg-day was based on a systemic toxicity study in mice exposed to anatoxin-a for 28 days (Fawell and James 1994, Fawell et al. 1999). Using the equation:

Guidance value (mg/L) = Short-term RfD X BW, where
IR

Short-term RfD = 0.003 mg /kg-day
BW = 15 kg child
IR = 0.05 L/h, assuming 2 h/d,

the resulting short-term recreational guidance value would be 0.45 mg/L (450 µg/L).

Additionally, EPA (2006b) derived a DRAFT subchronic RfD of 5 X 10-4 mg/kg-day by dividing the NOAEL by an uncertainty factor of 1000 (10 for interspecies extrapolation, 10 for interindividual variability, and 10 for database deficiencies). The NOAEL of 0.5 mg/kg-day was based on systemic toxicity in rats exposed to anatoxin-a for seven weeks (Astrachan and Archer 1981 in EPA 2006b, Astrachan et al. 1980). A short-term recreational guidance value was calculated as follows:

Guidance value (mg/L) = Subchronic RfD X BW, where
IR

Subchronic RfD = 0.0005 mg /kg-day
BW = 15 kg child
IR = 0.05 L/h, assuming 2 h/d.

The resulting subchronic recreational guidance value using the subchronic RfD would be 0.075 mg/L (75 µg/L).

As stated previously, neither guidance value calculated above is based on acute toxicity data. The endpoint of concern for anatoxin-a is the acute endpoint (frank effects or death). Acute toxicity data for anatoxin-a are limited to results of lethality assays in mice, with neurotoxicity identified as the cause of death and with a single dose LD_{50}

value of 13.3 mg anatoxin-a/kg (Fitzgeorge et al., 1994, Stevens and Krieger 1991). Fawell et al. (1999) conducted studies to assess the risk of effects on man of ingestion of anatoxin-a at low levels over longer periods. Their study used *in vivo* and *in vitro* experiments to investigate subacute toxicity, teratogenicity, and pharmacology of anatoxin-a in the mouse. Repeated sublethal oral administration over 28 days did not produce any reliable evidence of treatment-related toxicity and the authors conclude that anatoxin-a does not appear to be a developmental toxicant in mice. In their conclusions, the authors indicate that a guideline value for anatoxin-a in drinking water of 1µg/L would provide an adequate margin of safety of about three orders of magnitude (Fawell et al. 1999).

In summary, DOH recommends the use of 1 ug/L to provide an adequate margin of safety of about three orders of magnitude, based on Fawell et al. (1999). This recommendation is a protective approach for use in the absence of an acute anatoxin-a RfD and was accepted by DOH's Scientific Advisory Committee. The recommendation of 1 ug/L will be updated when an acute RfD for anatoxin-a is available to calculate a guidance value.

Saxitoxins

- (Originally published in October 2011: https://www.doh.wa.gov/portals/1/documents/4400/332-118-cylindrosax%20report.pdf)

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:

$$\textbf{Guidance value (µg/L)} = \frac{\textbf{Acute RfD x BW}}{\textbf{IR}}, \text{ where:}$$

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 BWSTX equivalents (Report of the Joint FAO/IOC/WHO2004). 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:

$$\textbf{Guidance value (µg/L)} = \frac{\text{Acute RfD X BW}}{\text{IR}},$$

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 for saxitoxins (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):

Health alert = (124 µg/60 kg X 70 kg X 0.5)/ (10 X 2L) µg/L = 3.6 µg/L, rounded down to 3 µg/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 (*Dolichospermum (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 2L).

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 0.8mg/kg= 0.8mg/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 2: Four options considered for use as saxitoxin recreational guidance values.

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

DOH recommends Option 1 as a recreational guidance value primarily because it is the most protective (lower) of the four options that were 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: Two-Tiered Approach to Managing Lakes with Cyanobacterial Blooms

The following updated framework for managing toxic or potentially toxic cyanobacterial blooms uses a two-tiered approach. Previously the state framework used a three-tiered approach but the framework was revised in 2021 as a result of stakeholder input based

on posting experience in the decade after the original approach was implemented. The two-tiered framework applies the recreational guidance values adopted by EPA for microcystins (8 µg/L) and cylindrospermopsins (15 µg/L) and recreational guidance values derived above for anatoxin-a (1 µg/L) and saxitoxins (75 µg/L). This framework is recommended for managing Washington lakes (Figure 1). DOH recommends that the state adopt EPA recreational guidance values in the future for anatoxin-a and saxitoxins after they are developed, reviewed and adopted by EPA.

