CYANOBACTERIA IN LAKES AND RESERVOIRS: TOXIN AND TASTE-AND-ODOR SAMPLING GUIDELINES 7.5

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Illustrations

Tables

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Cyanobacteria (also referred to as blue-green algae) cause a multitude of water-quality concerns, including the potential to produce toxins and taste-and-odor compounds. Toxins and taste-and-odor compounds may cause significant economic and public health concerns, and are of particular interest in lakes, reservoirs, and rivers that are used for drinking-water supply, recreation, or aquaculture. The purpose of NFM 7.5 is to provide guidelines for collecting, processing, and handling samples to be analyzed for cyanobacterial community composition (enumeration and identification) and total, particulate, and dissolved cyanobacterial toxins and taste-and-odor compounds in lakes and reservoirs (sections 7.5.5 through 7.5.9). Sections 7.5.1 through 7.5.4, however, are designed to provide some background information about cyanobacteria, including typical study designs and objectives related to the spatial and temporal occurrence of cyanobacteria (modified from Graham and others, 2008), in order to provide a useful context for sampling activities. The information presented here pertains to the occurrence of planktonic (free-floating) cyanobacteria in lakes and reservoirs.[1]

Cyanobacteria: True bacteria with a prokaryotic cell structure and containing chlorophyll-*a* (a photopigment characteristic of eukaryotic algae and higher plants).[2]

Algal groups other than cyanobacteria may cause taste-and-odor problems in drinking-water supplies, but the most frequent and severe events are associated with cyanobacteria (Wnorowski, 1992; Rashash and others, 1996; Taylor and others, 2005). One other freshwater/brackish water alga (the haptophyte *Prymnesium parvum*) is known to produce icthyotoxins under certain conditions; these icthyotoxins have been associated with fish kills only and not with human illness (Sager and others, 2007). All sampling guidelines presented in NFM 7.5 focus on planktonic cyanobacteria, but these guidelines also may apply to water-quality studies of other planktonic algal groups, depending on study objectives.

[1]Benthic cyanobacteria in lakes, reservoirs, streams, and rivers also may produce toxins and taste-and-odor compounds (Graham and others, 2008). Topics that address the sampling of benthic cyanobacteria in aquatic ecosystems are beyond the scope of this section of the *National Field Manual*.

[2]Terms related to cyanobacteria and algae are defined in the glossary, section 7.5.12.

Cyanobacterial toxins (also referred to as cyanotoxins) have been implicated in human and animal illness and death in more than 50 countries worldwide, and in at least 27 States in the United States (Graham, 2006). Human toxicoses associated with cyanobacterial toxins most commonly have occurred after exposure to the toxins through drinking water or through recreational activities (Yoo and others, 1995; Chorus and Bartram, 1999; Falconer, 2005; Huisman and others, 2005; Graham, 2006). Taste-and-odor compounds cause malodorous or unpalatable drinking water and fish flesh, resulting in increased treatment costs for drinking water and loss of recreational or aquacultural revenue. Federal and State agencies, resource managers, drinking-water treatment-facility operators, lake associations, and local officials increasingly are faced with decisions about managing cyanobacterial blooms that affect local economies and public health. Therefore, representative scientific data are needed to guide water management and public health decisions about cyanobacterial toxins and taste-and-odor compounds.

The study of cyanobacterial toxins and taste-and-odor compounds is an area of developing research. Although many approaches have been used in the design, sample collection, and analysis of studies addressing these cyanobacterial by-products, consistent guidelines for the development of such studies are necessary for making cross-comparisons among the data collected for these studies (Graham and Jones, 2007; Tillmanns and others, 2007). Collecting data that are comparable among a broad spectrum of studies is of particular importance in meeting the needs of the Nation and the mission of the U.S. Geological Survey (USGS).

7.5.1 LIGHT INTENSITY AND THERMAL STRATIFICATION IN LAKES AND RESERVOIRS

Lakes and reservoirs are characterized by vertical gradients caused by light and thermal stratification. An understanding of these gradients is required when designing water-quality studies and sampling guidelines for lakes and reservoirs.[3] More detail on light and thermal stratification in lakes and reservoirs can be found in Horne and Goldman (1994), Lampert and Sommer (2007), Wetzel and Likens (2000), and Wetzel (2001).

Light intensity decreases exponentially with depth, and a light gradient is present in all lakes and reservoirs (fig. 7.5–1). The terms photic (or euphotic) and aphotic zone are used to describe the major light gradients.

- **Photic (euphotic) zone –** The region where there is enough light to support photosynthesis; extends from the surface to the depth where light is approximately one percent of that at the surface.
- **Aphotic zone –** The region where there is not enough light to support photosynthesis; extends from below the photic zone to the bottom of the water body.

[3]The information and sampling approaches described herein are appropriate for lakes and reservoirs (the term "lakes", as used in this NFM section, applies also to reservoirs); however, lakes and reservoirs have distinct morphological and hydrological differences that influence water chemistry and biological communities. Topics that address the differences between lakes and reservoirs are beyond the scope of this report. For more information on the differences between lakes and reservoirs see Thornton and others (1990), Horne and Goldman (1994), and Wetzel (2001).

Thermal stratification creates isolated layers that result in thermal and chemical (such as nutrient) gradients; stratification is the result of differences in density associated with temperature (fig. 7.5–1). Stratification in deep lakes and reservoirs (referred to below as lakes) tends to be stable, whereas daily or continual mixing may occur in shallow lakes. Thermal stratification is a summer phenomenon in temperate lakes, but may occur year-round in subtropical and tropical lakes. Spatial variability of stratification within lakes and reservoirs is common because of changes in depth and other morphological features. Common terms that will be used throughout this guidance that relate to lake and reservoir stratification include:

- **Epilimnion –** The warm, buoyant upper layer of a stratified lake.
- **Metalimnion –** The middle layer of a stratified lake; the metalimnion is characterized by substantial decreases in temperature with depth.
- **Thermocline –** The region where temperature change is greater than or equal to 1°C per meter; the terms thermocline and metalimnion often are used synonymously.
- **Hypolimnion –** The cold, dense bottom layer of a stratified lake; the hypolimnion often becomes anoxic (little or no dissolved oxygen) in productive systems.
- **Turnover –** Complete isothermal mixing of a previously stratified lake.
- **Mixed Depth –** The depth of turbulent mixing; may include all or only a part of the water column, depending on stratification, solar irradiance, and wind.

During calm periods, temporary mixed layers (lasting hours to weeks) may form in shallow lakes, deeper lakes that do not typically stratify, or in the epilimnion of stratified lakes, creating greater complexity in vertical structure. Mixing in shallow and stratified lakes will vary daily and seasonally depending on solar irradiance and wind. Thermal stratification is most common, but gradients in water chemistry (often caused by submerged springs), such as salinity, also may cause stratification (Horne and Goldman, 1994; Lampert and Sommer, 2007; Wetzel and Likens, 2000; Wetzel, 2001).

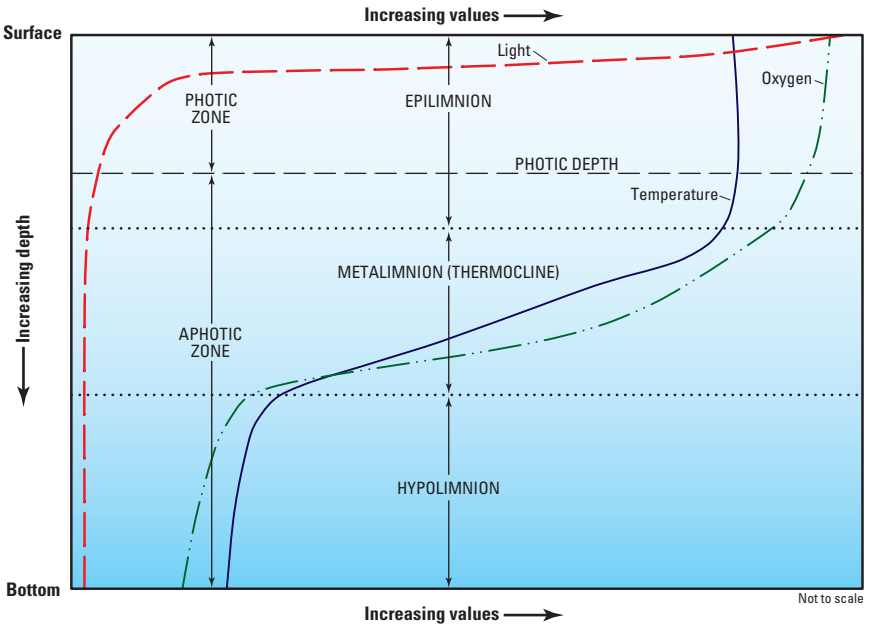


Figure 7.5–1. Stratification and light, temperature, and oxygen gradients that may develop in lakes and reservoirs. From Graham and others (2008).

7.5.2 CYANOBACTERIA, TOXINS, AND TASTE-AND-ODOR COMPOUNDS

Cyanobacteria (fig. 7.5–2) are true bacteria with a prokaryotic cell structure; however, cyanobacteria also have chlorophyll-*a*, a photopigment characteristic of eukaryotic algae and higher plants. Structurally the cyanobacteria are bacteria-like, but functionally the cyanobacteria are algae-like. Because of this photosynthetic functionality, cyanobacteria typically are sampled and analyzed as part of phytoplankton (algal) assemblages rather than bacterial assemblages in aquatic ecosystems (Wetzel, 2001; see NFM 7.4).

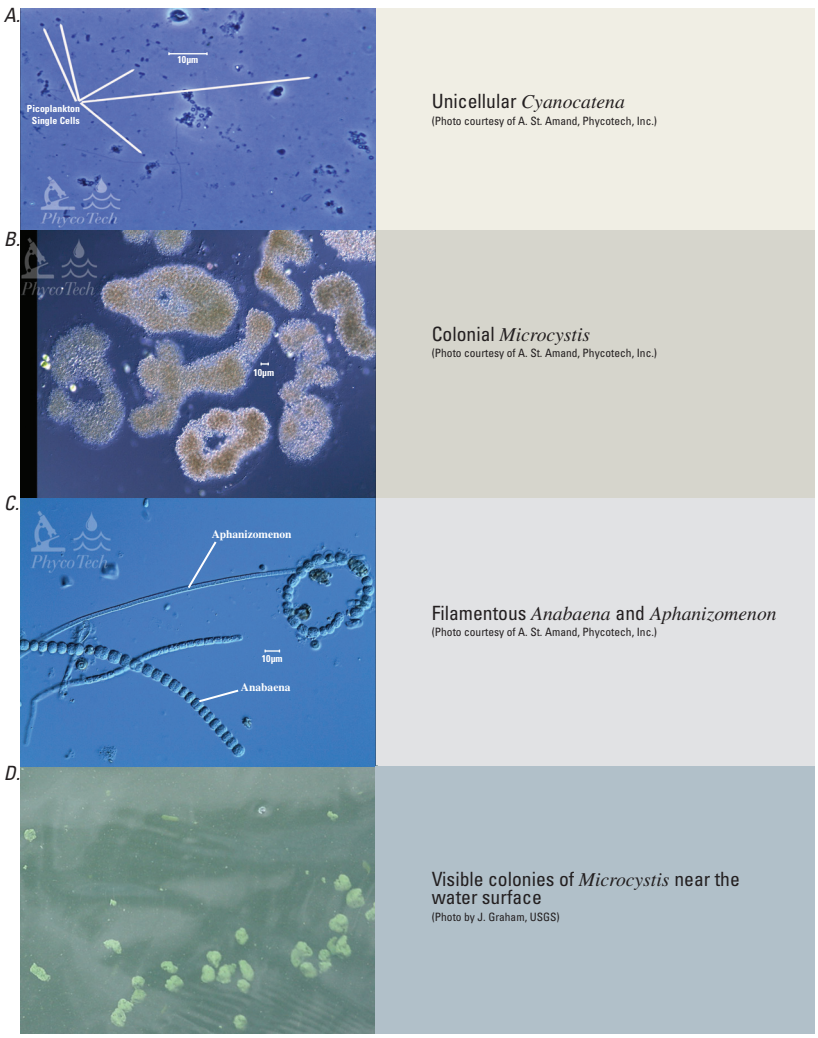


Figure 7.5–2. Examples of cyanobacteria. Cyanobacteria may be unicellular (***A***), colonial, (***B***), or filamentous (***C***) and range in size from 0.2 micrometers to greater than 1,600 micrometers. Larger filaments and colonies are often visible to the naked eye (***D***). From Graham and others (2008).

Cyanobacterial toxins and taste-and-odor compounds are naturally produced algal by-products; however, the function of these compounds currently (2008) is unknown. Hypothesized functions include primary roles in cellular processes, secondary metabolites, allelopathy, or defense mechanisms. Production of toxins and taste-and-odor compounds is strain, rather than species, dependent. Any one cyanobacterial species may have multiple strains, and toxic and non-toxic strains may occur simultaneously in an individual lake or reservoir (Vézie and others, 1998).

Most cyanobacterial taxa do not produce toxins or taste-and-odor compounds, but many of the common planktonic genera contain one or more toxin or (and) taste-and-odor producing strains (table 7.5–1). Whereas some strains may produce toxin and taste-and-odor compounds simultaneously, these compounds do not necessarily co-occur and the presence and concentration of one may not be used reliably to predict the presence and concentration of another (Chorus and Bartram, 1999).

Because toxin and taste-and-odor production is strain dependent, algal identification alone cannot be used to determine whether or not these by-products will be present, although genera that contain strains producing these compounds can be identified. Likewise, co-occurrence of potential producers and toxin and (or) taste-and-odor compounds does not positively identify the actual producer.

- Strain isolation and culture are required to conclusively determine the producer(s) of measured toxin and (or) taste-and-odor compounds.
- Genetic techniques that isolate specific gene clusters also are promising in identifying toxin producers in complex environmental samples.

Cyanobacteria typically are sampled and analyzed as part of phytoplankton (algal) assemblages rather than bacterial assemblages in aquatic ecosystems (NFM 7.4).

Table 7.5–1. Common genera of planktonic cyanobacteria that contain toxin and taste-and-odor producing strains.

[All data included in this table are based on documented production in laboratory cultures; data based on circumstantial evidence, such as co-occurrence of genera and toxin or taste-and-odor compounds in environmental samples, were not included. LYN, lyngbyatoxin-a; APL, aplysiatoxins; LPS, lipopolysaccharides; CYL, cylindrospermopsins; MC, microcystins; NOD, nodularins; ATX, anatoxins; BMAA, β-N-methylamino-L-alanine; NEO, neosaxitoxins; SAX, saxitoxins; GEOS, geosmin; MIB, 2-methylisoborneol]

	Dermatoxins				Hepatotoxins				Neurotoxins					Tastes and odors	
Cyanobacterial Genera	**LYN**	**APL**	**LPS**		**CYL**	**MC**	**NOD**		**ATX**	**BMAA**	**NEO**	**SAX**		**GEOS**	**MIB**
Colonial/Filamentous															
Anabaena			X		X	X			X	X	X	X		X	
Anabaenopsis			X			X									
Aphanizomenon			X		X				X	X	X	X		X	
Aphanocapsa			X			X									
Cylindrospermopsis			X		X					X		X			
Microcystis			X			X				X					
Nodularia			X				X			X					
Oscillatoria (Planktothrix)	X	X	X			X			X	X		X		X	X
Pseudanabaena			X			X			X					X	X
Raphidiopsis			X		X				X						
Unicellular															
Synechococcus			X			X				X				X	X
Synechocystis			X			X				X					

Sources: Wu and others (1991), Wnorowski (1992), Blevins and others (1995), Carmichael (1997), Bláha and Maršálek (1999), Chorus and Bartram (1999), Domingos and others (1999), Saadoun and others (2001), Oudra and others (2002), Watson (2003), Huisman and others (2005), and Taylor and others (2005). A comprehensive list of known cyanobacterial toxin and taste-and-odor producers is not currently (2008) available in the literature. Combined, the references used to create this table may be used to create a fairly complete list of planktonic and benthic producers.