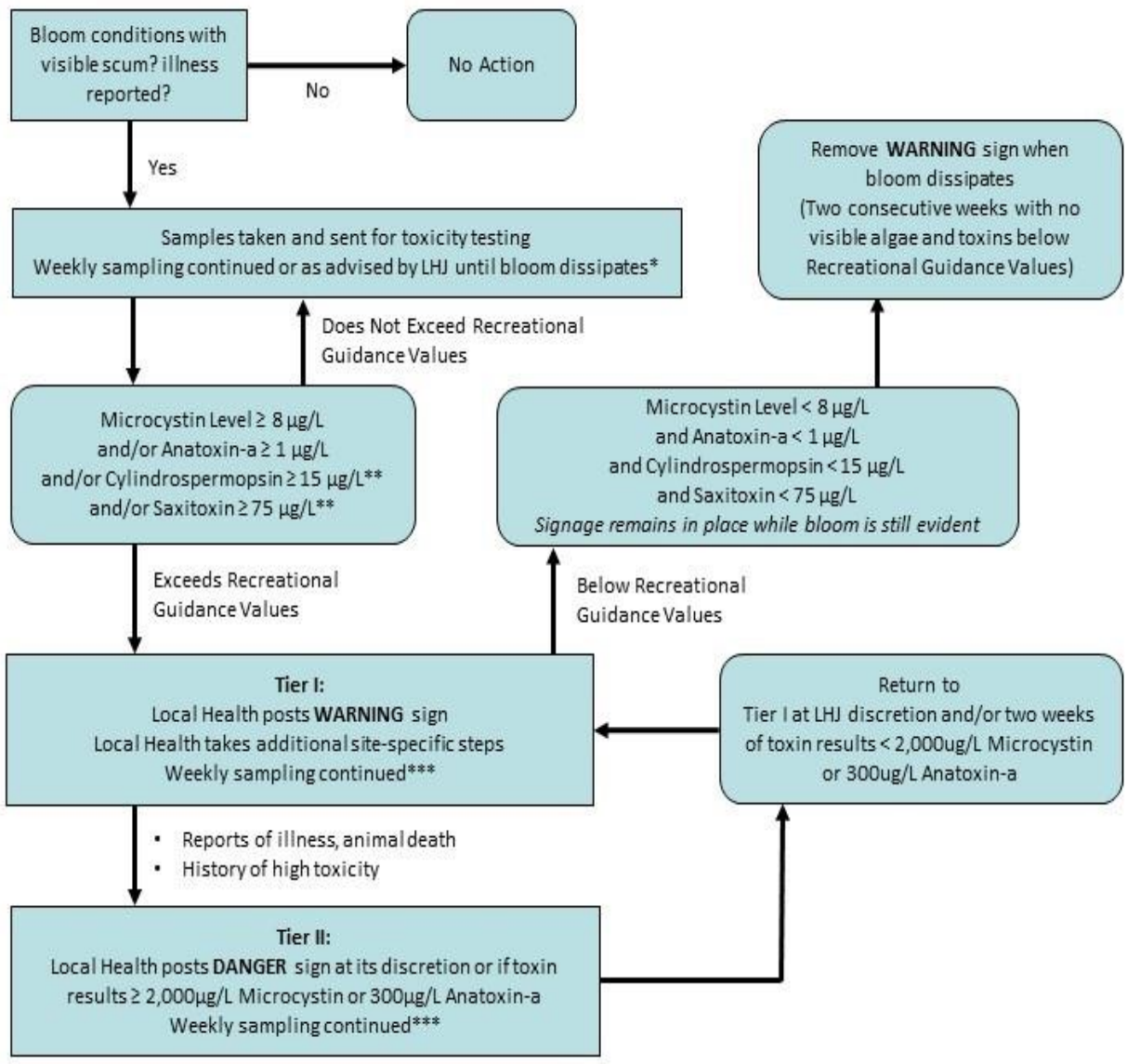
A unique feature of the Washington approach to cyanobacterial blooms in lakes is that bloom samples from most water bodies are eligible for toxicity testing. DOH and Ecology have incorporated outreach and educational efforts to encourage local health jurisdictions (LHJs), other agencies, lake residents, 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 via the nwtoxicalgae.org website, sample the water body experiencing the bloom, then send the sample to the laboratory for toxicity tests. Sampling and shipping directions are available from Washington State Toxic Algae freshwater algae bloom monitoring program:
https://www.nwtoxicalgae.org/ReportBloom.aspx

King County Environmental Lab (KCEL) is under contract with Ecology to test for microcystins, anatoxin-a, cylindrospermopsin, and saxitoxin. Results of toxicity analyses are incorporated into the toxic algae database and accessible via the link, above.

Figure 1: Two-tiered approach to managing Washington water bodies with cyanobacterial blooms.



**Informational Sign posted at LHJ's discretion (all year, June 1 – October 31, etc.).*
***Cylindrospermopsin and saxitoxin will be dropped from future analyses if not detected in the first sample.*
****Sampling every 2 weeks is an option if there is an ongoing dense bloom (at KCEL's suggestion in order to lower number of samples/season).*

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 Washington State Toxic Algae website and Ecology's list serve. In Washington, local jurisdictions have the authority to post advisories on water bodies within their districts (RCW 70.05.070).

If the bloom dissipates, no action is needed. If the bloom remains but microcystin levels are below 8 µg/L, anatoxin-a levels are below 1 µg/L, cylindrospermopsin levels are below 15 µg/L, and/or saxitoxin levels are below 75 µg/L, continue with weekly sampling until the bloom dissipates (sampling every two weeks is an option at KCEL's suggestion in order to lower the number of samples per season).

If microcystin levels are 8 µg/L or higher, anatoxin-a levels are 1 µg/L or higher, cylindrospermopsin levels are 15 µg/L or higher, and/or saxitoxin levels are 75 µg/L or higher, move to Tier I: the LHJ should post a WARNING sign (Figures 1, A1). The lake should be sampled weekly with the WARNING sign posted as long as microcystin concentrations are 8 µg/L or higher, anatoxin-a levels are 1 µg/L or higher, cylindrospermopsin levels are 15 µg/L or higher, and/or saxitoxin levels are 75 µg/L or higher. DOH recommends that LHJs sample two consecutive weeks with levels below recreational guidance values before retracting an advisory and removing the WARNING sign.

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 WARNING advisory and avoid areas with a moderate or heavier density of cyanobacteria, they should not be subject to any ill effects from cyanotoxins.

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.

If a lake has a history of high toxicity, if toxin concentrations are extremely high (> 2,000 µg/L microcystins or > 300 µg/L anatoxin-a) or if pet illnesses/death or human symptoms are reported, move to Tier II. Implementation of Tier II is based on judgment of the LHJ and local knowledge of the water body.

Tier II

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, A2). 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 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 I recommendations after retracting a lake closure until microcystin levels are less than 8 µg/L, anatoxin-a levels are less than 1 µg/L, cylindrospermopsin levels are less than 15 µg/L, and/or saxitoxin levels are less than 75 µg/L (Figure 1).

King County Environmental Lab results for microcystin, anatoxin-a, cylindrospermopsin, and saxitoxin will be added to the Washington State Toxic Algae database. For Tier I and Tier II, 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. However, 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 do 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 and cost of mailing sample kits to the laboratory.

Future Threats, Needs and Recommendations

A new threat from freshwater HABs in Washington is the contamination of reservoirs and lakes where surface water is used by residents for drinking water. In 2014, a 500-household lake community withdrew untreated drinking water during a period when anatoxin-a concentrations were low but still above state recreational guidelines. Previously, the drinking water source for Friday Harbor, an island town, had a toxic bloom that resulted in the need to ship in water for the community. Future efforts should focus on lakes used as drinking water sources and on coordination with managers of surface water systems that may develop toxic blooms.

Impacts of climate change on cyanobacteria need to be taken into consideration when sampling blooms to determine trends over time (i.e., annual variability), duration of blooms, and bloom size (i.e., spatial coverage). Warm temperatures and high nutrient concentrations promote cyanobacteria growth. If long-term climate projections for the Pacific Northwest are correct, rain events will increase, which may influence nutrient runoff from impervious surfaces, particularly as land is developed and regional populations increase. Long-term warming trends would also promote cyanobacteria (Plaas and Paerl 2021).

Another under-researched topic is the combined toxicity of blooms in lakes with more than one type of toxin. Further, as MC variants become easier to identify and quantify, toxicologists will need to determine actual toxicity of a water sample with MCs instead of assuming all variants are of equal toxicity to MC-LR, the current method.

In the future, our state will adopt national recreational guidance values for anatoxin-a and saxitoxin after each is developed by EPA. Collaboration with CDC on the OHHABS reporting system starting in 2021 will improve tracking animal and human health illness events at the state and national levels. Two additional areas of focus are incorporation

of risk guidance for animal exposures to cyanotoxins and evaluation of cyanotoxin aerosol risk to humans.

Recommendations for future work include ongoing collaborative work investigating the link of freshwater toxins with marine bivalve bioaccumulation of MCs. Further investigation of HAB genetics may help explain why some blooms are toxic and others are not. Pilot projects by LHJs using drones to determine effectiveness in assessing spatial and temporal extent of blooms have been proposed, and incorporation of EPA's CyAN application based on satellite imagery may provide early warning and evaluation of blooms in in state waterbodies.