7.5.2.A CYANOBACTERIAL ABUNDANCE, OCCURRENCE, AND DISTRIBUTION

Cyanobacteria are a natural part of phytoplankton assemblages in lakes and reservoirs and commonly are present in at least low abundances (Reynolds, 1984; Jones and Korth, 1995). Cyanobacterial abundance and community composition vary seasonally as a result of changes in water temperature, solar irradiance, meteorological conditions, hydrology, and nutrient supply. In temperate climates, cyanobacteria typically dominate the phytoplankton during midsummer to early fall, but may become dominant any time throughout the year, even under ice during winter. In subtropical and tropical climates, cyanobacteria may dominate at any time, and dominance may persist year-round (Chorus and Bartram, 1999; Wetzel, 2001; Falconer, 2005; Huisman and others, 2005).

Eutrophication results in conditions that are favorable for cyanobacterial growth (elevated nutrients, reduced light penetration), and cyanobacterial blooms frequently occur in eutrophic (nutrient rich, high productivity) lakes and reservoirs. Cyanobacterial blooms also may occur in oligotrophic (nutrient poor, low productivity) systems, although not as frequently as in eutrophic systems. Blooms in oligotrophic systems often are associated with benthic cyanobacteria or favorable nutrient and light conditions at depth in a stably stratified water column (Reynolds, 1984; Chorus and Bartram, 1999; Wetzel, 2001; Falconer, 2005; Huisman and others, 2005).

Many, although not all, cyanobacteria have gas vacuoles that allow them to maintain a favorable position in the water column by regulating buoyancy. Light (the photic depth), nutrients, carbon availability, stratification, and mixed depth (depth of turbulent mixing in the water column) all affect cyanobacterial position in the water column. Optimal water-column position is species specific. Three distinct patterns in distribution may develop (Reynolds and Walsby, 1975; Reynolds, 1987; Humphries and Lyne, 1998):

1. Cyanobacteria may maintain a position in the photic zone, regardless of mixed depth. Populations typically are distributed uniformly throughout the photic zone. This distribution may develop in well-mixed lakes or reservoirs (referred to in this list as lakes) or in the epilimnion of stably stratified lakes. Many cyanobacterial genera, including *Anabaena*, *Aphanizomenon*, *Microcystis* (fig. 7.5–2) and *Cylindrospermopsis* display this type of buoyancy regulation.
2. Cyanobacteria may migrate to different locations in the photic zone throughout the day. Populations generally move toward the surface at night or in the early morning and downward later in the day. This diel movement of cyanobacterial populations typically is linked with the development of diel mixed layers in shallow lakes or in the epilimnion of stably stratified lakes. Regardless of movement or location, cyanobacteria typically maintain a position in the photic zone. In shallow lakes, where light penetrates to the bottom, cyanobacteria may spend part of the day on the sediment surface. Diel movement most commonly is associated with species of *Microcystis*, although *Anabaena* and other gas-vacuolate taxa also may migrate on a diel basis.
3. Cyanobacteria may maintain a position at a particular depth, typically at the interface between the epilimnion and metalimnion. This phenomenon is referred to as a metalimnetic bloom, and most commonly is associated with species of *Planktothrix* (*Oscillatoria*). Metalimnetic cyanobacterial blooms occur most frequently in mesotrophic lakes. For metalimnetic blooms to develop, the water column must be stably stratified and photic depth must penetrate into the metalimnion; metalimnetic cyanobacterial blooms can exist at light levels at or below one percent of incident light.

When referring to cyanobacteria, the term "bloom" commonly is associated with the accumulation of cyanobacteria at the water surface; however, cyanobacterial blooms are more typically mixed throughout the photic zone, epilimnion, or water column. Surface accumulations, or scums, may develop when cyanobacteria float to the surface during unusually calm conditions that cause a sudden change in turbulent mixing (sudden calm will not affect metalimnetic populations) or when cyanobacteria begin to senesce (age) and are no longer able to regulate buoyancy effectively. Surface accumulations occur most frequently after periods of calm, sunny weather and may develop and dissipate within a matter of hours as conditions change. Because the entire cyanobacterial population may accumulate within 1 to 2 centimeters (cm) of the water surface, cell densities may be extremely large (more than a million cells per milliliter), even if water-column densities were relatively small (less than 20,000 cells per milliliter). Surface accumulations of cyanobacteria may concentrate even further when wind blows to leeward areas (for example, near-shore areas, bays, and inlets), often resulting in the development of dense accumulations that may extend from the surface to depths of more than 1 meter (m) (Graham and others, 2008).

7.5.2.B TOXINS

Cyanobacterial toxins are chemically and bioactively diverse (Sivonen, 1996; Carmichael, 1997; Chorus and Bartram, 1999), all targeting fundamental cellular processes and thereby affecting a wide range of organisms (Falconer, 1993; Christofferson, 1996). **The cyanobacteria are the only member of freshwater phytoplankton communities known to produce toxins that also may affect terrestrial organisms.**

The most common cyanobacterial toxin groups are described in figure 7.5–3 and table 7.5–2. The three main classes of cyanobacterial toxins, defined by their effects on human health, are neurotoxins, hepatotoxins, and dermatoxins (Graham and others, 2008). Many of the cyanobacterial toxins have multiple variants, with some variants being more toxic than others. For example, the microcystins, currently believed to be the most common group of cyanobacterial toxins, have more than 80 known variants with LD_{50}'s (lethal dose required to kill 50 percent of the test population; determined by intraperitoneal mouse assays) ranging from 25 to greater than 1,000 micrograms per kilogram (µg/kg) (most variants fall within the 50 to 300 µg/kg range). The adverse health effects caused by contact with, or ingestion or inhalation of, large concentrations of cyanobacterial toxins (acute exposure) are well documented (table 7.5–2). The effects of exposure to low levels of cyanobacterial toxins for an extended period of time (chronic exposure), however, are not well understood (Chorus and Bartram, 1999; Graham and others, 2008).

- The greatest risk of exposure to elevated concentrations of cyanobacterial toxins probably is through accidental ingestion and inhalation during recreational activities. The World Health Organization (WHO) has defined the following low, moderate, high, and very high risk categories for adverse health effects that occur through recreational exposure to microcystins (Chorus and Bartram, 1999):
  - **Low risk**: less than 10 micrograms per liter (µg/L)
  - **Moderate risk**: 10–20 µg/L
  - **High risk**: 20–2,000 µg/L
  - **Very high risk**: greater than 2,000 µg/L
- The WHO has developed a provisional guideline of 1 µg/L for microcystin-LR in treated drinking water (Chorus and Bartram, 1999) and currently (2008) is developing a guideline for cylindrospermopsin. Falconer (2005) has proposed a guideline of 1 µg/L for cylindrospermopsins in finished drinking water.
- Several countries have set national standards or guidelines for microcystins in drinking water based on the WHO guideline, including Australia, Brazil, Canada, the Czech Republic, France, Poland, and Spain (Huisman and others, 2005). In the United States the cyanobacterial toxins microcystin, cylindrospermopsin, and anatoxin currently are on the U.S. Environmental Protection Agency (2005) drinking-water contaminant candidate list.

CAUTION: **Inhalation or ingestion of large concentrations of cyanobacterial toxins can result in adverse health effects.**

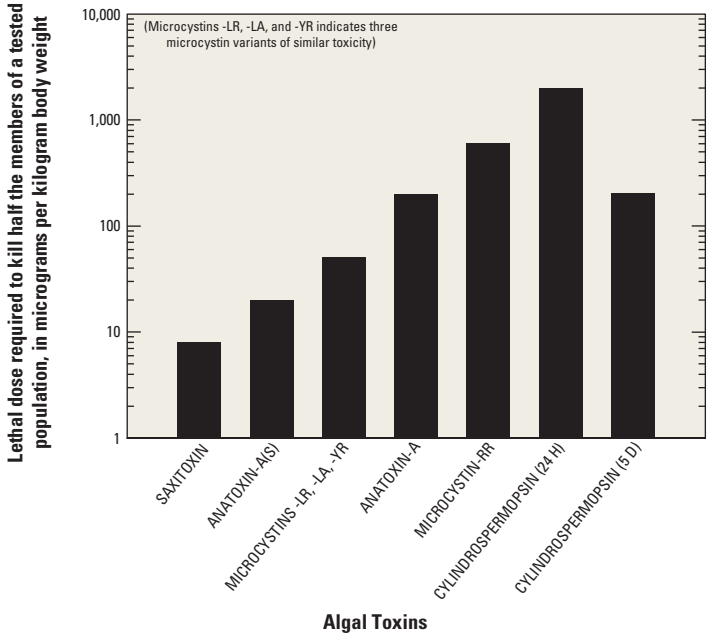


Figure 7.5–3. Toxicity of several cyanobacterial toxins (based on intraperitoneal mouse assays). From Graham and others (2008); figure based on data from Chorus and Bartram (1999).

Table 7.5–2. Common cyanobacterial toxins, toxicity (based on intraperitoneal mouse assays), and common effects of exposure.

[Most toxin groups have several variants with a range of toxicities. Although known chronic effects are listed, the chronic effects of exposure to cyanotoxins currently (2008) are not well understood. LD_{50}, lethal dose required to kill half of the members of a tested population; µg/kg, micrograms per kilogram of body weight; --, no data available; >, greater than]

Class	Toxin	Toxicity (LD_{50})	Acute effects	Chronic effects
Neurotoxins	Anatoxins	20–250 µg/kg	Seizure, paralysis, respiratory failure, death	Unknown
	Saxitoxins[1]	10 µg/kg	Tingling or numbness in extremities, paralysis, respiratory failure, death	Unknown
	β-N-methylamino-L-alanine (BMAA)	--	--	Neurodegenerative disease
Hepatotoxins	Microcystins	25 to > 1,000 µg/kg	Acute hepatoenteritis, shortness of breath, interhepatic hemorrhage, hemorrhagic shock, heart failure, death	Chronic liver injury, tumor promoter
	Cylindrospermopsins	200–2,100 µg/kg	Acute hepatoenteritis; renal, lung, heart, spleen, thymus, and adrenal damage; death	Potential carcinogen, mutagen
	Nodularins[2]	50 µg/kg	Similar to microcystins	Tumor promoter
Dermatoxins	Lyngbyatoxins	300 µg/kg	Severe dermatitis, gastroenteritis	Tumor promoter
	Aplysiatoxins	300 µg/kg	Severe dermatitis, gastroenteritis	Tumor promoter
	Lipopolysaccharides	--	Dermatitis, gastroenteritis	Unknown

[1]Also known as paralytic shellfish poisons (PSPs).
[2]To date, nodularins have been detected only in brackish waters.
Sources: Chorus and Bartram (1999), Falconer and Humpage (2006), and Stewart and others (2006).

7.5.2.C TASTE-AND-ODOR COMPOUNDS

There are many potential sources of taste and odor in finished drinking water, including biological activity in the source water, chemical contamination (natural and anthropogenic) of the source water, chemicals used in the treatment processes, and biological activity or materials present in the distribution system. Biological activity associated with naturally occurring algae in source water is among the most common causes of tastes and odors in finished drinking water. Many groups of algae produce taste-and-odor compounds, with approximately 200 compounds identified to date; however, most taste-and-odor problems in drinking water are associated with cyanobacterial production of geosmin and 2-methylisoborneol (MIB) (Wnorowski, 1992; Rashash and others, 1996; Watson, 2003; American Water Works Association, 2004; Taylor and others, 2005).

- The taste-and-odor compounds geosmin and MIB cause earthy and musty tastes and odors and are detectable by humans at concentrations between 5 and 10 nanograms per liter; therefore, these compounds may be detectable in the environment before potential cyanobacterial producers are detected. In addition, the producer may be a relatively small component of the phytoplankton community.
- Taste-and-odor episodes caused by cyanobacteria have occurred even when cyanobacteria are not at detectable levels in the water column (Wnorowski, 1992; Jones and Korth, 1995; Rashash and others, 1996; Watson, 2003; Taylor and others, 2005).
- Most taste-and-odor problems associated with geosmin and (or) MIB are caused by cyanobacteria, but bacteria in the actinomycetes group also may produce geosmin and MIB (Wnorowski, 1992; Rashash and others, 1996; Watson, 2003; Taylor and others, 2005).

Actinomycetes bacteria are not photosynthetic and are not part of the phytoplankton community in lakes and reservoirs. The actinomycetes bacteria largely are terrestrial organisms associated with soils; taste-and-odor compounds typically are produced terrestrially and washed into lakes or reservoirs with or without the bacteria that produced them. Inflow events with high suspended-sediment loads may result in taste-and-odor episodes caused by geosmin and (or) MIB produced by actinomycetes bacteria (Zaitlin and others, 2003; Zaitlin and Watson, 2006). When washed in, actinomycetes bacteria generally are associated with the sediment. However, taste-and-odor compounds produced by actinomycetes bacteria can be located in the water column and throughout the lake or reservoir, depending on whether the compounds are dissolved or associated with the bacteria, hydrology of inflows and mixing with surrounding water, and sediment settling rates. Because of settling, actinomycetes may accumulate in the hypolimnion or near the bottom of lakes or reservoirs. It currently (2008) is unknown if or for how long terrestrial actinomycetes bacteria remain metabolically active in aquatic environments (Wnorowski, 1992; Rashash and others, 1996; Watson, 2003; Zaitlin and others, 2003; Taylor and others, 2005; Zaitlin and Watson, 2006).

Bacteria in the actinomycetes group, in addition to cyanobacteria, may produce geosmin and MIB.

The actinomycetes bacteria are difficult to adequately sample, identify, and measure. Like cyanobacteria, the actinomycetes may produce geosmin and MIB simultaneously, but production is strain specific (Wnorowski, 1992; Watson, 2003; Zaitlin and others, 2003; Zaitlin and Watson, 2006). Total actinomycetes typically are measured, rather than being identified at the genera or strain level. Strain isolation and culture are required to conclusively determine actinomycetes production of geosmin and (or) MIB.

TEMPORAL AND SPATIAL VARIABILITY OF CYANOBACTERIA 7.5.3

The temporal and spatial variability of cyanobacterial populations depend on lake or reservoir hydrology, morphology, geography, water chemistry, and biological interactions. Spatiotemporal occurrence patterns are unique to individual lakes, and generalizations are difficult because of the large degree of variability among lakes. Likewise, a diverse range of physical, chemical, and biological factors may potentially limit algal growth, and no one variable is an unequivocal link to cyanobacterial bloom formation. Long-term patterns may emerge in lakes that have been studied for several years for when, where, and under what conditions cyanobacterial blooms typically develop; or cyanobacterial blooms may occur only sporadically (Reynolds, 1984, 1998; Chorus and Bartram, 1999; Wetzel, 2001; Falconer, 2005).

- Local knowledge of general lake limnology and cyanobacterial bloom occurrence will enhance the ability to select appropriate sampling times and locations to meet study objectives.
- If historical data are unavailable, initial sampling schedules may be more intensive until general patterns in occurrence begin to emerge.

Cyanobacterial toxins and taste-and-odor compounds occur in a particulate phase and in a dissolved phase. Combined, the particulate (intracellular) and dissolved (extracellular) phases comprise total toxin or taste-and-odor concentrations. Particulate, dissolved, and total toxin and taste-and-odor compound concentrations may all be measured; however, measured total and particulate concentrations are dependent on the efficacy of extraction methods (Graham and others, 2008).

- The **particulate phase** comprises compounds that are maintained intracellularly by the cyanobacteria. Intracellular toxin and taste-and-odor concentrations are strain dependent and may vary by several orders of magnitude between strains. Laboratory studies with individual strains indicate that spatial and temporal variability in toxin and taste-and-odor concentrations are likely due to changes in cyanobacterial abundance and strain composition rather than changes in intracellular content (Rashash and others, 1996; Orr and Jones, 1998; Chorus, 2001; Long and others, 2001; Oudra and others, 2002). Because production of toxins and taste-and-odor compounds is strain specific, cyanobacterial abundance in mixed field populations typically is not a good indicator of toxin and (or) taste-and-odor occurrence and concentration.
- The **dissolved phase** comprises extracellular compounds that have been released into the water column either actively by healthy cells or passively upon cell lysis and death. Cyanobacterial toxins generally are maintained intracellularly until cell lysis and death. Thus, dissolved toxin concentrations typically remain low until populations either begin to senesce naturally or are lysed by management practices, such as application of algaecides.