Outreach efforts on freshwater HABs have met some needs but other educational needs remain. Attention and funding for outreach work is a continued necessity. Outreach to veterinary clinics regarding differential diagnoses and distribution of posters for pet owner education has been effective in the state; annual outreach to the public and to hunters owning dogs should continue. However, a major outreach and education gap in the state is for physicians who treat those exposed to toxic blooms. More recently, DOH has included outreach to drinking water operators about available toxicity tests, bloom identification, and options for treatment when blooms occur, and funding mechanisms for emergency cyanobacteria illness response in the case of drinking water contamination should be investigated. Finally, periodic webinars regarding blooms, toxicity testing, lake postings, and illness investigations are advised to ensure all areas of the state are aware of the program and knowledgeable about state-level technical support.

Summary

DOH offers freshwater recreational guideline values for microcystin, anatoxin-a, cylindrospermopsin, and saxitoxin (Table 5). In this report, DOH incorporated EPA's recommended freshwater recreational guidance values for microcystins and cylindrospermopsins into the state's Lake Management Protocol. Until EPA adopts national recreational guidance values for anatoxin-a and saxitoxin, provisional guidance values for both cyanotoxins were developed as described in this document and recommended for the state's Lake Management Protocol.

Table 3: Washington State recreational guidance for freshwater cyanotoxins.

Toxin	Recreational Guidance Value
Microcystins	8 µg/L
Anatoxin-a	1 µg/L
Cylindrospermopsin	15 µg/L
Saxitoxin	75 µg/L

DOH recommends s a recreational guidance value of 8 µg/L microcystins, based on EPA's recommended human health recreational ambient water quality criteria or swimming advisory for microcystins (EPA 2019). This recommended recreational guidance value is used in a two-tiered framework for LHJs or the appropriate local agency to follow in the event of a toxic bloom.

DOH recommends an interim (provisional) recreational guidance value of 1 µg/L anatoxin-a. This recommendation provides an adequate margin of safety of about three orders of magnitude and is based on a systemic toxicity study in mice exposed to anatoxin-a for 28 days (Fawell et al. 1999). When EPA adopts a human health recreational ambient water quality criteria or swimming advisory for anatoxin-a or when an acute RfD becomes available, DOH will reassess this interim anatoxin-a guidance value. The (provisional) recreational guidance value of 1 µg/L anatoxin-a is also used in a two-tiered lake management framework.

DOH recommends s a recreational guidance value of 15 µg/L cylindrospermopsin, based on EPA's recommended human health recreational ambient water quality criteria or swimming advisory for cylindrospermopsin (EPA 2019). This recommended recreational guidance value is used in a two-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 of 75 µg/L saxitoxin. This recommendation uses an acute reference dose (acute RfD) 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 incorporated into a two-tiered lake management framework.

When a cyanobacterial bloom is developing or a bloom or scum is observed, LHJs may sample the waterbody and wait for results from the King County Environmental Lab. If microcystin levels remain below 8 µg/L, anatoxin-a levels remain below 1 µg/L, cylindrospermopsin levels remain below 15 µg/L and/or saxitoxin levels remain below 75 µg/L, no action is taken.

If cyanotoxin levels are above those shown in Table 5, the LHJ posts WARNING signs until levels fall below guidance values for at least two consecutive weeks (Tier I). LHJs may conduct additional outreach efforts as needed. Under rare circumstances, a LHJ may need to post DANGER signs (Lake Closed) when toxin results > 2,000 µg/L microcystins or 300 µg/L anatoxin-a or at the LHJ's discretion. DANGER signs revert to WARNING signs at the LHJ's discretion or when toxin results are less than 2,000 µg/L microcystins or 300 µg/L anatoxin-a, and weekly sampling is continued.

Ecology and DOH personnel are available to discuss results of lake testing and consult with LHJs during their decision-making process. Ecology and King County Department of

Natural Resources will update and maintain lake toxicity data on the Washington State Toxic Algae Freshwater Algae Bloom Monitoring Program website (https://www.nwtoxicalgae.org/) and Ecology's list serve. Finally, each agency has additional information on freshwater algae and toxicity issues available on each website as a public education and risk communication resource.

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Appendix A. Warning and Danger Signs: A Two-Tiered Approach

Figure A1: Warning sign for use in Tier I.

Figure A2: Danger sign for use in Tier II.

Appendix B. Informational Sign: "When in Doubt, Stay Out"

Figure B1: Informational sign for use in Two-Tier Lake Management Protocol.

Appendix C. Veterinary Informational Poster

Figure C1: Veterinary Poster for clinics and for lake posting.