Because cyanobacterial toxins typically are maintained intracellularly, toxins generally have a similar spatial distribution as the cyanobacteria (Chorus and Bartram, 1999; Falconer, 2005). Theoretically, the partitioning of cyanobacterial toxins between the particulate and dissolved phases will change with time as population density increases and declines; particulate concentrations will track overall changes in cyanobacterial population density, whereas dissolved concentrations will remain relatively low until population density begins to decline.

The general pattern in the temporal distribution of total, particulate, and dissolved cyanobacterial toxin concentrations, with respect to cyanobacterial population density, is illustrated in figure 7.5–4. Field observations indicate that this pattern occurs in lakes and reservoirs (Jones and Orr, 1994; Heresztyn and Nicholson, 1997; Chiswell and others, 1999; Chorus and Bartram, 1999); however, many other patterns also may occur because toxic and non-toxic strains of cyanobacteria can co-occur and will not necessarily have the same population dynamics.

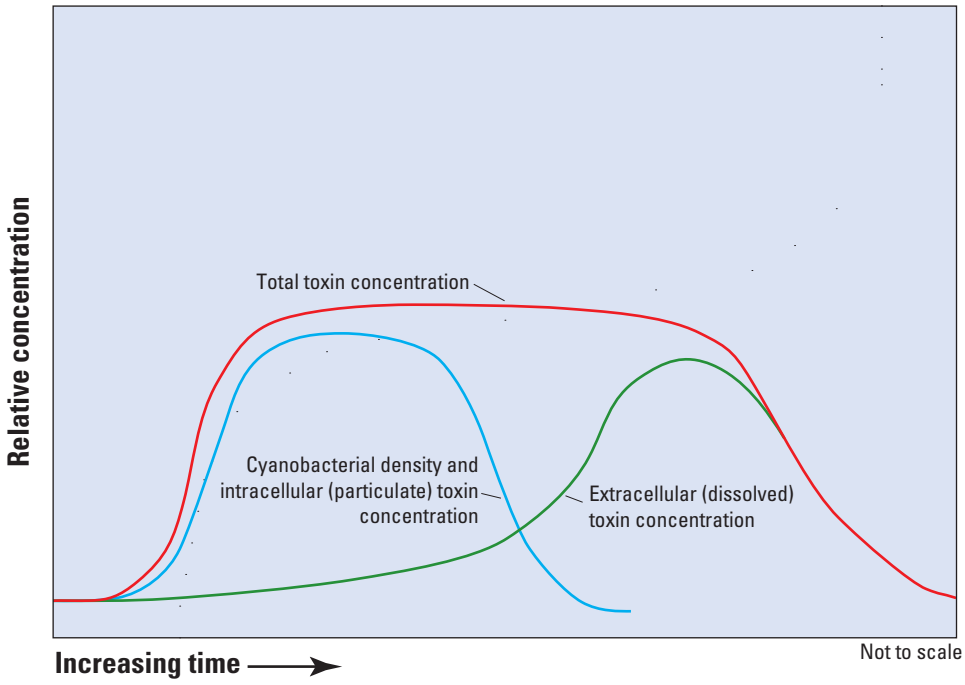


Figure 7.5–4. Theoretical temporal distribution of total, particulate, and dissolved cyanobacterial toxin concentrations with respect to cyanobacterial population density. From Graham and others (2008).

The partitioning of taste-and-odor compounds between the particulate and dissolved phases is more complex because healthy cyanobacterial cells either can maintain these compounds intracellularly or release them into the environment; therefore, taste-and-odor compounds are not necessarily associated with the cyanobacteria and may or may not have similar spatial distributions (Rashash and others, 1996; Watson, 2003; Taylor and others, 2005).

The environmental fate and transport of cyanobacterial toxins and taste-and-odor compounds under natural lake or reservoir conditions have not been well studied. Available laboratory data indicate that photolysis and biodegradation are the most likely mechanisms for the degradation of cyanobacterial by-products in lakes and reservoirs (Graham and others, 2008). Based on field observations, dissolved-phase toxins and taste-and-odor compounds may persist for several days to weeks after the decline of a cyanobacterial population (Jones and Orr, 1994; Heresztyn and Nicholson, 1997; Chorus and Bartram, 1999). The persistence of toxins and taste-and-odor compounds, coupled with the potential for transport, may lead to spatial or temporal uncoupling of cyanobacteria and their by-products.

TEMPORAL VARIABILITY 7.5.3.A

Seasonal patterns in cyanobacterial abundance and community composition are affected substantially by temperature, solar irradiance, and nutrient supply (Wetzel, 2001). Cyanobacterial populations tend to peak between midsummer and early fall when water temperatures are at seasonal maxima and nutrient concentrations are at seasonal minima; however, cyanobacteria may remain abundant year-round, even in temperate lakes or reservoirs, or peak under ice during winter. Cyanobacterial populations also may vary on much shorter time scales (hours or days). Whereas cyanobacterial abundance may increase fairly rapidly (days), vertical migration, entrainment in temporary circulation cells, or wind movement of surface accumulations may rapidly change the areal distribution or water-column location of cyanobacteria within the lake or reservoir, but not its overall abundance. Knowledge of local conditions, including patterns in circulation, mixing, and prevailing winds, will enhance the overall design of a cyanobacteria study and aid in the interpretation of observed changes in cyanobacterial abundance and community structure (Graham and others, 2008).

SPATIAL VARIABILITY 7.5.3.B

Some knowledge of the distribution of cyanobacteria, as affected by hydrology, morphology, and typical conditions throughout a lake or reservoir is required before sites are selected for toxin and taste-and-odor studies. Cyanobacterial movement affects distribution in the water column, and wind movement of surface accumulations may have a substantial effect on where cyanobacteria are located within a lake or reservoir; however, the general location of cyanobacteria largely is affected by lake or reservoir hydrology and morphology and their effects on circulation patterns. Cyanobacterial populations may be distributed evenly throughout a lake or reservoir or have an irregular distribution because of currents and prevailing winds (fig. 7.5–5). Statistically significant differences in toxin concentrations have been detected within sampling distances of 10 m (Carmichael and Gorham, 1981; Lanaras and others, 1989; Kotak and others, 2000), although extreme spatial variation is not necessarily typical (Jungmann and others, 1996; Graham and others, 2006).

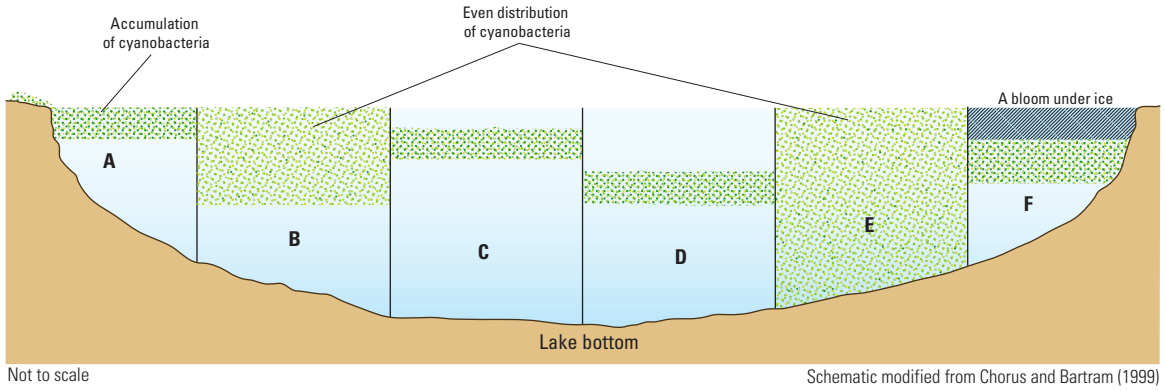
The spatial distribution of cyanobacteria may change rapidly because of changing hydrologic conditions, such as new circulation patterns or inflow events. After heavy rains, cyanobacteria in the reservoir shown in figure 7.5–5 were washed from up-reservoir areas into the main body of the reservoir. The cyanobacterial population in this case was restricted to the old river channel (near the tree line). In larger lakes or reservoirs, certain areas (shallow bays and coves, sites directly affected by nutrient-rich inflows, or structures that affect flow such as dikes, piers, or intake towers) may be more prone to the development of cyanobacterial blooms and surface accumulations. These isolated blooms may remain localized or become spread throughout the lake or reservoir because of inflow events or circulation patterns (Reynolds, 1984; Chorus and Bartram, 1999; Wetzel, 2001; Falconer, 2005).

Figure 7.5–5. Example of the spatial variability of cyanobacteria within a reservoir. From Graham and others (2008).

The ability of many toxin and taste-and-odor producing cyanobacteria to control their position in the water column needs special consideration when sampling for these compounds. The vertical distribution of cyanobacteria may vary widely over relatively short (hours or days) periods of time (Reynolds, 1984; Chorus and Bartram, 1999; Falconer, 2005). Sample location in the water column relative to cyanobacterial distribution may substantially affect cyanobacterial community composition, toxin, and (or) taste-and-odor results. For example, if cyanobacteria are maintaining a position at depth in the water column and surface samples are collected, toxins or taste-and-odor compounds may not be detected even when present. There are six general water-column distributions of cyanobacteria; detailed descriptions and occurrence information are given in figures 7.5–6 and 7.5–7:

1. Shoreline, near-shore, or open-water surface accumulations and scums (fig. 7.5–6*A* and fig. 7.5–7*A*). Surface accumulations and scums are of greatest concern for exposure to high concentrations of cyanobacterial toxins during recreational activities.
2. Even distribution throughout the photic zone or epilimnion (fig. 7.5–6*B* and fig. 7.5–7*B*).
3. Specific depth in the photic zone (fig. 7.5–6*C* and fig 7.5–7*C*). In shallow lakes or reservoirs where light penetrates to the bottom (photic depth equals maximum depth), cyanobacteria may be located on or near the bottom.

4. Metalimnetic bloom (fig. 7.5–6*D*, fig. 7.5–7*D*, fig. 7.5–8). This is a special case of a population maintaining a specific depth in the photic zone. Metalimnetic blooms are evident by sharp increases in oxygen at depth, typically near the interface of the epilimnion and metalimnion (figs. 7.5–1 and 7.5–8). Metalimnetic blooms are of particular concern in drinking-water supplies because the populations are not visibly evident and may be located at the same depth as the drinking-water intake.
5. Even distribution throughout the water column (fig. 7.5–6*E*; fig. 7.5–7*E*).
6. Under ice (fig. 7.5–6*F*; fig. 7.5–7*F*).

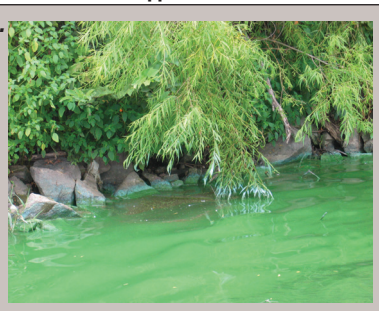


EXPLANATION

Potential water column distributions of cyanobacteria

- **A** Shoreline, near-shore, and open water accumulations and scums
- **B** Even distribution throughout the photic zone or epilimnion
- **C** Specific depth in the photic zone
- **D** Metalimnetic bloom (special case of **C**)
- **E** Even distribution throughout the water column
- **F** Under ice bloom

Figure 7.5–6. Potential water-column distributions of cyanobacteria. From Graham and others (2008).

Appearance	Location	Description	Occurrence
A.  	Shoreline, near-shore and open-water surface accumulations and scums.	Discoloration of the water surface; typically blue-green or bright green but may also be brown or red[1]. May be a thin layer near the surface or be thick or paint-like. Accumulations may extend from the surface to depths of one meter or more. Dissolved oxygen may be supersaturated near the surface.	Develop when existing cyanobacterial population accumulates near the water surface. May occur in open-water or near-shore areas. May rapidly move or dissipate with changes in wind speed and direction. May occur during calm periods or during the decline of a cyanobacterial population. May be widespread or have a patchy distribution depending on wind and circulation patterns.
B. 	Dispersed in photic zone.	Water likely has an obvious color; typically blue-green or bright green but may also be brown or red. Large colonies and filaments may be visible.	Relatively calm conditions. May occur in well-mixed or stably stratified water column.
	Dispersed in epilimnion. Appearance will vary depending on the type and abundance of cyanobacteria.	Water may or may not have obvious color; typically blue-green or bright green but may also be brown or red. Large colonies or filaments may or may not be visible.	Stably stratified water column. Breezy, windy conditions.

[1]From a distance, duckweed *(Lemna)* may be confused with a cyanobacterial bloom; duckweed has a bright green color and may cover large areas. Duckweed is a plant with visible leaves, and up close, duckweed is easily distinguished from cyanobacteria. Duckweed typically proliferates in shallow, nutrient rich ponds, bays, and small lakes/reservoirs. Examples of duckweed appearance are available on the internet at http://www.mobot.org/jwcross/duckweed/duckpix.htm and http://aquaplant.tamu.edu/database/floating_plants/common_duckweed_pics.htm.

All photographs by J.L. Graham, U.S. Geological Survey, with the exception of (***E***), courtesy of Kansas Department of Health and Environment, and (***F***), courtesy of an anonymous photographer.

Figure 7.5–7. The appearance, location, description, and occurrence of cyanobacteria in lake and reservoir water: (***A***) shoreline, near-shore, and open-water surface accumulations or scums, (***B***) dispersed in photic zone and the epilimnion, (***C***) specific depth in photic zone, (***D***) metalimnetic bloom, (***E***) even distribution throughout the water column, and (***F***) under ice. From Graham and others (2008).

	Appearance	Location	Description	Occurrence
C.		Specific depth in photic zone. Appearance will vary. Here the cyanobacteria were near the water surface during early morning.	Water may or may not have obvious color. Large colonies and filaments may or may not be visible. Specific location in the photic zone may be difficult to determine without discrete samples.	Relatively calm conditions. May occur in well-mixed or stably stratified water column.
			Caution: In shallow lakes where light penetrates to the bottom (photic depth is greater than maximum depth) cyanobacteria may be located on or near the bottom.	
D.		Metalimnetic bloom (special case of a population maintaining a specific depth in the photic zone). Metalimnetic blooms are not visible at the water surface; they can be detected by a sharp increase in dissolved oxygen at depth.	Not visible from the surface. Evident by a sharp increase in dissolved oxygen at depth, typically near the interface of the epilimnion and the metalimnion.	Stably stratified water column. Intermediate trophic status (mesotrophic). Photic zone penetrates into the metalimnion.
E.		Even distribution throughout the water column. Appearance will vary depending on the type and abundance of cyanobacteria.	Water may or may not have obvious color; typically blue-green or bright green but may also be brown or red. Large colonies or filaments may or may not be visible.	Well-mixed conditions in: Shallow lakes/reservoirs; Deeper lakes/reservoirs that do not typically stratify; Autumn, winter, and spring conditions in temperate lakes/reservoirs that stratify.
F.		Under ice.	May be visible under ice depending on ice thickness; color is typically blue-green or red. Typically visible when ice plug is removed.	Ice clarity and thickness allows adequate light penetration.

All photographs by J.L. Graham, U.S. Geological Survey, with the exception of (***E***), courtesy of Kansas Department of Health and Environment, and (***F***), courtesy of an anonymous photographer.

Figure 7.5–7. The appearance, location, description, and occurrence of cyanobacteria in lake and reservoir water: (***A***) shoreline, near-shore, and open-water surface accumulations or scums, (***B***) dispersed in photic zone and the epilimnion, (***C***) specific depth in photic zone, (***D***) metalimnetic bloom, (***E***) even distribution throughout the water column, and (***F***) under ice. From Graham and others (2008).—Continued

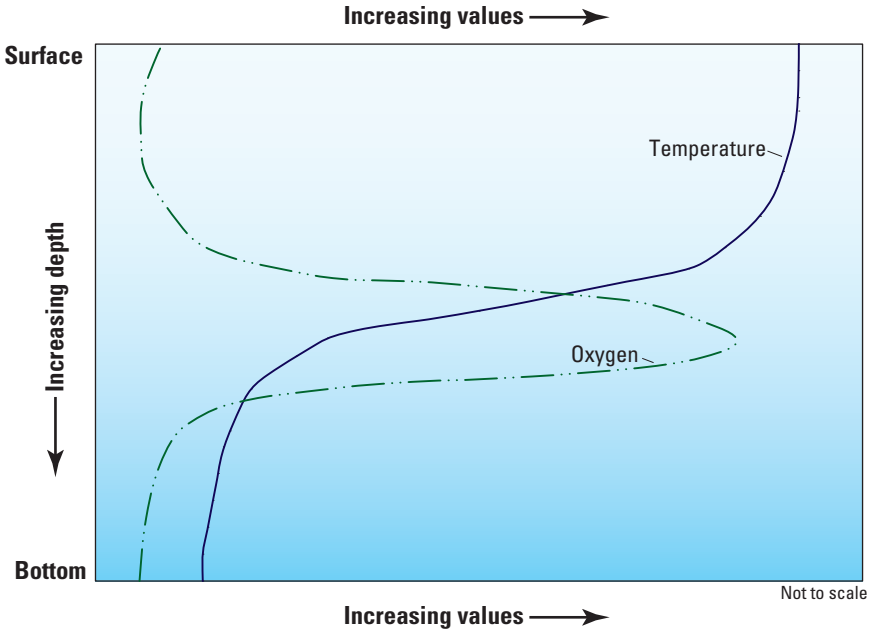


Figure 7.5–8. Graphical representation of the temperature and oxygen gradients that will develop in the water column when a metalimnetic cyanobacterial bloom is present. From Graham and others (2008).

Once toxins and taste-and-odor compounds are released by cyanobacteria into the dissolved phase, they will become uniformly distributed throughout the mixed layer in which they are released; this typically is a rapid process, but large localized concentrations may develop in hydraulically isolated areas such as coves (Jones and Orr, 1994). Generally, the distribution of dissolved compounds in the water column will depend on the layer in which the cyanobacteria were located when the compounds were released. In stratified lakes or reservoirs, cyanobacteria most likely are in the epilimnion or, less frequently, the metalimnion. Dissolved toxin and taste-and-odor samples usually can be collected from the same water column location as samples for intracellular (particulate) analysis (Chorus and Bartram, 1999; Falconer, 2005); however, high taste-and-odor concentrations may occasionally occur in the hypolimnion of stably stratified lakes or reservoirs (Carpenter, 2002; Mau and others, 2004).

7.5.4 STUDY OBJECTIVES AND DESIGNS

Averett and Schroder (1994) describe three general types of surface-water-quality studies: reconnaissance studies, monitoring studies, and interpretive studies. Cyanobacterial toxin and taste-and-odor studies are discussed with respect to these three study types. The keys to a well-designed study include using the scientific method, a well-defined problem, clear objectives, and an appropriate approach.[4] Study objectives will dictate what variables need to be measured; the ancillary data to be collected (7.5.5.F), and when, where, and how samples will be collected.

[4]A comprehensive treatment of the fundamentals of how to design water-quality studies is beyond the scope of NFM 7.5 but can be found in reports such as Friedman and Erdmann, 1982; Shampine and others, 1992; and Averett and Schroder, 1994. The information provided in NFM 7.5 is intended to be used in conjunction with these and other reports that specifically address the design of surface-water-quality studies.

The study of cyanobacterial toxins and taste-and-odor compounds is an active area of developing research, but many fundamental questions remain about the occurrence, environmental causes, and ecological consequences of these compounds (Chorus, 2001). General study objectives and guidelines on when, where, and how to sample are described below for reconnaissance, monitoring, and interpretive studies.[5] The information given is intended to illustrate how the information presented on the spatial and temporal variability of cyanobacteria and cyanobacterial blooms can be used to guide and enhance the design of field studies.

- **Reconnaissance studies**. Reconnaissance studies often assess the occurrence, distribution, and concentration of cyanobacteria and associated toxins and taste-and-odor compounds. Such studies may focus on spatial and (or) temporal variability at a range of scales, from single systems to States or regions. Studies of lakes or reservoirs used for recreation typically emphasize cyanobacterial toxins, whereas studies of lakes or reservoirs used for drinking-water supply may emphasize taste-and-odor compounds and toxins. General study objectives and guidelines describing when, where, and how samples typically are collected for reconnaissance studies are presented in table 7.5–3. An example of a study design, approach, and field form for a reconnaissance study is given in Appendix 7.5–A.

Table 7.5–3. Objectives and guidelines describing when, where, and how samples typically are collected for reconnaissance studies.

General objective	Site location	Sampling frequency	Sample type
Regional Studies			
Spatial Variability • Emphasis on presence/absence	• Single representative site, typically an open, deep-water site • Site will be determined based on the location of surface accumulations and scums	• Single point in time when most cyanobacterial-related issues occur • During known surface bloom events	• Integrated photic zone • Integrated epilimnion • Surface sample • Surface sample
Spatial and Temporal Variability • Emphasis on presence/absence and changes in concentration with time	• Single representative site, typically an open, deep-water site	• Multiple times during the period when most cyanobacterial-related issues occur – Weekly – Bi-weekly – Monthly – Annually	• Integrated photic zone • Integrated epilimnion • Surface sample
Single-System Studies			
Spatial Variability • Emphasis on presence/absence	• Multiple sites	• Single point in time when a cyanobacterial bloom is occurring	• Integrated photic zone • Integrated epilimnion • Integrated water column • Surface sample
Spatial and Temporal Variability • Emphasis on presence/absence and changes in concentration over time	• Multiple sites	• Multiple times during the period when most cyanobacterial-related issues occur – Weekly – Bi-weekly – Monthly	• Integrated photic zone • Integrated epilimnion • Integrated water column • Surface sample
• Emphasis on spatial changes within the water body or water column over relatively short periods of time	• Single representative site • Multiple sites	• Multiple points in time when a cyanobacterial bloom is occurring – Hourly – Daily	• Integrated photic zone • Integrated epilimnion • Integrated water column • Surface sample • Discrete depth

Sources: Chorus and Bartram (1999), Falconer (2005), and Wetzel and Likens (2000).

[5]A comprehensive list of all possible types, sampling approaches and methods, or site-selection criteria for studies of toxins and taste-and-odor compounds in lakes and reservoirs is beyond the scope of NFM 7.5.

- **Monitoring studies**. Monitoring studies often are conducted to evaluate the potential for human health risks and taste-and-odor events associated with cyanobacterial toxins and taste-and-odor compounds. Monitoring studies typically determine the concentration of cyanobacterial toxins and taste-and-odor compounds in areas where exposure is most likely to occur, such as popular swimming areas or drinking-water intakes. Recreational areas are monitored for cyanobacterial toxins to assess human health risks; results often are used to make decisions about posting warnings and closing recreational areas. Drinking-water intakes are monitored for taste-and-odor compounds and (or) toxins to assess the potential for taste-and-odor events and human health risks; results often are used to guide drinking-water treatment processes, such as use of activated carbon. Guidelines describing when, where, and how samples typically are collected for monitoring studies are presented in table 7.5–4. An example of a study design, approach, and field form for a monitoring study is given in Appendix 7.5–B.

Table 7.5–4. Objectives and guidelines describing when, where, and how samples typically are collected for monitoring studies.

General objective	Site location	Sampling frequency	Sample type
Recreational Areas	• Beaches • Open water areas used for full-body contact recreation • Bay or cove areas used for full-body contact recreation • Public access sites	• Routine basis during periods of peak recreational use – Daily – Weekly	• Surface sample • Integrated photic zone
Drinking-Water Supplies	• Location relevant to the drinking-water intake(s)	• Routine basis – Daily – Weekly • During periods when events have historically occurred • During events	• Discrete depth • Integrated photic zone • Integrated epilimnion • Integrated water column

Sources: Chorus and Bartram (1999), Falconer (2005), and Wetzel and Likens (2000).

- **Interpretive studies**. Interpretive studies often are conducted to assess the processes that affect the spatial and temporal distribution and abundance of cyanobacterial toxins and taste-and-odor compounds. Interpretive studies of toxins and (or) taste-and-odor compounds may include, but are not limited to, the assessment of the physical, chemical, and biological factors affecting occurrence and concentration, environmental fate and transport studies, and toxicological studies.

  — **Occurrence and concentration studies** assess the environmental factors affecting cyanobacteria and often are focused on developing empirical models (Mau and others, 2004; Christensen and others, 2006). Several years of study, with data collection over a range of hydrologic and meteorologic conditions, often are required before true patterns begin to emerge. Long-term studies are required to develop real-time water-quality and other predictive models to provide early warning of the potential occurrence and (or) concentration of cyanobacterial toxins and taste-and-odor compounds. Reliable real-time water-quality models and other predictive tools will allow resource managers to respond more effectively to cyanobacterial blooms. Table 7.5–5 presents general study objectives and guidelines describing when, where, and how samples typically are collected for studies that assess the environmental factors affecting the occurrence and concentration of cyanobacterial by-products. An example of a study design, approach, and field form for an interpretive study is given in Appendix 7.5–C.

— **Fate-and-transport and toxicological studies** are crucial to the overall understanding of environmental and public health risks associated with cyanobacterial toxins. Determination of the environmental fate and transport of cyanobacterial toxins and taste-and-odor compounds generally involves examining development, movement, persistence, and degradation of cyanobacterial populations, toxins, taste-and-odor compounds, and associated degradates within the lake or reservoir environment (for example, movement to downstream drinking-water intakes) and among its ecosystem compartments. Fate-and-transport and toxicological studies frequently require collection of water-column samples from lakes and reservoirs. In addition, these studies often involve the sampling of sediments, benthic organisms, and tissues from fish and other aquatic and terrestrial organisms; however, few data are available regarding the partitioning of cyanobacterial toxins and taste-and-odor compounds in these ecosystem compartments and sampling protocols and analytical methods have not been, or currently (2008) are being, developed. Discussion or examples of how to collect the samples needed from these media is beyond the scope of NFM 7.5.

Table 7.5–5. Objectives and guidelines describing when, where, and how samples typically are collected for interpretive studies assessing the physical, chemical, and biological factors affecting the occurrence and concentration of cyanobacteria and associated toxins and taste-and-odor compounds.

General objective	Site location	Sampling frequency	Sample type
• Environmental factors influencing spatial and (or) temporal occurrence • Real-time estimation of occurrence/concentration • Predictive models	• Single representative site, typically an open, deep-water site – Sites for drinking-water studies are typically located near intakes • Multiple sites – Sites where cyanobacterial blooms are known to initiate – Sites where cyanobacteria are typically abundant – Inflow sites[1]	• Routine basis – Weekly – Bi-weekly – Monthly	• Integrated photic zone • Integrated epilimnion • Integrated water column • Discrete depth
	• Sites where surface accumulations/scums are located	• Event samples – Sampling plans need to be flexible enough to respond to events	• Surface sample

[1]Monitoring of major inflows is essential in the development of predictive models and beneficial in the development of models for real-time estimation of occurrence/concentration. Standard U.S. Geological Survey protocols for sampling streams and rivers should be used when sampling inflows (NFM 4). Cyanobacterial samples and samples for toxin and taste-and-odor analysis may or may not be analyzed at inflow sites, depending on conditions; however, actinomycetes samples (NFM 7.1) must be collected.

Sources: Chorus and Bartram (1999), Christensen and others (2006), Falconer (2005), and Wetzel and Likens (2000).

7.5.5 SAMPLE COLLECTION

The sample collection approaches described below are appropriate for samples to be analyzed for cyanobacterial community composition (enumeration and identification) and total, dissolved, and particulate cyanobacterial toxins and taste-and-odor compounds. Site selection and sampling approach (sampling frequency, location, time of day samples are collected, sample types, samplers used, and sample holding times) need to be carefully considered with respect to the specific objectives of the study. In this regard, special consideration needs to be given to the areal and water-column distribution of the cyanobacteria in the lake or reservoir when deciding where and how to collect samples; the sampling location needs to be relevant to where the cyanobacterial community is located.

In general, **toxin and taste-and-odor samples must be shipped within 24 to 48 hours**, and sample collection should be planned accordingly. Additional considerations to be incorporated in the study sampling plan include the maintenance of sample quality under adverse conditions of site logistics, climate, or weather. When planning the project, project staff should be aware, for example, of sample holding times when shipping to or from areas of the country where adverse weather conditions, such as severe thunderstorms, flooding, or blizzards, may delay shipping or receipt of samples. Facilities may need to be modified to hold samples for cyanobacterial analysis under controlled conditions to avoid sample exposure to extreme temperatures in the event of shipment delays. If shipping delays are anticipated, contact the analyzing laboratory for information on how to hold samples. Data storage and management also are an essential component for which appropriate plans need to be made, as well as arranging for an adequate level of database development and management.

Three general types of water-quality samples are typically collected from lakes and reservoirs: surface samples, discrete-depth samples, and depth-integrated samples. For each sample type, a single grab sample may be collected or multiple grab samples may be composited. The types of sampling devices (samplers) commonly used to collect each type of sample are listed in table 7.5–6. Detailed descriptions of the samplers and proper uses are presented in NFM 2.1.1.B, NFM 4.1.3.B, NFM 4.1.3.C, Britton and Greeson (1987), and U.S. Environmental Protection Agency Standard Methods Sections 1060 and 10200 (American Public Health Association, 2005) and the advantages and disadvantages of each sampler type are presented in NFM 7.4, table 7.4–8. It is important that samples be collected in a manner that does not rupture or deform cyanobacterial cells, particularly when analyzing for species composition and particulate and dissolved toxin and taste-and-odor concentrations. In general, sampling devices and churns have a minimal impact on cyanobacterial cell integrity; exceptions are discussed in more detail below and in NFM 7.4, table 7.4–8. **Because toxins and taste-and-odor compounds are organic compounds, the samplers and churns used to collect and composite samples must be made of fluorocarbon polymers, such as Teflon®; metals, such as stainless steel; or glass (NFM 2.0.1).**

Table 7.5–6. Samplers commonly used to collect surface, discrete-depth, and depth integrated samples in lakes and reservoirs.

Sample type	Sampler
Surface	• Hand-held open mouth bottle sampler • Weighted bottle sampler (US WBH-96)
Discrete depth	• Kemmerer bottle • Van Dorn bottle – horizontal or vertical • Pump – diaphragm or peristaltic[1]
Depth integrated	• Kemmerer bottle • Van Dorn bottle – vertical • Teflon stop-cock bailer • Pump – diaphragm or peristaltic[1]

[1]This sampling method may rupture or deform cyanobacterial cells and the advantages and disadvantages of this method (discussed in NFM 7.4, table 7.4–8) must be carefully considered when collecting samples for the analysis of species composition or particulate and (or) dissolved toxin and taste-and-odor concentrations.

General field procedures are the same, regardless of whether surface, discrete-depth, or depth-integrated grab or composite samples are collected. Sample location and the method used to collect samples may be dependent on water-column stratification and the location of cyanobacteria. Where and how to collect samples often are decided in the field after an initial assessment of the presence of phytoplankton (including cyanobacteria) in the water column.

- The distribution of phytoplankton (and cyanobacteria) in the water column often can be determined by a combination of visual assessment, light profiles to determine photic depth, and temperature, dissolved oxygen, pH, and *in vivo* fluorescence (chlorophyll and (or) phycocyanin, a light-gathering pigment unique to cyanobacteria, NFM 7.4.2) profiles to determine stratification, mixed depth, and photosynthetic activity (figs. 7.5–6, 7.5–7, and 7.5–8).
- Signs of photosynthetic activity and phytoplankton (including cyanobacteria) location in the water column include:
  - Sharp increases in pH and dissolved-oxygen (DO) concentration (for example, see fig. 7.5–8).
  - Increased fluorescence of chlorophyll and (or) phycocyanin.

Because pH and DO may increase as a result of photosynthesis, these measurements will give more distinct signals under bright, sunny conditions. Conversely, fluorescence is inhibited by light (NFM 7.4.2.C) and signals will be more distinct during overcast or dark conditions (such as apparent increases in fluorescence in the aphotic zone or at night). **Profile results must be carefully interpreted with respect to weather conditions and time of sampling.** Cyanobacteria often will maintain a position in the photic zone, regardless of mixed depth; therefore, if cyanobacterial distribution in the water column cannot be conclusively determined, an integrated photic-zone sample will likely be fairly representative (Graham and others, 2008).

Signs of phytoplankton activity often are used to select sampling depths. The location of decomposing phytoplankton also may be discernable, particularly after a substantial bloom.

- Increased pH and DO during daylight hours or *in vivo* fluorescence (an estimate of chlorophyll or phycocyanin) are signs of phytoplankton activity.
- Sharp declines in pH or DO, particularly after a substantial bloom, are signs of phytoplankton decomposition.
- Intracellular compounds, including toxins and taste-and-odor compounds, may be released to the water during periods of senescence.

To collect samples for analysis of cyanobacterial community composition (enumeration and identification), toxins, and (or) taste-and-odor compounds follow these guidelines:

1. Before departing for field work, ensure that the field team is familiar with the USGS parts-per-billion protocol (for example, equipment-selection guidelines (NFM 2), equipment decontamination (NFM 3), clean hands/dirty hands (CH/DH) techniques and quality control (NFM 4 and NFM 5), safety requirements (NFM 9), and all other relevant guidance described in the study's sampling, quality-assurance, and work plans). Check that field personnel are prepared to implement such required and recommended procedures.
2. Document in field notes the water color and the presence of any visible cyanobacteria or cyanobacterial accumulations near the surface (fig. 7.5–7), the location and extent of surface accumulations, and any odors that may be associated with cyanobacterial accumulations (commonly earthy/musty, sulfurous, or septic odors).
3. Take photographs of the site, particularly areas with obvious cyanobacterial accumulations.
4. Measure and record a vertical light profile throughout the photic zone (NFM 7.4.1.B). Ideally, photic depth is measured with a light sensor; however, photic depth can be estimated as approximately 2 ½ times the Secchi disc depth (Horne and Goldman, 1994; Wetzel and Likens, 2000).
5. Measure and record vertical profiles of field properties in situ—water temperature, dissolved oxygen, pH, specific conductance, turbidity, and (or) in situ fluorescence (an estimate of chlorophyll or phycocyanin). To measure a vertical profile in a lake or reservoir, lower the sensor into the water and take readings about 1 cm below the water surface. Continue lowering the sensor and take readings every 0.5 to 1 m until the sensor reaches the bottom. More detail on measuring profiles in lakes and reservoirs is given in NFM 7.4.1.B and Wetzel and Likens (2000).
6. Use photic depth and profile data to assess the location of cyanobacteria in the water column (figs. 7.5–6, 7.5–7, and 7.5–8) and select sampling locations. Remember that if the distribution of cyanobacteria in the water column cannot be determined, a depth-integrated photic-zone sample will generally be representative. Record sampling depths and locations on the field sheet.
7. Field rinse all sampling equipment with native water immediately before the equipment is used (NFM 4.1.3).
8. Collect samples while wearing clean gloves; use parts-per-billion CH/DH techniques (NFM 4.0.2). More detail on collecting grab, composite, surface, discrete-depth, and depth-integrated samples is provided in sections 7.5.5.A–7.5.5.D. **Because cyanobacteria may be toxic, always wear gloves and avoid having sample water contact the face**. Other sample collection safety considerations are discussed in section 7.5.8.

9. Process samples (section 7.5.6 and NFM 5). Place samples on ice in the dark immediately after sample collection. As long as samples remain chilled and in the dark, they may be held for several hours before processing. Sample processing procedures typically will depend on instructions from the laboratory at which the samples will be analyzed.
10. Clean equipment. If the sampler will not be reused during a field trip, rinse the sampler components thoroughly with deionized water before they dry and place the sampler in a plastic bag for transport to the office laboratory for cleaning. If the sampler will be reused during the field trip, rinse the sampler components with deionized water before they dry. Field-clean the sampler at the next sampling site (NFM 3) and rinse with native water before use.

If the distribution of cyanobacteria in the water column cannot be determined, collect a depth-integrated photic-zone sample and record this in the field notes.

SINGLE-GRAB AND COMPOSITE SAMPLES 7.5.5.A

Whether a single grab or composite sample is collected will depend on study objectives and the volume of water needed. Compared with grab samples, composite samples are more representative of overall lake or reservoir conditions; however, because of the generally irregular distribution of cyanobacterial communities, concentrations of toxins and taste-and-odor compounds may be diluted when using a sample-composite method (American Public Health Association, 2005). Thus it is important to understand that data interpretation can be biased by the sample-collection and -processing methods used.

Grab Samples

Grab samples are collected from a specific location in the water column. Single grab samples typically are collected when:

- Spatial variability is not a concern, or multiple grab samples are being collected and analyzed separately to describe spatial variability, and the volume of water required for analyses does not exceed the volume of the sampling device.

  Example—When collecting a sample from a single representative location in a lake or reservoir.

- Temporal variability is not a concern, or multiple grab samples are being collected and analyzed separately to describe temporal variability, and the volume of water required for analyses does not exceed the volume of the sampling device.

  Example—When collecting samples on a weekly basis.

Composite samples

Composite samples are collected by combining multiple grab samples. Composite samples typically are collected when:

- The volume of water required for analyses exceeds the volume of the sampling device. In this case, multiple grab samples typically are collected from the same location.

  Example—A composite sample composed of five integrated photic zone samples collected from a single open-water location in a lake or reservoir.

- Spatial variability is a concern. Spatial composites are comprised of multiple grab samples collected from different locations either within the water column or throughout a given area of a lake or reservoir.

  Example—A composite sample composed of single grab samples, collected every meter from the lake or reservoir surface to the bottom; or single grab samples collected from 20 locations within a recreational area.

- Temporal variability is a concern. Temporal composites are composed of multiple grab samples collected during different times.

  Example—A composite sample composed of single grab samples collected every 15 minutes over a period of 2 hours. Temporal composites typically are not collected for cyanobacterial toxin and taste-and-odor studies.

7.5.5.B SURFACE SAMPLES

Open-mouth samplers typically are used to collect surface samples (NFM 2.1.1.B, 4.1.1.B, table 7.5–6). Wide-mouthed Teflon or glass bottles are used to collect samples for analysis of toxins and (or) taste-and-odor compounds.[6] Samples for cyanobacterial (phytoplankton) community composition (enumeration and identification) can be collected into polyethylene bottles, but the bottle must be triple-rinsed with native water before use (table 7.5–7). If surface samples are collected in open-water areas, samples need to be collected 0.5 to 1.0 m below the lake or reservoir surface to avoid substances in the surface film that may interfere with analyses (American Public Health Association, 2005). An exception to this would be if surface accumulations or scums are being sampled; then collection at the water surface is appropriate. Samples are collected with an open-mouth sampler and composited into a Teflon churn (see NFM 2) if the volume of water required for analyses exceeds the volume of the sampling bottle.

Study objectives must be considered when determining how to sample surface accumulations and scums, since cyanobacterial density can vary widely across the area of accumulation.

- If a general idea of maximum toxin and (or) taste-and-odor compound concentration is desired, collect a sample from the thickest part of the accumulation or scum.

[6]Some laboratories may recommend using polyethylene bottles for toxin samples, especially when samples are being frozen. Small amounts of toxins, particularly the microcystins, are known to sorb to polyethylene bottles; nevertheless, use of polyethylene still is common.

- If an average concentration in the accumulation or scum is desired, composite multiple surface samples from throughout the area into a churn. A grid or transect approach often is used for this type of collection (American Public Health Association, 2005). The number of samples used for a composite will depend on study objectives and the size of the accumulation or scum.
- If information on spatial heterogeneity is desired, collect surface grab samples throughout the accumulation or scum area, keeping them separate. Do not composite these samples.

Table 7.5–7. Bottle types and volumes commonly used for toxin, taste-and-odor, and cyanobacterial (phytoplankton) community composition (enumeration and identification) samples.

[The bottle types and volumes described here are for general information; bottle type and volume will ultimately depend on instructions from the laboratory at which the samples will be analyzed. The specific instructions from the analyzing laboratory on sample bottles and volumes should be followed carefully.]

Analysis	Bottle type(s)	Bottle volume(s), in milliliters
Toxin	• Baked amber glass • Teflon • Polyethylene[1]	500–1,000
Taste and odor	• Baked amber glass • Amber glass septum vials	125–250 10–50
Cyanobacterial community composition	• Amber glass[2] • Polyethylene[1]	125–1,000

[1]Small amounts of toxins, particularly the microcystins, are known to sorb to polyethylene bottles, but polyethylene is still commonly used. **If a polyethylene bottle is used for toxin sample collection it should be triple rinsed with native water prior to use.**
[2]Amber bottles are preferred but clear bottles may be used as long as the samples are immediately preserved and kept in the dark.

DISCRETE-DEPTH SAMPLES 7.5.5.C

Discrete-depth samples typically are collected when the location of the cyanobacterial community is known, when there is a structure of interest at depth (such as a drinking water intake), or when the vertical water-column distribution of the cyanobacterial community and associated toxins and taste-and-odor compounds is being studied in detail. Thief samplers, such as Kemmerer or Van Dorn bottles, are commonly used to collect discrete-depth samples, although pumps also may be used (table 7.5–6; NFM 2.1.1.B, Thief samplers; NFM 4.1.3).

Kemmerer and Van Dorn bottles are cylindrical tubes that have stoppers at each end and a mechanism that closes the sampler at depth (Britton and Greeson, 1987); these samplers come in a range of sizes from approximately 0.5 L to 10 L, and are made from a variety of materials including Teflon and stainless steel. The key difference between Kemmerer and Van Dorn bottles is the location of the closure mechanism: the closure mechanism of a Kemmerer bottle is inside the bottle (NFM 2.1.1.B, fig. 2–2*A*) and the closure mechanism of a Van Dorn bottle is outside the bottle (NFM 2.1.1.B, fig. 2–2*B*). Because the closure mechanism of the Van Dorn bottle is on the outside, these bottles may be used in either the vertical or horizontal position (operation in the horizontal position captures a narrower depth range than operation in the vertical position).

To operate a Kemmerer or Van Dorn bottle:

1. Check to ensure the closure mechanism is working.
2. Open the sampler and slowly lower (do not drop) the bottle to the desired depth.
3. Trip the closure mechanism and bring the sample to the surface.
4. Draw off water into a Teflon churn by means of the nozzle in the lower stopper.
5. Repeat the above steps until the volume of water required for analyses has been collected.

Pumps (NFM 2.1.1.B and 4.1.1.B) allow greater speed of collection and are particularly useful when large quantities of water are required; however, pumps may damage cyanobacteria and other organisms and are not the best option when analyzing for species composition and particulate and dissolved toxin and taste-and-odor concentrations. The advantages and disadvantages of pumps (NFM 7.4, table 7.4–8) need to be considered carefully before use. Select a diaphragmatic or peristaltic pump in order to minimize damage to cyanobacteria during sample collection (American Public Health Association, 2005). Sample tubing used with pumps needs to be made of Teflon.

To use a pump to collect a discrete water sample:

1. Lower the pump intake to the desired depth.
2. Allow three sample-tubing volumes to rinse the sample tubing with native water before collecting the sample (NFM 4.1.1.B).
3. Pump the sample directly into a Teflon churn until the volume of water required for analyses has been collected. The use of a churn may not be necessary for a discrete sample if a relatively small volume of water is required for analyses; in this case, pump water directly into a Teflon or glass bottle.

7.5.5.D DEPTH-INTEGRATED SAMPLES

Depth-integrated samples may encompass the photic zone, epilimnion, metalimnion, hypolimnion, entire water column, or other layers of interest, depending on study objectives. Depth-integrated samples typically are collected when the cyanobacterial community is evenly distributed throughout a layer of interest, or the interest is primarily in dissolved concentrations. An exception may be when the distribution of cyanobacteria in the water column cannot be determined; in this case an integrated photic-zone sample generally is considered representative. There are two types of depth-integrated samples: continuous and discontinuous.

Continuous Depth-Integrated Samples

Continuous samples incorporate the entire depth of interest and are collected using bailers or pumps (pumps may not be the best option for sampling—see discussion of pumps in 7.5.5.C above and in NFM 7.4, table 7.4–8). Several continuous samples may be composited to obtain the necessary sample volume.

Bailers are cylindrical tubes with a check valve at the bottom that allows water inflow, but prevents outflow when raised. Bailers can be open or have check valves also at the top. Bailers are available in a variety of lengths and in a variety of materials. Teflon should be used when collecting samples for cyanobacterial toxin and taste-and-odor analyses.

To collect a continuous sample using a bailer:

1. Select a Teflon bailer that is long enough to incorporate the entire portion of the water column that is of interest. For example, if the photic zone extends to a depth of 2 m and an integrated photic-zone sample is being collected, then the bailer needs to be at least 2 m in length.
2. Lower the bailer slowly, until the bottom (the check-valve intake) is at the bottom of the desired depth interval.
3. Bring the bailer to the surface.
4. Draw off the water into a Teflon churn by means of a bottom-emptying device that releases the check valve.
5. Repeat the above steps until the volume of water required for analyses has been collected.

To collect a continuous sample using a pump (NFM 4.1.1.B):

1. Lower the pump intake to the bottom of the desired depth interval.
2. Allow three sample-tubing volumes to rinse the sample tubing with native water before collecting the sample (NFM 4.1.1.B).
3. Slowly raise the sampler through the vertical while pumping continuously at a constant rate.
4. Pump the sample directly into a Teflon churn until the volume of water required for analyses has been collected. This may require raising and lowering the sampler several times; turn off the pump either at the fully raised or fully lowered position, not in the middle of the range of sampling depths.

Discontinuous Depth-Integrated Samples

Discontinuous samples do not incorporate the entire depth of interest, and typically are collected using Kemmerer or Van Dorn bottles. The percent of the water column represented can be increased by using a vertical sampler rather than a horizontal sampler (table 7.5–6; NFM 2.1.1.B, Thief samplers; NFM 4.1.3). Pumps also may be used to collect discontinuous samples. Kemmerer and Van Dorn bottles and pumps are used in the same manner as described in section 7.5.5.C, except the composite is composed of samples collected from different depths, rather than the same depth.

- A depth-integrated sample may be obtained by collecting and compositing several discontinuous samples from multiple depths within the layer of interest.
- The sampling intervals must be equal (for example, every meter within the photic zone) when collecting discontinuous depth-integrated samples (Chorus and Bartram, 1999; American Public Health Association, 2005).

7.5.5.E QUALITY CONTROL

Quality-control samples such as blanks, replicates, and splits are an integral component of well-designed water-quality studies (NFM 4.3). Replicate samples (independent or 'true' replicate samples, and split replicates) are used to assess total variability in sample collection, processing, shipping, handling, and analysis of toxins, taste-and-odor compounds, and cyanobacterial (phytoplankton) community composition. The types, number, and distribution of quality-control samples to be collected are specified in the quality-assurance plan for the study and depend on study design and objectives. General information on different types of quality-control samples are discussed elsewhere (Friedman and Erdmann, 1982; U.S. Environmental Protection Agency, 2008; NFM 4.3).

- Equipment and field blanks are collected to verify the adequacy of cleaning procedures and the influence of equipment cleaning and sample handling on analyte concentrations (NFM 4.3.1). Field blanks are particularly important when sampling equipment is being cleaned in the field after collecting samples from surface accumulations of cyanobacteria (fig. 7.5–7*A*). To ensure there is no carry-over contamination, field blanks are best collected at the end of the day after the last sample (NFM 7.4.1.D). Organic-grade blank water (that is, pesticide-grade blank water (PBW)) is used for collecting equipment and field blanks. USGS equipment and field-blank data are stored in the National Water Information System (NWIS) QWDATA database 2.
- Cyanobacterial communities tend to have an irregular distribution within the water column and throughout a lake or reservoir; therefore, field replicates (NFM 4.3.2) to assess variability among samples are particularly important in studies of cyanobacterial toxins and taste-and-odor compounds. Concurrent field replicates may be used to assess the variability introduced from sample collection as well as inherent system variability at a single location within a lake or reservoir. Because of the patchy nature of cyanobacterial communities, particularly surface accumulations, concurrent field replicates cannot be used to evaluate laboratory variability. Concurrent replicates in lakes/reservoirs are collected as described in NFM 4.3.2.A with the appropriate modifications, depending on whether surface, discrete-depth, or depth-integrated samples are being collected.

Example—To collect concurrent replicates of a composite discontinuous integrated photic-zone sample with samples collected every 1 m from the surface to a depth of 5 m, using a horizontal Van Dorn bottle:

1. Using clean equipment, complete equipment field-rinsing procedures.
2. At 0.5 m, collect a sample and draw off the sample into a field-rinsed Teflon churn splitter.
3. Resample at 0.5 m and draw off the sample into a second field-rinsed Teflon churn splitter.
4. At 1.0 m collect a sample and draw off the sample into the second churn splitter.
5. Resample at 1.0 m and draw off the sample into the first churn splitter.
6. Collect and draw off the sample into each churn splitter in this manner for each of the remaining depths (2.0 m, 3.0 m, 4.0 m, and 5.0 m), alternating churn splitters as described in steps 2 through 5 listed above.
7. Process and preserve a sample (a) from the first churn, and (b) from the second churn (see section 7.5.6).

▶ Sequential field replicates may be used to assess inherent system variability and can be designed to assess spatial variability by collecting samples from two or more locations within a given sampling area; for example, two discontinuous integrated photic-zone samples collected 10 m apart. Sequential replicates also may be used to assess temporal variability (NFM 4.3.2.B); however, because cyanobacteria may change location in the water column, use caution when interpreting data from temporal replicates that are collected hours or more apart.

▶ Split samples (NFM 4.3.2.C) may be used to evaluate intra- and inter-laboratory variability. Evaluating inter-laboratory variability is particularly important when using multiple laboratories for toxin and taste-and-odor analyses because methods for the extraction and analysis of toxins and taste-and-odor compounds are evolving rapidly and are not consistent among laboratories. Likewise, there are several accepted methods for cyanobacterial (and phytoplankton) enumeration and identification.

Use of a single laboratory throughout the study is recommended. Methods for the extraction and analysis of toxins and taste-and-odor compounds and cyanobacterial identification and enumeration often are inconsistent among laboratories.

7.5.5.F ANCILLARY DATA

Ancillary data collected during cyanobacterial toxin and taste-and-odor studies will depend on the study objectives. Chlorophyll samples commonly are collected as part of cyanobacterial toxin and taste-and-odor studies; chlorophyll is an indicator of algal biomass (NFM 7.4) and frequently is included in general water-quality monitoring programs. Other commonly measured variables are listed in table 7.5–8. Generally, all subsamples for laboratory analyses, including cyanobacterial community composition and toxin and taste-and-odor analyses, are collected from the same grab or composite sample. The volume of the grab or composite sample must be sufficient for all planned analyses.

Table 7.5–8. Commonly collected ancillary data for studies of cyanobacterial toxins and taste-and-odor compounds.

[NFM, National Field Manual for the Collection of Water-Quality Data; GPS, global positioning system]

Ancillary data	Description	References for additional information
Observational data	• General field observations such as water color and clarity, current meteorological conditions, presence of visible algae, presence of surface accumulations or scums of cyanobacteria • Meteorological conditions several days before sampling • Occurrence of recent inflow events • Water residence time • Lake level	NFM 4 Wetzel and Likens, 2000
Field measurements	• Photographs of current conditions • GPS coordinates • Vertical profiles of light, temperature, dissolved oxygen, specific conductance, pH, turbidity, and in situ fluorescence • Secchi disc depth	NFM 4 NFM 6 NFM 7.4.1B Wetzel and Likens, 2000
Laboratory analyses	• Phytoplankton community composition, abundance, and biovolume	Procedures described in this NFM section 7.5 Britton and Greeson, 1987 Standard Methods for the Examination of Water and Wastewater, Sections 1060 and 10200
	• Chlorophyll	NFM 7.4
	• Nutrients - total nitrogen and phosphorus, total Kjeldahl nitrogen (ammonia plus organic nitrogen), nitrate, nitrite, ammonia, and orthophosphorus	NFM 4.0, 4.1 NFM 5
	• Suspended sediment	NFM 4.0, 4.1 NFM 5
	• Actinomycetes bacteria (taste-and-odor studies)	NFM 7.1
	• Alkalinity	NFM 4. 0, 4.1 NFM 5 NFM 6.6
	• Cations and anions	NFM 4.0, 4.1 NFM 5
	• Carbon: total, dissolved, and particulate	NFM 4.0, 4.1 NFM 5

SAMPLE HOLDING TIME, PROCESSING, AND SHIPPING 7.5.6

Several cyanobacterial toxins and taste-and-odor compounds commonly are measured, including the toxins microcystin, cylindrospermopsin, anatoxin, and saxitoxin, and the taste-and-odor compounds geosmin and MIB.

A key decision when designing toxin and taste-and-odor studies is whether total, particulate, or dissolved concentrations will be analyzed, as this affects the procedures to be used for sample preparation, processing, and preservation. Some laboratories may request whole-water samples and do all processing in-house; others will provide specific instructions on sample bottle type and preferred preservation, whereas others may provide sample bottles and preservatives. There also may be restrictions on delivery days that can affect analyses of unpreserved samples. Clear communication with the laboratories performing the analyses is required to ensure that sample analyses will provide relevant results. Although sample preparation, processing, and shipping largely may be determined by the analyzing laboratory, general guidelines are provided below. **The guidelines below provide common processing procedures; however, if the analyzing laboratory provides contradictory or alternative instructions, those should be followed instead.**

The specific instructions from the analyzing laboratory on sample processing, preservation, and shipping should be followed carefully. Contact the laboratory with any questions.

SAMPLE HOLDING TIME 7.5.6.A

Ideally, all samples are processed on-site. If samples must be transported to the office laboratory before being processed, place the samples on ice and keep in the dark, because heat and light can degrade cyanobacterial cell integrity, toxins, and taste-and-odor compounds. **If shipping is delayed, contact the analyzing laboratory for guidance.**

- If the analyzing laboratory is processing the samples, then samples must be shipped on the same day they are collected.
- For samples being processed on-site:
  - **Phytoplankton samples** must be processed and preserved as soon as possible after collection. Once preserved, they may be stored for extended periods of time (weeks to months), although it is preferable to ship the samples to the laboratory on the day of collection or as soon as possible thereafter.

— **Toxin samples** should be processed and shipped to the laboratory on the day of collection. (Although current understanding does allow toxin samples to be held for up to 24 hours before being processed if the samples are kept cold and stored in the dark, shipping them to the laboratory on the same day as collection is preferable.)

— **Taste-and-odor samples** must be shipped on the same day they are collected, regardless of whether the samples are processed on-site or by the laboratory.

Samples should not be frozen without guidance from the analyzing laboratory. Many cyanobacterial toxins do not degrade when frozen, and samples may be stored in a freezer for several months or years (Chorus and Bartram, 1999). Keep in mind that if whole-water samples are frozen, cyanobacterial cells will lyse and release intracellular toxins. Freezing and thawing whole-water samples frequently is used to determine total toxin concentration (Graham and others, 2008). Taste-and-odor samples generally are not frozen.

Once whole-water samples are frozen, only total toxin concentrations can be measured.

7.5.6.B SAMPLE PROCESSING

How samples are processed depends on the targeted analytes and study objectives. Processing samples for cyanobacteria, toxins, and taste-and-odor compounds consists primarily of preparing composite samples, withdrawing subsamples from the composite, filtering those samples to be analyzed for dissolved-phase concentrations, and preserving the samples as dictated by the analyzing laboratory. In general, sample processing will have a minimal impact on cyanobacterial cells when implemented properly.

- The rate for churning a composite sample should not exceed the recommended 9 inches per second (NFM 5.1.1.A) and the disk should not break the surface of the water; as long as these recommendations are followed, churning should not lyse cyanobacterial cells.
- Sample bottle type and volume will usually be determined by the analyzing laboratory. Common bottle types for each analysis are listed in table 7.5–7.

Procedures are described below for processing toxin and taste-and-odor samples, and for cyanobacterial (phytoplankton) samples.

Toxin and Taste-and-Odor Samples

The procedures used for processing toxin and taste-and-odor samples depend on whether the samples are to be analyzed for total, particulate, or dissolved concentration. **Analysis of taste-and-odor compounds most commonly is performed on dissolved samples, but total and particulate concentrations also are determined regularly** (van der Ploeg and others, 1992; Wnorowski and Scott, 1992; Jones and Korth, 1995; Yen and others, 2007). Taste-and-odor compounds are semi-volatile; however, the traditional volatile organic carbon sampler does not need to be used when processing samples unless specified by the analyzing laboratory. The procedures for each are described as follows.

- **Total-Concentration Samples**. Total concentration samples measure both particulate (intracellular) and dissolved (extracellular) phases of cyanobacterial toxins and (or) taste-and-odor compounds. Total concentrations are measured in whole-water samples (fig. 7.5–9). Alternatively, total concentrations also may be calculated by summing particulate and dissolved concentrations (fig. 7.5–9).

  To process total-concentration samples:

  1. Follow the general USGS procedures for processing raw (unfiltered) samples (NFM 5.1), using CH/DH protocols (NFM 4.0.2 and NFM 5.0.1).
  2. Wear disposable, powderless gloves throughout sample collection and processing.
  3. Prelabel the sample bottle. The label must include the station name and number, date, time, type of sample (for example, integrated photic zone), sample depth (for example, 0 to 5 m), and type of analysis (for example, total toxin).
  4. Homogenize the water sample by churning as described above, and withdraw (subsample) enough water to fill the sample bottle.
     - If samples will be frozen, bottles should be filled only to two-thirds full to allow for expansion.
     - Bottle type and volume will depend on instructions from the laboratory at which the samples will be analyzed (table 7.5–7).
  5. Protect the sample from light and immediately place it on ice. If the sample bottle is glass, first put it into a protective foam sleeve to prevent breakage. Loose ice is preferred over block ice to reduce the chance of breakage during shipping.

Clear communication with the analyzing laboratory is critical to ensuring proper sample handling for the analysis to be performed.

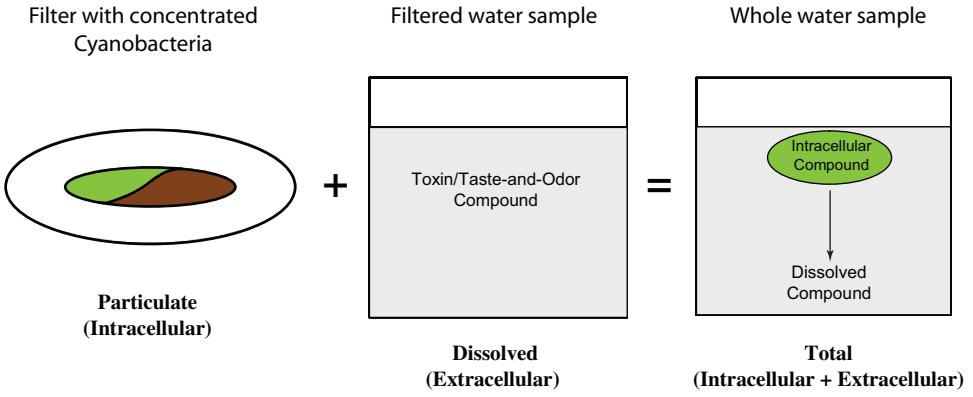


Figure 7.5–9. Schematic showing the partitioning of cyanobacterial toxins and taste-and-odor compounds among the total, particulate, and dissolved phases in different types of samples. From Graham and others (2008).

▶ **Particulate-Concentration Samples**. Particulate samples measure only the intracellular phase of cyanobacterial toxins and (or) taste-and-odor compounds. Particulate concentrations are measured by concentrating cyanobacteria from a known volume of water onto a filter. Alternatively, particulate concentrations also may be calculated by taking the difference between total and dissolved concentrations (fig. 7.5–9).

To process particulate-concentration samples:

1. Follow the general USGS procedures for processing raw (unfiltered) samples (NFM 5.1), using CH/DH protocols (NFM 4.0.2 and NFM 5.0.1).
2. Wear disposable, powderless gloves throughout sample collection and processing.
3. Homogenize the water sample by churning as directed above, and withdraw a subsample.
4. Process the subsample in the same manner as phytoplankton chlorophyll samples (the procedure is described in NFM 7.4, section 7.4.5.A) using 0.7-micrometer (μm) glass fiber filters and a glass or metal filter funnel and glass receiving flask. The maximum pressure of the filtering apparatus must not exceed 15 pounds per square inch (NFM 7.4.5); greater pressure may rupture cyanobacterial cells. For particulate analysis, the material retained on the filter is kept for analysis. The sample filtrate may be discarded or analyzed for dissolved concentration.
5. Record the filtered volume on the field sheet and on the sample label.
6. Label the sample. The label must include the station name and number, date, time, type of sample (for example, integrated photic zone), sample depth (for example, 0 to 5 m) type of analysis (for example, particulate toxin), and volume filtered.
7. Keep the sample chilled until it is shipped by immediately placing it on ice. Pack the sample on dry ice for shipping (it is not necessary to place the sample immediately on dry ice after processing). If samples are not shipped immediately upon return to the office laboratory, place them in the freezer.

▶ **Dissolved-Concentration Samples.** Samples for analysis of dissolved concentrations are used to measure only the extracellular phase of cyanobacterial toxins and (or) taste-and-odor compounds.

To process dissolved-concentration samples:

1. Follow the general USGS procedures for processing raw (unfiltered) samples (NFM 5.1), using CH/DH protocols (NFM 4.0.2 and NFM 5.0.1).
2. Wear disposable, powderless gloves throughout sample collection and processing.
3. Prelabel the sample bottle. The label must include the station name and number, date, time, type of sample (for example, integrated photic zone), sample depth (for example, 0 to 5 m) and type of analysis (for example, dissolved toxin).
4. Homogenize the water sample by churning as directed by USGS protocol, and withdraw a subsample.
5. Filter the subsample in the same manner as phytoplankton chlorophyll samples (see the procedure described in NFM 7.4, section 7.4.5.A) using 0.7-µm glass fiber filters and a glass or metal filter funnel and glass receiving flask. The filtrate from preparation of particulate toxin samples or chlorophyll samples may be used for dissolved-concentration analysis.
6. Pour the filtrate from the glass receiving flask into the sample bottle. Be sure to filter enough volume to fill the sample bottle. If samples will be frozen, bottles should be filled only to two-thirds full to allow for expansion. Bottle type and volume will depend on instructions from the laboratory at which the samples will be analyzed (table 7.5–7).
7. Protect the sample from light and immediately place on ice. If the sample bottle is glass, first put it into a protective foam sleeve to prevent breakage. Loose ice is preferred over block ice to reduce the chance of breakage during shipping.

Toxin and taste-and-odor concentrations typically are expressed volumetrically as micrograms per liter (µg/L) because volumetric concentrations are easily related to drinking-water and recreational- guideline values. Concentrations also may be expressed gravimetrically as micrograms per gram (µg/g) ash-free dry weight.

To estimate ash-free dry weight:

1. Prepare an additional sample for particulate analysis using the same volume of water.
2. Immediately place the sample on ice. Samples should be shipped on dry ice (it is not necessary to place the sample immediately on dry ice after processing if it is being shipped the same day). If samples are not shipped immediately upon return to the office laboratory place them in the freezer.
3. USGS employees send the sample to the National Water Quality Laboratory for phytoplankton ash-free dry weight analysis (Lab Code 2190).
4. Use ash-free dry weight to calculate gravimetric concentrations:
   Particulate toxin (µg) ÷ ash-free dry weight (g) = µg toxin/g of ash-free dry weight.

TECHNICAL NOTE: Gravimetric concentrations must be interpreted with caution. When collected from dense accumulations of cyanobacteria, gravimetric expression of toxin or taste-and-odor concentration often is directly related to cyanobacterial abundance; however, when collected in open-water areas, ash-free dry weight will incorporate all suspended organic material including other phytoplankton, zooplankton, and detritus (Chorus and Bartram, 1999).

Cyanobacterial (Phytoplankton) Samples

Cyanobacteria are considered to be part of the phytoplankton community, and there is no difference between samples collected for cyanobacterial analysis and phytoplankton analysis. Cyanobacteria and phytoplankton samples typically are analyzed for community composition, abundance (cells or natural units per milliliter or liter), and biovolume (cubic micrometers per milliliter or liter) (Blomqvist and Herlitz, 1998; Olrik and others, 1998). Because there can be great variability among analysts, it is important to confirm counting and processing methodologies before the analysis, including subsample preparation, counting threshold, taxonomic references, and the experience of the analyst. This is particularly important when using multiple laboratories for analysis. Because of variability among laboratories, any interpretation based on data from different laboratories must be undertaken with great caution and several split replicate samples collected from a range of environmental conditions must be analyzed to assess analytical variability. **Switching laboratories during the course of a study is strongly discouraged.**

TECHNICAL NOTE: Some cyanobacterial colonies or filaments may exceed the maximum particle size (250 micrometers) recommended for use of a churn splitter (NFM 2.2.1); however, particle size is not an important criterion for churn-splitter use when dealing with cyanobacteria. In contrast to sediment processing, settling is not an issue for cyanobacteria or algae. Churning velocity will maintain all cyanobacterial and algal size classes within the sample, regardless of buoyancy. Although some colonies and filaments may break apart during the churning process, individual cell integrity should not be affected by churning. Use of the churn splitter is inappropriate, however, when collecting samples from thick surface accumulations with little water content.

To process cyanobacterial (phytoplankton) samples for identification and enumeration:

1. Follow the general USGS procedures for processing raw (unfiltered) samples (NFM 5.1), using CH/DH protocols (NFM 4.0.2 and NFM 5.0.1).
2. Wear disposable, powderless gloves throughout sample collection and processing.
3. Prelabel the sample bottle. The label must include the station name and number, date, time, type of sample (for example, integrated photic zone), sample depth (for example, 0 to 5 m), and type of analysis (for example, dissolved toxin). Bottle type and volume will depend on instructions from the laboratory at which the samples will be analyzed (table 7.5–7).
4. Homogenize the water sample by churning as directed by USGS protocol and fill the sample bottle, leaving enough room to add the preservative.

5. Add the required amount of preservative to the sample. The type of preservative selected depends on the requirements of the laboratory doing the analysis. Lugol's iodine and glutaraldehyde are commonly used preservatives. Because of its toxicity (see ***CAUTION*** below), handle glutaraldehyde with care and follow all safety precautions.
   - Handle these preservatives only under good ventilation, wearing gloves and safety glasses.
   - Keep the Material Satety Data Sheets (MSDS) for Lugol's iodine and glutaraldehyde close at hand.
   - Disposal of these chemicals must conform to local ordinance and governmental regulations.
6. Protect sample from light and immediately place on ice. If sample bottle is glass, first put it into a protective foam sleeve to prevent breakage. Loose ice is preferred over block ice to reduce the chance of breakage during shipping.

CAUTION: **Glutaraldehyde is highly toxic. Handle glutaraldehyde only as directed and at the recommended concentration. For a 5 to 25 percent aqueous solution, the MSDS states that glutaraldehyde is corrosive; causes eye burns; is harmful if inhaled, absorbed through skin, or swallowed; causes severe skin irritation and irritation to the respiratory tract (*http://www.jtbaker.com/msds/englishhtml/g4404.htm*, accessed 05/06/2008). Inhalation can be fatal at higher concentrations (*http://msds.chem.ox.ac.uk/GL/glutaric_dialdehyde.html*, accessed 05/06/2008).**

Detailed information on how to collect and preserve phytoplankton samples can be found in Britton and Greeson (1987), American Public Health Association (2005), Wetzel and Likens (2000), and from the laboratory at which samples will be analyzed.

Because the variability among laboratories can be substantial, any interpretation of data base on data from a variety of laboratories must be undertaken with great caution, and only if the appropriate quality-control measures have been incorporated so that analytical variability can be assessed.

7.5.6.C SAMPLE SHIPPING

Before shipping, check the samples to ensure that labels include:

1. Station name and number
2. Date
3. Time
4. Type of sample (for example, integrated photic zone)
5. Sample depth (for example, 0 to 5 m)
6. Type of analysis (for example, particulate toxin)
7. Volume filtered (for particulate toxin analysis only)

Place clear tape over the completed sample label to protect it during shipping from direct contact with cube or loose ice (not block ice) or dry ice.

Samples must be kept chilled (on ice or refrigerated) and in the dark until shipped. Ideally, samples should be shipped on the same day they are collected, following the instructions provided by the analyzing laboratory.

- Samples, other than those for particulate analysis, typically are placed in a cooler, packed in double bags on ice, and shipped using priority overnight mail to arrive at the analyzing laboratory the next morning.
- Particulate samples are packed with dry ice and double bagged, and should be shipped separately from other samples. (For guidance on shipping samples on dry ice see NFM 7.4, section 7.4.6.B.) Appropriate sample documentation should be placed in a separate resealable plastic bag and attach to the inside lid of the shipping cooler. The documentation required will depend on the requirements specified by the analyzing laboratory.

Be aware of sample holding times when shipping to areas of the country that may be experiencing adverse weather. It is better to hold samples under controlled conditions than to have samples exposed to extreme temperatures if shipments get delayed; however, **unprocessed toxin samples need to be shipped within 24 hours**. Once preserved, samples for cyanobacterial and phytoplankton analysis may be stored for longer periods of time (weeks to months); nevertheless, they should be shipped to the analyzing laboratory as soon as possible.

ANALYTICAL TECHNIQUES 7.5.7

A variety of analytical techniques can aid in the determination of cyanobacterial toxins (Graham and others, 2008). Relative advantages and disadvantages of common analytical techniques utilized for analysis of cyanobacterial toxins and taste-and-odor compounds are shown in table 7.5–9. Bioassays, such as enzyme-linked immunosorbent assays (ELISA) typically are easy to learn and use, and are relatively cost effective when used as a screening tool or, when appropriate, for toxicity assessment (Chorus and Bartram, 1999; Msagati and others, 2006); however, cross-reactivity can lead to a lack of specificity for the target analyte(s) (Metcalf and others, 2002). If toxin-specific information is required, the chromatographic techniques are more appropriate, but the cost is greater per sample in comparison to bioassays. Researchers are utilizing multi-toxin liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) methods more frequently because of the ability to distinguish a larger variety of individual toxins more readily, and avoid the derivatization that would be required for any gas chromatography (GC)-based technique. LC/MS and LC/MS/MS can suffer from matrix effects (the influence of other chemical constituents in a sample that impacts quantitation of the analyte of interest leading to signal enhancement or suppression) more than the other techniques, but it is possible to compensate for this problem through standard addition (a laboratory "spike" sample).

Fewer analytical options are available for determining taste-and-odor compounds. Closed- and open-loop stripping techniques combined with a GC flame ionization detector or GC-MS have been used previously, but stripping techniques largely have been replaced by solid-phase microextraction (SPME) where phase transfer of the semivolatile compounds to the gas phase is followed by separation and detection by GC/MS. Typical method reporting levels range from 1 to 5 nanograms per liter (ng/L), the lower threshold for human detection of these compounds through smell (Zimmerman and others, 2002; Taylor and others, 2005). Currently, there are no commercially available ELISA methods for taste-and-odor analysis sensitive enough to be of practical use.

Table 7.5–9. Advantages and disadvantages of common analytical techniques used for the analysis of cyanobacterial toxins and taste-and-odor compounds.

Analytical techniques	Advantages	Disadvantages
Bioassays		
Enzyme-linked immunosorbent assays (ELISA), inhibition assays, and radioassays	• Relatively easy to use • Cost per analysis lowest of all techniques • Can be useful as screening tools • Can indicate toxicity in some cases	• Data interpretation can be difficult • Inhibition assays and radioassays not always available • Bioassays frequently possess some reactivity towards compounds other than the intended target • Radioassays require permits to work with radioisotopes • Research objectives may require a chromatographic technique for compound-specific quantitation
Gas Chromatography (GC)		
Flame ionization detector (GC/FID) and mass spectrometry (GC/MS)	• Compound specific • Cost per analysis is intermediate • Compound identification by GC/MS is superior to GC/FID	• Toxins will most likely require derivitization[1] • Not all compounds are amenable to derivitization • GC/FID may require further confirmation • Sample concentration techniques may be necessary
Liquid Chromatography (LC)		
Ultraviolet-Visible (LC/UV-Vis), fluorescence (LC/Fluorescence), mass spectrometry (LC/MS), tandem mass spectrometry (LC/MS/MS), and ion trap mass spectrometry (LC/ITMS)	• Derivitization typically not necessary • Compound specific • Greatest number of toxins are amenable to LC techniques • Cost per analyte can be lowest in a multi-analyte method • Compound identification is superior by LC/MS/MS or LC/ITMS	• Matrix effects can be substantial • Cost per sample most expensive • Spectroscopic techniques may require further confirmation • Sample concentration techniques may be necessary

[1]Derivitization is the chemical modification of an analyte to improve identification, to enhance analyte response, and (or) improve compatibility with a particular analytical technique.

SAFETY CONSIDERATIONS 7.5.8

Cyanobacterial toxins are known to cause human illness, and skin contact may result in irritation and rash.

- Always wear gloves when collecting samples for cyanobacterial toxin and taste-and-odor analysis. If dense surface accumulations are going to be sampled, gloves that extend to the shoulder are recommended.
- Avoid skin and eye contact with dense surface accumulations. If contact with dense surface accumulations occurs, wash the affected area with soap and water and rinse immediately with clean water.
- Inhalation of aerosols may be a problem for those with respiratory illness. Personnel having a recent history of asthma or respiratory disease should take the necessary precautions when collecting or processing samples.
- General safety considerations for water sampling are described in NFM 9.

REPORTING OF CYANOBACTERIAL POPULATIONS, TOXINS, AND TASTE-AND-ODOR COMPOUNDS 7.5.9

Many of the cyanobacterial toxin and taste-and-odor compounds already have parameter codes available for entry into the USGS National Water Information System (NWIS) and updates are made as new compounds and analyses become available.

- USGS personnel should check the NWIS parameter code dictionary to see what codes are available for toxin and taste-and-odor compounds. The parameter code dictionary is available online at: *http://waterdata.usgs.gov/*nwis/help?codes_help #Table8, accessed 7/15/2008.
- Analyzing agency and method codes also need to be entered into NWIS because of the many different extraction and analysis techniques used for toxins and taste-and-odor compounds.

Most cyanobacterial and phytoplankton data are not entered into NWIS, because the system is not amenable for use with biological data. A system for storing cyanobacterial/phytoplanktonic data (for example, possibly using a MicroSoft Access Database) is necessary, and should be developed for each project until a centralized database is available.

7.5.10 SELECTED REFERENCES

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7.5.11 ACKNOWLEDGMENTS

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7.5.12 GLOSSARY

Actinomycetes bacteria—A group of aerobic, gram-positive bacteria that are largely terrestrial organisms associated with soils. The actinomycetes bacteria play a major role in the mineralization of organic matter in soils. Actinomycetes bacteria are not photosynthetic and are not part of the phytoplankton community in lakes and reservoirs.

Acute—Single exposure to a relatively high dose of a toxic substance.

Algae—Unicellular or simple multicellular photosynthetic organisms containing chlorophyll-*a*; there are numerous groups of algae, most of which are eukaryotic aquatic organisms.

Allelopathy—Inhibition or suppression of growth by one species of algae or plant by chemicals produced by another species.

Aphotic zone—Region where there is not enough light to support photosynthesis; extends from below the photic zone to the lake or reservoir bottom.

Benthic—Associated with the bottom of a lake or reservoir. After benthos, a term used to describe the bottom of a lake or reservoir.

Bioactive—Indicates that a substance has an effect on living tissue.

Biovolume—The volume of an organism or group of organisms.

Bloom—The term bloom, not specific to cyanobacteria, is inexact and subjective. Common definitions include (1) a large population or extremely high cell densities of phytoplankton (extremely high densities are typically defined as greater than 20,000 to 100,000 cells per milliliter); (2) a proliferation of phytoplankton dominated by a single or a few species; and (3) a visible accumulation of phytoplankton at the water surface.

Blue-green algae—See cyanobacteria.

Cell lysis—The death of a cell due to rupture of the cellular membrane; cell lysis may be caused by natural processes, such as viruses, or artificial processes, such as application of algicides.

Chronic—Repeated exposure to relatively low doses of a toxic substance over an extended period of time.

Cyanobacteria—Cyanobacteria are true bacteria with a prokaryotic cell structure; however, cyanobacteria also have chlorophyll-*a*, a photopigment characteristic of eukaryotic algae and higher plants. Structurally the cyanobacteria are bacteria-like but functionally the cyanobacteria are algae-like. Cyanobacteria are typically sampled and analyzed as part of phytoplankton (algal) assemblages rather than bacterial assemblages in aquatic ecosystems. Cyanobacteria are often called blue-green algae.

Cyanotoxins—Any of a number of toxins produced by the cyanobacteria. The cyanotoxins include dermatoxins, hepatotoxins, and neurotoxins and impact a wide range of aquatic and terrestrial organisms, including humans.

Derivitization—A chemical modification of an analyte to improve identification, to enhance analyte response, and (or) improve compatibility with a particular analytical technique.

Dermatoxin—A toxin that affects the skin and membrane tissues.

Diel—Regular cycle over a 24-hour period.

Epilimnion—The warm, buoyant upper layer of a stratified lake.

Enzyme-linked immunosorbent assay (ELISA)—A biological assay that is based on immunological principles where antibodies specific to an analyte are utilized in a competitive manner with a corresponding antigen resulting in a measureable response. ELISA's typically are not specific to a single compound, but respond to a class of compounds to varying extents and therefore are frequently used to screen samples with further confirmation by an independent technique with specificity such as chromatography.

Eukaryotic—Having cells with a distinct membrane-bound nucleus; characteristic of all organisms except bacteria, cyanobacteria, and other primitive organisms.

Eutrophic—High nutrient content and levels of production.

Eutrophication—Nutrient enrichment (particularly nitrogen and phosphorus) in aquatic ecosystems leading to increased productivity.

Extracellular—Occurring outside of a cell.

Hepatotoxin—A toxin that affects the liver.

Hypereutrophic—Very high nutrient content and levels of production.

Hypolimnion—The cold, dense bottom layer of a stratified lake; the hypolimnion often becomes anoxic (little or no dissolved oxygen) in productive systems.

Icthyotoxin—A toxin that affects fish or is specific to fish.

Incident light—The light that actually falls on a surface.

Intracellular—Occurring within a cell.

Intraperitoneal—Within the abdominal cavity.

Kemmerer bottle sampler—A type of thief sampler used for collecting water samples at discrete depths. The sampler is held open at both ends and lowered vertically through the water column. At the desired depth a mechanism triggers the sampler and it closes, capturing the water at depth. The main difference between a Kemmerer bottle and a Van Dorn bottle is the mechanism used to open and close the bottle. See figure 2–2*A* in NFM 2.

Matrix effects—A general term used to describe the influence of other chemical constituents in a sample that impacts quantitation of the analyte of interest leading to signal enhancement or suppression. This is typically discussed when analyses are conducted by liquid chromatography with spectroscopic detection (fluorescence and ultraviolet visible wavelengths) and mass spectrometry.

Mesotrophic—Moderate nutrient content and levels of production.

Metalimnetic bloom—An algal population that develops at the interface between the epilimnion and metalimnion in a stably stratified lake. Metalimnetic blooms most commonly occur in mesotrophic lakes where light penetrates into the metalimnion.

Metalimnion—The middle layer of a stratified lake; the metalimnion is characterized by substantial decreases in temperature with depth.

Mixed depth—The depth of turbulent mixing; may include all or only a portion of the water column depending on stratification, solar irradiance, and wind.

Neurotoxin—A toxin that affects the central nervous system.

Oligotrophic—Low nutrient content and levels of production.

Photic (euphotic) zone—Region of water column where there is enough light to support photosynthesis; extends from the surface to the depth were light is approximately one percent of that at the surface.

Phycocyanin—An accessory pigment to chlorophyll that is unique to the cyanobacteria.

Phytoplankton—Algae, including the cyanobacteria, suspended in the water column.

Plankton, planktonic—Drifting or weakly swimming organisms (phytoplankton, zooplankton, or bacteria) that are suspended in the water column in lakes, reservoirs, and other fresh and marine water bodies.

Prokaryotic—Having cells that lack a distinct membrane-bound nucleus; characteristic of bacteria, cyanobacteria, and other primitive organisms.

Secondary metabolite—Cellular products that are not directly involved in primary cellular processes that support growth and development.

Senesce (senescence)—The process of growing old; aging.

Species—A distinct kind of organism; the major division of genus.

Standard addition—A quantitative technique used to minimize the impacts of matrix effects where the response of a known mass of the analyte of interest is amended into a split of the sample. This allows for matrix effect compensation against a known mass of analyte.

Strain—A group of organisms of the same species that have distinctive characteristics but are not considered a separate species.

Taste-and-odor compounds—Compounds that produce objectionable tastes and odors in finished drinking water.

Thermocline—The region where temperature change is greater than or equal to 1°C per meter; the terms thermocline and metalimnion often are used synonymously.

Trophic status—Level of productivity in an ecosystem.

Turnover—Complete isothermal mixing of a previously stratified lake.

Van Dorn bottle sampler—A type of thief sampler used for collecting water samples at discrete depths. The sampler is held open at both ends and lowered either vertically or horizontally through the water column. At the desired depth a mechanism triggers the sampler and it closes, capturing the water at depth. The main difference between a Van Dorn bottle and a Kemmerer bottle is the mechanism used to open and close the bottle. In addition, the Van Dorn bottle may be held either vertically or horizontally, while the Kemmerer bottle can be used only in the vertical position. See figure 2–2*B* in NFM 2.

APPENDIXES

APPENDIX 7.5–A. Example Design and Approach for a Regional Reconnaissance Study to Determine the Occurrence of Cyanobacterial Toxins and Potential Toxin Producers

APPENDIX 7.5–B. Example Design and Approach for a Study to Monitor a Recreational Beach for Cyanobacterial Toxins

APPENDIX 7.5–C. Example Design and Approach for an Interpretive Study to Develop a Real-Time Model to Estimate Geosmin and 2-methylisoborneol (MIB) Concentrations

For all lake and reservoir field work:

- **Wear a personal floatation device (PFD) and appropriate protective clothing when sampling in water or from a boat.**
- **Use sampling methods appropriate for the collection of samples to be analyzed at parts-per-billion to parts-per-trillion concentrations.**

APPENDIX 7.5–A. Example Design and Approach for a Regional Reconnaissance Study to Determine the Occurrence of Cyanobacterial Toxins and Potential Toxin Producers

Objective

Document the occurrence of cyanobacterial toxins and potential toxin producers in all primary recreational and drinking-water-supply lakes and reservoirs within a State.

Design and Approach

Sampling frequency. Samples will be collected monthly during June through September, the period when cyanobacterial populations typically peak.

Site location. In small lakes and reservoirs, samples will be collected at a single, representative open-water site near the deepest part of the lake or reservoir. In large lakes and reservoirs, samples will be collected at representative open-water sites in each of the main basins (lakes) or tributary arms, including a site near the outlet or dam (for manmade reservoirs). If present, samples from surface accumulations of cyanobacteria also will be collected.

Sample type. Integrated photic-zone samples will be collected from open-water sites. Surface grab samples will be collected from surface accumulations.

Sampler used. Samples will be collected every meter throughout the photic zone using a vertical Van Dorn bottle. The samples from each depth will be composited in a churn. An open-mouth bottle sampler will be used for surface grab samples.

Type of toxin analysis. Cyanobacterial toxin samples will be analyzed for total (particulate plus dissolved) toxin concentrations for anatoxins, cylindrospermopsins, and microcystins.

Ancillary data. At each sampling site, photographs and global positioning system (GPS) coordinates will be recorded and Secchi disc depth and vertical profiles of light (irradiance), water temperature, dissolved oxygen, specific electrical conductance, pH, turbidity, and in situ fluorescence will be measured. In addition to cyanobacterial toxins, each sample will be analyzed for cyanobacterial (phytoplankton) community composition (enumeration, biovolume, and identification); chlorophyll; total phosphorus and nitrogen concentrations; and suspended sediment.

Quality-control samples. Equipment blanks and field blanks will be collected during each monthly sampling trip. Sequential replicates (true field replicates) will be collected for 20 percent of all samples. Split replicates will be collected for 10 percent of all samples.

Results. The results from this study can give an indication of how frequently cyanobacterial toxins occur in the State, which toxins are most common, and the range of typical concentrations. Potential cyanobacterial toxin producers also will be identified. General water-quality conditions in the State during peak periods of cyanobacterial abundance will be described. **This study will not, however, give a good indication of maximum toxin concentrations; monthly samples may miss periods of peak cyanobacterial toxin abundance.**

Field Form for a Regional Reconnaissance Study for Occurrence of Cyanobacterial Toxins and Potential Toxin Producers

Station Information		
Station Name:	Date: (MM/DD/YY) _____/_____/_____	Time: ___ ___ ___ ___
Station Identification Number:		
Project Name:		
Sampled By:	Samples Shipped By:	Date: (MM/DD/YY) _____/_____/_____

Sampling Information
Sample Type: Regular Concurrent Replicate Sequential Replicate Split Replicate Field Blank Laboratory Blank Other:_____
Sample Collection: Integrated Photic Zone Sample Surface Accumulation Sample Photic Depth: Sample Depths: Sampling Device: Vertical Van Dorn Open Mouth Bottle Sampler Sampling Method: Multiple Vertical Grab Sample

Physical Site Conditions
Lake Color: Brown Green Blue Clear Other_____
Surface Accumulation Present: Yes No Accumulation Sample Collected: Yes No Location:
Sky: Clear Cloudy _____% Precipitation: Light Medium Heavy Rain Mist Fog
Wind: Calm Light Breeze Gusty Windy
Other Observations:

Related Sampling Activities		
Variable	Supporting Information	Collected (Check)
Total Cyanobacterial Toxins	Bottle Type: HDPE Amber Glass	
Phytoplankton	Preservative: Preservative volume:_____	
Chlorophyll	Volume Filtered:	
Nutrients		
Suspended Sediment		
Remarks:		

Field Form for a Regional Reconnaissance Study for Occurrence of Cyanobacterial Toxins and Potential Toxin Producers—*continued*

Field Measurements		
Latitude: __ __°__ __' __ __" N	Secchi Disc Depth:	Air Temperature:
Longitude: __ __°__ __' __ __" W	Surface Irradiance:	Barometric Pressure:
	Photic Depth:	Other:

Vertical Profile

Depth	Irradiance	Water Temperature	Dissolved Oxygen	Specific Conductance	pH	Turbidity	In situ Fluorescence

Photographs/Observations:

APPENDIX 7.5–B.
Example Design and Approach for a Study to Monitor a Recreational Beach for Cyanobacterial Toxins

Objective

Monitor a recreational beach to determine if cyanobacterial toxins pose an acute exposure risk.

Design and Approach

Sampling frequency. Samples will be collected weekly between Memorial Day and Labor Day, the periods of peak recreational use. If cyanobacteria accumulate in the beach area, daily samples will be collected until the accumulation dissipates.

Site location. Samples will be collected from nine locations within the designated recreational area and composited. The nine locations will be determined by evenly dividing the recreational area into three transects that begin at the beach and extend into the water. Samples will be collected from three locations (ankle, knee, and chest deep) along each transect. Sample collection starts at the waters edge in ankle-deep water and continues into the water to approximately chest depth. Ankle-deep water samples will be collected approximately 0.15 meters (m) below the surface. Knee- and chest-deep water samples will be collected approximately 0.30 m below the surface. If dense cyanobacterial accumulations are present outside of transect locations, an additional sample will be collected from the accumulation.

Sample type. Surface grab samples will be collected. At knee- and chest-deep locations, samples will be collected approximately 15 centimeters below the water surface. Dense cyanobacterial accumulations will be sampled at the water surface.

Sampler used. Grab samples will be collected with an open-mouth bottle at each transect location and composited into a churn.

Type of toxin analysis. Cyanobacterial toxin samples will be analyzed for total (particulate plus dissolved) toxin concentrations for anatoxins, cylindrospermopsins, and microcystins.

Ancillary data. At each sample transect, global positioning system (GPS) coordinates will be recorded. In addition, photographs will be taken, and Secchi disc depth, water temperature, dissolved oxygen, specific electrical conductance, pH, turbidity, and in situ fluorescence will be measured at one central location in the recreational area.

Quality-control samples. Equipment blanks and field blanks will be collected monthly. Sequential replicates (true field replicates) will be collected for 20 percent of all samples. Split replicates will be collected for 10 percent of all samples.

Results. The results from this study will give an indication of when cyanobacterial toxin concentrations are elevated enough to cause human health concerns. Data collected may be used to post warnings or close recreational areas. Study results also will give an indication of the range of cyanotoxin concentrations in the recreational area and when peak values typically occur.

Field Form for a Study to Monitor a Recreational Beach for Cyanobacterial Toxins

Station Information		
Station Name:	Date: (MM/DD/YY) _____/_____/_____	Time: ___ ___ ___ ___
Station Identification Number:		
Project Name:		
Sampled By:	Samples Shipped By:	Date: (MM/DD/YY) _____/_____/_____

Sampling Information
Sample Type: Regular Concurrent Replicate Sequential Replicate Split Replicate Field Blank Laboratory Blank Other:_____
Sample Collection: Surface Grab Sample Surface Accumulation Sample Sampling Device: Open Mouth Bottle Sampler Sampling Method: Grab Sample

Physical Site Conditions
Lake Color: Brown Green Blue Clear Other_____
Surface Accumulation Present: Yes No Accumulation Sample Collected: Yes No Location:
Sky: Clear Cloudy _____% Precipitation: Light Medium Heavy Rain Mist Fog
Wind: Calm Light Breeze Gusty Windy
Other Observations:

Field Measurements		
Latitude: __ __°__ __' __ __" N	Secchi Disc Depth:	pH:
Longitude: __ __°__ __' __ __" W	Water Temperature:	Turbidity:
Air Temperature:	Dissolved Oxygen:	In Situ Fluorescence:
Barometric Pressure:	Specific Conductance:	Other:
Photographs/Observations:		

Sample Locations		
Transect 1	Transect 2	Transect 3
Ankle Depth: Latitude: __ __°__ __' __ __" N Longitude: __ __°__ __' __ __" W	Ankle Depth: Latitude: __ __°__ __' __ __" N Longitude: __ __°__ __' __ __" W	Ankle Depth: Latitude: __ __°__ __' __ __" N Longitude: __ __°__ __' __ __" W
Knee Depth: Latitude: __ __°__ __' __ __" N Longitude: __ __°__ __' __ __" W	Knee Depth: Latitude: __ __°__ __' __ __" N Longitude: __ __°__ __' __ __" W	Knee Depth: Latitude: __ __°__ __' __ __" N Longitude: __ __°__ __' __ __" W
Chest Depth: Latitude: __ __°__ __' __ __" N Longitude: __ __°__ __' __ __" W	Chest Depth: Latitude: __ __°__ __' __ __" N Longitude: __ __°__ __' __ __" W	Chest Depth: Latitude: __ __°__ __' __ __" N Longitude: __ __°__ __' __ __" W
Remarks:	Remarks:	Remarks:

Related Sampling Activities		
Variable	Supporting Information	Collected (Check)
Total Cyanobacterial Toxins	Bottle Type: HDPE Amber Glass	
Remarks:		

APPENDIX 7.5–C.
Example Design and Approach for an Interpretive Study to Develop a Real-Time Model to Estimate Geosmin and 2-methylisoborneol (MIB) Concentrations

Objective

Describe the environmental factors that are associated with taste-and-odor episodes caused by cyanobacteria in a drinking-water supply reservoir and develop a real-time model to estimate the probability of geosmin and MIB concentrations exceeding the human detection threshold of 10 nanograms per liter.

Design and Approach

Sampling frequency. Samples will be collected monthly from November through April and bi-weekly from May through October, when cyanobacteria typically are abundant in the drinking-water supply reservoir. The sampling regime may need to be adjusted depending on potential ice cover. During a taste-and-odor event, sampling frequency will be increased to weekly or semi-weekly. Sample collection will continue for 5 years.

Site location. The main study site will be located within the vicinity of the drinking-water intake. Other locations also may be sampled if surface accumulations of cyanobacteria develop.

Sample type. The type of samples collected will depend on the vertical structure and distribution of cyanobacteria. Generally, when the water column is stratified, integrated samples will be collected from the epilimnion, metalimnion, and hypolimnion; when the water column is not stratified, integrated photic-zone samples will be collected. Surface samples and discrete-depth samples also may be collected depending on cyanobacterial distribution.

Sampler used. A pump will be used to collect depth-integrated samples and discrete-depth samples. An open-mouth bottle sampler will be used to collect surface samples. All sample types will be composited in a churn for sample processing.

Type of taste-and-odor analysis. Cyanobacterial taste-and-odor samples will be analyzed for total and dissolved concentrations of geosmin and MIB.

Ancillary data. Real-time water-quality monitors will be installed in the photic zone near the drinking-water intake, and will measure lake level, light (irradiance), water temperature, dissolved oxygen, specific electrical conductance, pH, turbidity, and in situ fluorescence. An anemometer (wind meter) also will be installed at the site. Other meteorological data will be obtained daily from a local weather station and the timing of inflow events will be recorded.

During each sampling, site photographs and global positioning system (GPS) coordinates will be recorded and Secchi disc depth and vertical profiles of light (irradiance), water temperature, dissolved oxygen, specific electrical conductance, pH, turbidity, and in situ fluorescence will be measured. In addition to taste-and-odor compounds, each sample will be analyzed for total and dissolved cyanobacterial toxins, cyanobacterial (phytoplankton) community composition (enumeration, biovolume, and identification), chlorophyll, actinomycetes bacteria, nutrients, suspended sediment, major ions, and alkalinity.

Quality-control samples. Equipment blanks and field blanks will be collected quarterly. Sequential replicates (true field replicates) will be collected for 20 percent of all samples. Split replicates will be collected for 10 percent of all samples.

Results. The results of this study will describe the seasonal patterns in the occurrence of taste-and-odor compounds and give an indication of when taste-and-odor episodes are most likely to occur. In addition, the relations between environmental variables, cyanobacterial community composition, and the occurrence of taste-and-odor compounds will be described, and may indicate potential management options. Real-time data and discrete water-quality samples will allow the development of a real-time model to estimate the probability that taste-and-odor compound concentrations will exceed the human detection threshold. This model can allow the drinking-water treatment facility to adjust treatment accordingly and minimize the effects of taste-and-odor occurrences on drinking-water quality.

Field Form for a Study to Develop a Real-Time Model to Estimate Geosmin and 2-methylisoborneol (MIB) Concentrations

Station Information		
Station Name:	Date: (MM/DD/YY) _____/_____/_____	Time: ___ ___ ___ ___
Station Identification Number:		
Project Name:		
Sampled By:	Samples Shipped By:	Date: (MM/DD/YY) _____/_____/_____

Sampling Information

Sample Type: Regular Concurrent Replicate Sequential Replicate Split Replicate Field Blank
Laboratory Blank Other:_____

Sample Collection: Integrated Epilimnion Integrated Metalimnion Integrated Hypolimnion
Integrated Photic Zone Discrete Depth Surface Accumulation

Photic Depth:
Integrated Sample Depths:
Discrete Sample Depth:

Sampling Device: Pump Open Mouth Bottle Sampler

Sampling Method: Multiple Vertical Point Sample Grab Sample

Physical Site Conditions

Lake Color: Brown Green Blue Clear Other_____

Surface Accumulation Present: Yes No Accumulation Sample Collected: Yes No

Location:

Sky: Clear Cloudy _____% Precipitation: Light Medium Heavy Rain Mist Fog

Wind: Calm Light Breeze Gusty Windy

Other Observations:

Related Sampling Activities

Variable	Supporting Information	Collected (Check)
Total Taste-and-Odor		
Dissolved Taste-and-Odor		
Total Cyanobacterial Toxins	Bottle Type: HDPE Amber Glass	
Dissolved Cyanobacterial Toxins	Bottle Type: HDPE Amber Glass	
Phytoplankton	Preservative: Preservative volume:_____	
Chlorophyll	Volume Filtered:	
Actinomycetes Bacteria		
Nutrients		
Suspended Sediment		
Major Ions		
Alkalinity		

Remarks:

Field Form for a Study to Develop a Real-Time Model to Estimate Geosmin and 2-methylisoborneol (MIB) Concentrations—*continued*

Field Measurements		
Latitude: __ __°__ __' __ __" N	Secchi Disc Depth:	Air Temperature:
Longitude: __ __°__ __' __ __" W	Surface Irradiance:	Barometric Pressure:
	Photic Depth:	Other:

Vertical Profile

Depth	Irradiance	Water Temperature	Dissolved Oxygen	Specific Conductance	pH	Turbidity	In situ Fluorescence

Photographs/Observations: