CHAPTER 8
Algae and cyanobacteria in fresh water

The term algae refers to microscopically small, unicellular organisms, some of which form colonies and thus reach sizes visible to the naked eye as minute green particles. These organisms are usually finely dispersed throughout the water and may cause considerable turbidity if they attain high densities. Cyanobacteria are organisms with some characteristics of bacteria and some of algae. They are similar to algae in size and, unlike other bacteria, they contain blue-green and green pigments and can perform photosynthesis. Therefore, they are also termed blue-green algae (although they usually appear more green than blue).

Human activities (e.g., agricultural runoff, inadequate sewage treatment, runoff from roads) have led to excessive fertilization (eutrophication) of many water bodies. This has led to the excessive proliferation of algae and cyanobacteria in fresh water and thus has had a considerable impact upon recreational water quality. In temperate climates, cyanobacterial dominance is most pronounced during the summer months, which coincides with the period when the demand for recreational water is highest.

Livestock poisonings led to the study of cyanobacterial toxicity, and the chemical structures of a number of cyanobacterial toxins (cyanotoxins) have been identified and their mechanisms of toxicity established. In contrast, toxic metabolites from freshwater algae have scarcely been investigated, but toxicity has been shown for freshwater species of Dinophyceae and also the brackish water Prymnesiophyceae and an ichthyotoxic species (*Peridinium polonicum*) has been detected in European lakes (Pazos et al., in press; Oshima et al., 1989). As marine species of these genera often contain toxins, it is reasonable to expect toxic species among these groups in fresh waters as well.

Although many species of freshwater algae proliferate quite intensively in eutrophic waters, they do not accumulate to form dense surface scums (often termed blooms) of extremely high cell density, as do some cyanobacteria. The toxins that freshwater algae may contain are therefore not accumulated to concentrations likely to become hazardous to human health or livestock. For these reasons, this chapter will focus primarily on the health impacts of cyanobacteria. More detailed coverage of cyanobacteria and human health is available in *Toxic Cyanobacteria in Water* (Chorus & Bartram, 1999).

8.1 Occurrence of toxic cyanobacteria

Toxic cyanobacteria are found worldwide in inland and coastal water environments. At least 46 species have been shown to cause toxic effects in vertebrates (Sivonen & Jones, 1999). The most common toxic cyanobacteria in fresh water are *Microcystis* spp., *Cylindrospermopsis raciborskii*, *Planktothrix* (syn. *Oscillatoria*) *rubescens*, *Synechococcus* spp., *Planktothrix* (syn. *Oscillatoria*) *agardhii*, *Gloeotrichia* spp., *Anabaena* spp., *Lyngbya* spp., *Aphanizomenon* spp., *Nostoc* spp., some *Oscillatoria* spp., *Schizothrix* spp. and *Synechocystis* spp. Toxicity cannot be excluded for further species and genera. As research broadens and covers more regions over the globe, additional toxic species are likely to be found. Therefore, it is prudent to presume a toxic potential in any cyanobacterial population.

The most widespread cyanobacterial toxins are microcystins and neurotoxins (see section 8.3). Some species contain neurotoxin and microcystin simultaneously. Field populations of the most common bloom-forming genus, *Microcystis*, are almost always toxic (Carmichael, 1995), but non-toxic strains do occur. Generally, toxicity is not a trait specific for certain species; rather, most species comprise toxic and non-toxic strains. For microcystins, it has been shown that toxicity of a strain depends on whether or not it contains the gene for microcystin production (Rouhiainen et al., 1995; Dittmann et al., 1996) and that field populations are a mixture of both genotypes with and without this gene (Kurmayer et al., 2002). Experience with cyanobacterial cultures also shows that microcystin production is a fairly constant trait of a given strain or genotype, only somewhat modified by environmental conditions (see various contributions in Chorus, 2001). While conditions leading to cyanobacterial proliferation are well understood (the physiological or biochemical function of toxins for the cyanobacteria is the subject of many hypotheses—Chorus & Bartram, 1999), the factors leading to the dominance of toxic strains over non-toxic ones are not.

Worldwide, about 60% of cyanobacterial samples investigated contain toxins (see section 8.4). The toxicity of a single bloom may, however, change in both time and space. Demonstrations of toxicity of the cyanobacterial population in a given lake do not necessarily imply an environmental or human hazard as long as the cells remain thinly dispersed. Mass developments and especially surface scums pose the risks.

8.2 Formation of cyanobacterial blooms

In contrast to true algae, many species of planktonic cyanobacteria possess specialized intracellular gas vesicles. Stacks of these minute (<300 nm) proteinaceous hollow cylinders maintain a gas-filled space in the cell, which enables the organism to regulate its buoyancy and thus to actively seek water depths with optimal growth conditions. However, regulation of buoyancy by changing the amount of gas in the vesicles is slow. Cells adapted to turbulent mixing by enlarged gas vesicles will take a few days to reduce their buoyancy in order to adapt to more quiescent conditions. Thus, especially when the weather changes from stormy to fine (i.e., mixing conditions in the water change from turbulent to strongly stratified), many excessively

buoyant cells or colonies may accumulate at the surface. Light winds drive them to leeward shores and bays, where they form scums (Figure 8.1). In extreme cases, such agglomerations may become very dense and even acquire a gelatinous consistency. More frequently, they are seen as streaks or slimy scums that may even look like blue-green paint or jelly. Such situations may change rapidly, within hours, or may remain unchanged for weeks (Chorus & Bartram, 1999).

Scums can be quickly broken by wave action and redispersed by renewed wind mixing. However, especially in shallow bays, scum material may take a long time to disperse, as a result of either wave wash or, ultimately, disintegration of the cells. Dying and lysing cells release their contents into the water, where pigments may adopt a copper-blue colour. Bacterial decomposition leads to rapid putrefaction of the material. The in-shore deposits are often repulsive and potentially very toxic.

Whereas agglomerations of cyanobacteria are usually caused by planktonic species in eutrophic waters, benthic mats in oligotrophic waters (which are relatively poor in plant nutrients) occasionally also cause problems; these surface-covering mats can grow only in clear water, in which sunlight penetrates to the bottom. During sunny days, their photosynthesis may lead to high rates of oxygen production, forming bubbles that loosen parts of the mats and drive them to the surface. Mats of benthic cyanobacteria washed to the shore and scavenged by dogs have been lethal (Edwards et al., 1992), and cattle deaths on Swiss alpine meadows may also be caused by benthic cyanobacteria (Mez et al., 1997, 1998). Although relevant for pets and live-stock, the human health impact of these cyanobacteria on beaches will be considerably lower than that of scums in the water. Awareness of the potential toxicity of such beached mats is, however, important, because they accumulate along shores of clear waters usually not recognized as potentially producing harmful cyanobacteria or algae.

8.3 Cyanotoxins

Progress in analytical chemistry has enabled the isolation and structural identification of three neurotoxins with somewhat different modes of blocking neuronal signal transmission (anatoxin-a, anatoxin-a(s) and saxitoxins), one general cytotoxin, which inhibits protein synthesis (cylindrospermopsin), and a group of toxins termed microcystins (or nodularins, found in brackish waters), which inhibit protein phosphatases. Phosphatase inhibition is generally cytotoxic, but microcystins are primarily hepatotoxic, because they use the bile acid carrier to pass through cell membranes. These toxins were named after the organism from which they were first isolated, but most of them have been found in a wider array of genera, and some species contain more than one toxin or both microcystins and neurotoxins.

Although the toxins listed in Table 8.1 are assumed to be the substances most significant for human health, it is unlikely that all of the important cyanotoxins have been discovered. Yoo et al. (1995) pointed out that an increasing variety of individual toxins is continually being discovered. Numerous pharmacological working groups are conducting research for pharmacologically active substances from cyanobacteria (e.g., Mundt & Teuscher, 1988; Falch et al., 1995). Fastner et al.

Lake profile

moderate risk level:
- 50 µg/l chlorophyll-a
- or 100 000 cells/ml
- possibly 20 µg/l of microcystin in top 4 m of water body

4 m

4 cm

Buoyancy leads to 100-fold accumulation of cells

100-fold accumulation to high risk level scum:
- 5 000 µg/l chlorophyll-a
- or 10 000 000 cells/ml
- possibly 2 000 µg/l of microcystin in top 4 cm of water body

fetch of wind 100 m

very thick scum

1000-fold accumulation if wind sweeps scums from 100 m into 10 m:
- 50 000 µg/l chlorophyll-a
- or 100 000 000 cells/ml
- possibly 20 000 µg/l of microcystin concentrated in one bay of the water body

Lake bird's eye view

direction of wind

direction of wind

FIGURE 8.1. SCHEMATIC ILLUSTRATION OF SCUM FORMATION CHANGING THE CYANOTOXIN RISK FROM MODERATE TO HIGH (CHORUS & BARTRAM, 1999)

(2001) showed that primary rat hepatocytes reacted to microcystins in crude extracts of some strains of cyanobacteria in close correlation to their content of microcystins, but that this reaction was further enhanced by an unknown factor. Oberemm et al. (1997) demonstrated substantial toxicity of cyanobacterial crude extracts to fish eggs, the effects not being due to the content of any of the known cyanotoxins.

TABLE 8.1. CYANOBACTERIAL TOXINS AND THEIR ACUTE TOXICITY[a]

Cyanotoxins	LD_{50} (i.p. mouse)[b] of pure toxin (µg/kg)	Taxa known to produce the toxin(s)	Mechanism of toxicity
Protein phosphatase blockers (cyclic peptides with the amino acid ADDA)			
Microcystins in general (~60 known congeners)	45–>1000	*Microcystis, Planktothrix, Oscillatoria, Nostoc Anabaena, Anabaenopsis Hapalosiphon*	all block protein phosphatases by covalent binding and cause haemorrhaging of the liver; cumulative damage may occur
Microcystin-LR	60 (25–125)		
Microcystin-YR	70		
Microcystin-RR	300–600		
Nodularin	30–50	*Nodularia spumigena*	
Neurotoxins			
Anatoxin-a (alkaloid)	250	*Anabaena, Oscillatoria, Aphanizomenon, Cylindrospermum*	blocks post-synaptic depolarization
Anatoxin-a(s) (unique organophosphate)	40	known only from two species of *Anabaena*	blocks acetylcholinesterase
Saxitoxins (carbamate alkaloids)	10–30	*Aphanizomenon, Anabaena, Lyngbya, Cylindrospermopsis raciborskii*	block sodium channels
Cytotoxin			
Cylindrospermopsin (alkaloid)	2100 in 1 day 200 in 5–6 days	*Cylindrospermopsis raciborskii*	blocks protein synthesis; substantial cumulative toxicity

[a] derived from Turner et al., 1990; Kuiper-Goodman et al., 1999; Sivonen & Jones, 1999.

[b] LD_{50} = lethal dose$_{50}$ (the dose of a chemical that will, on average, kill 50% of a group of experimental animals); i.p. = intraperitoneal.

8.3.1 Microcystins

Microcystins are the most frequently occurring and widespread of the cyanotoxins. They are cyclic heptapeptides containing a specific amino acid (ADDA) side chain which, to date, has been found only in microcystins and nodularin (a cyclic pentapeptide toxin of cyanobacteria from brackish waters). About 70 structural analogues of microcystin have been identified (Rinehart et al., 1994; Sivonen & Jones, 1999). They vary with respect to methyl groups and two amino acids within the ring. This has consequences for the tertiary structure of the molecule and results in pronounced differences in toxicity as well as in hydrophobic/hydrophilic properties. Microcystins block protein phosphatases 1 and 2a (which are important molecular switches in all eukaryotic cells) with an irreversible covalent bond (MacKintosh et al., 1990).

The chief pathway for microcystins entry into cells is the bile acid carrier, which is found in liver cells and, to a lesser extent, in intestinal epithelia (Falconer, 1993). For vertebrates, a lethal dose of microcystin causes death by liver necrosis within hours up to a few days. Evidence for the permeability of other cell membranes to microcystins is controversial. It is possible that hydrophobic structural analogues can penetrate into some cell types even without the bile acid carrier (Codd, 1995). In addition, Fitzgeorge et al. (1994) published evidence for disruption of nasal tissues by the common hydrophilic analogue microcystin-LR. While toxicity by oral uptake is generally at least an order of magnitude lower than toxicity by intraperitoneal (i.p.) injection, intranasal application in these experiments was as toxic as i.p. injection, and membrane damage by microcystin enhanced the toxicity of anatoxin-a. This uptake route may be relevant for water sports activities that lead to inhalation of spray and droplets, such as waterskiing.

Microcystins are found in most populations of *Microcystis* spp. (which frequently form surface scums) and in strains of some species of *Anabaena* (which may also form scums). High microcystin content has also been observed in *Planktothrix* (syn. *Oscillatoria*) *agardhii* and *P. rubescens* (Fastner et al., 1999). *P. agardhii*, however, never forms scums, and where it occurs *P. rubescens* does not usually form scums during the recreational water use season, thus reducing the hazard to swimmers.

Fitzgeorge et al. (1994) demonstrated that microcystin toxicity is cumulative: a single oral dose resulted in no increase in liver weight (which is a measure of liver damage), whereas the same dose applied daily over seven days caused an increase in liver weight of 84% and thus had the same effect as a single oral dose 16 times as large. This may be explained by the irreversible covalent bond between microcystin and the protein phosphatases and subsequent substantial damage to cell structure (Falconer, 1993). Healing of the liver probably requires growth of new liver cells. Subacute liver injury is likely to go unnoticed for two reasons:

- liver injury results in externally noticeable symptoms only when it is severe;
- acute dose–response curves for microcystins are steep. Therefore, little acute damage may occur until levels close to severe acute toxicity are reached. As a result of the lack of apparent symptoms at moderate exposure, exposure is likely to be continued by people uninformed of the risk (e.g., for consecutive days of a holiday or a hot spell), which will increase the risk of cumulative liver damage.

There are two aspects of chronic microcystin damage to the liver—progressive active liver injury (Falconer et al., 1988) and the potential for promotion of tumour growth. Tumour-promoting activity of microcystins is well documented, although microcystins alone have not been demonstrated to be carcinogenic. Promotion of mouse skin tumours has been shown after initiation by topical exposure to a carcinogen (dimethylbenzanthracene) followed by ingestion of a *Microcystis aeruginosa* extract (Falconer & Buckley, 1989; Falconer & Humpage, 1996). In rat liver studies, the appearance of pre-neoplastic liver foci and nodules was promoted by pure microcystin-LR in a protocol involving one i.p. dose of diethylnitrosamine and i.p. doses of microcystin-LR over several weeks (Nishiwaki-Matsushima et al., 1992).

Studies on the mechanism of cell toxicity showed that microcystin interferes with cell structure and mitosis, and this may help to explain the tumour-promoting activity (Falconer & Yeung, 1992; Kaja, 1995). It has been suggested that, in China, cases of liver tumours in humans may be associated with the presence of cyanotoxins in drinking water (Ueno et al., 1996).

8.3.2 Neurotoxins

Irrespective of somewhat different modes of action, all three neurotoxins (Table 8.1) have the potential to be lethal by causing suffocation—anatoxin-a and a(s) through cramps, saxitoxins through paralysis. However, no human deaths from exposure to neurotoxins associated with recreational use of water are known.

Anatoxin-a(s) is the only known naturally occurring organophosphate cholinesterase inhibitor and causes strong salivation (the 's' in its name stands for salivation), cramps, tremor, diarrhoea, vomiting and an extremely rapid death (within minutes). Saxitoxins and anatoxin-a(s) are among the most neurotoxic substances known. However, evidence is accumulating that in lakes and rivers they do not occur as frequently as microcystins. This applies especially to anatoxin-a(s): to date, it has been found only in a small number of *Anabaena* blooms in North America. Furthermore, concentrations even of these highly toxic substances in scums will scarcely reach levels acutely neurotoxic to a human ingesting a mouthful. In contrast, neurotoxicity may be experienced by livestock that drink many litres of contaminated water and pets—especially dogs—that gather scum material in their fur and ingest it through grooming with the tongue.

After ingestion of a sublethal dose of these neurotoxins, recovery appears to be complete, and no chronic effects have been observed to date. For these reasons, the neurotoxins are a hazard to be aware of when using waters populated with cyanobacteria for recreation. On the basis of current knowledge, however, it is reasonable to consider them less dangerous than microcystins or cylindrospermopsin, which may cause ongoing injury.

8.3.3 Cylindrospermopsin

Cylindrospermopsin is an alkaloid isolated from *Cylindrospermopsis raciborskii* (Ohtani et al., 1992). It is a general cytotoxin that blocks protein synthesis, the first clinical symptoms being kidney and liver failure. In contrast to the pure toxin, crude extracts of the organism also cause injury to the lungs, adrenals and intestine, indicating further, unknown toxins in the organism. Clinical symptoms may become manifest only several days after exposure, so it will often be difficult to determine a cause-effect relationship. Patients intoxicated with cylindrospermopsin via drinking-water in an incident in Australia escaped death only through skilled and intensive hospital care (Falconer, 1996). *Cylindrospermopsis raciborskii* is considered to be a tropical and subtropical species, but has been reported to form blooms as far north as Vienna (Roschitz, 1996). Substantial populations have been reported from north-eastern Germany (C. Wiedner, personal communication), and generally *C. raciborskii*

appears to be invading temperate regions (Padisák, 1997). Thus, cylindrospermopsin may become relevant in temperate zones in future.

8.3.4 *Analysis*

From the 1960s to the end of the 1980s, detection of cyanotoxin was primarily performed with the mouse bioassay (outlined in section 7.4), conducted to assess the safety of drinking-water supplies. Due to the high cost and lack of approved laboratories as well as ethical limitations of applicability, this method is not suitable for large screening or monitoring programmes. However, effective methods of chemical analysis are now available for the known cyanotoxins, and sensitive immunoassays as well as enzyme assays have become commercially available for the most important ones (e.g., microcystins and saxitoxins). This opens new possibilities for screening programmes targeted at assessment of the potential risk, as well as for regular surveillance (Chorus & Bartram, 1999).

8.4 Evidence for toxicity of cyanobacteria

Observations of lethal poisoning of animals drinking from water with mass developments of cyanobacteria are numerous. The first documented case of a lethal intoxication of livestock after drinking water from a lake heavily populated with cyanobacteria was published in the 1800s (Francis, 1878), and cases recorded since have included sheep, cattle, horses, pigs, dogs, fish, rodents, amphibians, waterfowl, bats, zebras and rhinoceroses (Codd et al., 1989). Dogs have died after grooming accumulations of cyanobacteria out of their fur or after ingesting beached mats of benthic cyanobacteria.

A number of human deaths have been reported through exposure to cyanobacterial toxins through renal dialysis (Carmichael, 1996; Jochimsen et al., 1998), and also implicated in drinking-water (Teixera et al., 1993). Health impairments are also seen from numerous anecdotal reports of irritations of the skin and/or mucous membranes and from documented cases of illness after exposure through drinking-water as well as accidental swallowing or aspiration of scum material. Other sources of information include toxicological data from animal experiments and data on concentrations of cyanobacterial toxins in waters used for drinking-water purposes and recreation.

Human health risk from exposure to cyanobacteria and their toxins during recreational water use arises through three routes of exposure:

- direct contact of exposed parts of the body, including sensitive areas such as the ears, eyes, mouth and throat, and the areas covered by a bathing suit (which may collect cell material);
- accidental uptake of water containing cells by swallowing; and
- uptake of water containing cells by aspiration (inhalation).

Different cyanobacterial metabolites are likely to be involved in evoking symptoms associated with these exposure routes.

8.4.1 *Exposure through dermal contact*

Allergic or irritative dermal reactions of varying severity have been reported from a number of freshwater cyanobacterial genera (*Anabaena*, *Aphanizomenon*, *Nodularia*, *Oscillatoria*, *Gloeotrichia*) after recreational exposure. Bathing suits and particularly wet suits tend to aggravate such effects by accumulating cyanobacterial material and enhancing disruption of cells and liberation of cell content. Reports from the USA have recorded allergic reactions from recreational exposure, and the cyanobacterial pigment phycocyanin has been shown to be responsible in one case (Cohen & Reif, 1953). In addition, cutaneous sensitization to cyanobacteria has been documented. Skin irritations were a frequent symptom found in an epidemiological study by Pilotto et al. (1997) on health effects after recreational exposure to cyanobacteria. This study showed correlation to cyanobacterial cell density and duration of exposure, but not to microcystin concentrations. It is probable that these symptoms are not due to the recognized cyanotoxins listed in Table 8.1, but rather to currently largely unidentified substances.

Allergic reactions to cyanobacteria are frequently reported at the level of "anecdotal evidence" from eutrophic recreational waters, and it has been claimed that "allergic reactions to cyanobacteria are relatively common" (Yoo et al., 1995, p. 77). However, these have been rarely investigated in scientific studies or published. Among the small number of publications available, Heise (1949) described ocular and nasal irritations in swimmers exposed to Oscillatoriaceae. McElhenny et al. (1962) applied extracts from four different algal species, including cyanobacteria and Chlorophyceae (as intracutaneous skin tests), to 20 non-allergic children, none of who responded, and to 120 children with respiratory allergies, 98 of who showed clear positive reactions to at least one of the test strains. Mittal et al. (1979) tested 4000 patients in India with respiratory allergies, 25% of who showed positive reactions to either cyanobacteria or Chlorophyceae, or to both.

Allergic reactions are not confined to cyanobacteria, but may also be evoked by planktonic algae. However, allergic reactions require elevated cell densities in water used for swimming, and mass developments in fresh waters are most frequently due to cyanobacteria. Furthermore, other groups of algae do not accumulate as surface scums, and therefore their metabolites will not occur in comparably high concentrations. Thus, cyanobacteria are likely to be the most frequently occurring cause of such reactions.

8.4.2 *Exposure through ingestion or aspiration*

Swallowing or aspiration was the exposure route in most of the documented cases of human illness that have been associated with cyanobacteria (Box 8.1). In contrast to dermal contact, uptake of cyanobacteria involves a risk of intoxication by the cyanotoxins listed in Table 8.1. This risk may be estimated from cell density, cellular toxin content and known mechanisms of toxicity. Acute mechanisms of toxicity are well known for the neurotoxins and microcystins, and some information is available to estimate risks due to repeated or chronic exposure.

BOX 8.1 HUMAN ILLNESS ASSOCIATED WITH EXPOSURE TO CYANOBACTERIA

ILLNESS ATTRIBUTED TO CYANOTOXINS IN RECREATIONAL WATER

1959: **Canada:** In spite of a kill of livestock and warnings against recreational use, people still swam in a lake infested with cyanobacteria. Thirteen persons became ill (headaches, nausea, muscular pains, painful diarrhoea). In the excreta of one patient—a medical doctor who had accidentally ingested water—numerous cells of *Microcystis* spp. and some trichomes of *Anabaena circinalis* could be identified (Dillenberg & Dehnel, 1960).

1989: **England:** Ten out of 20 soldiers became ill after swimming and canoe training in water with a heavy bloom of *Microcystis* spp.; two developed severe pneumonia attributed to the inhalation of a *Microcystis* toxin and needed hospitalization and intensive care (Turner et al., 1990). Swimming skills and the amount of water ingested appear to have been related to the degree of illness.

1995: **Australia:** Epidemiological evidence of adverse health effects after recreational water contact from a prospective study involving 852 participants showed elevated incidence of diarrhoea, vomiting, flu symptoms, skin rashes, mouth ulcers, fevers, and eye or ear irritations within 2–7 days after exposure (Pilotto et al., 1997). Symptoms increased significantly with duration of water contact and density of cyanobacterial cells, but were not related to the content of known cyanotoxins.

ILLNESS ATTRIBUTED TO CYANOTOXINS IN DRINKING-WATER

1931: **USA:** A massive *Microcystis* bloom in the Ohio and Potomac rivers caused illness of 5000–8000 people whose drinking-water was taken from these rivers. Drinking-water treatment by precipitation, filtration and chlorination was not sufficient to remove the toxins (Tisdale, 1931).

1968: **USA:** Numerous cases of gastrointestinal illness after exposure to mass developments of cyanobacteria were compiled by Schwimmer & Schwimmer (1968).

1979: **Australia:** Combating a bloom of *Cylindrospermopsis raciborskii* in a drinking-water reservoir on Palm Island with copper sulfate led to liberation of toxins from the cells into the water and resulted in serious illness (with hospitalization) of 141 people supplied from this reservoir (Falconer, 1993, 1994).

1981: **Australia:** In the city of Armidale, liver enzyme activities (a sign of exposure to toxic agents) were found to be elevated in the blood of the population supplied from surface water polluted by *Microcystis* spp. (Falconer et al., 1983).

1985: **USA:** Carmichael (1994) compiled case studies on nausea, vomiting, diarrhoea, fever and eye, ear and throat infections after exposure to mass developments of cyanobacteria.

1988: **Brazil:** Following the flooding of the Itaparica Dam in Bahia State, some 2000 cases of gastroenteritis were reported over a 42-day period, of which 88 resulted in death. Investigation of potential causes of this epidemic eliminated pathogens and identified a very high population of toxic cyanobacteria in the drinking-water supply in the affected areas (Teixera et al., 1993).

1993: **China:** The incidence of liver cancer was related to water sources and was significantly higher for populations using cyanobacteria-infested surface waters than for those drinking groundwater (Yu, 1995).

Continued

1994: **Sweden:** Illegal use of untreated river water in a sugar factory led to an accidental cross-connection with the drinking-water supply for an uncertain number of hours. The river water was densely populated by *Planktothrix agardhii* and samples taken a few days before and a few days after the incident showed these cyanobacteria to contain microcystins. In total, 121 of 304 inhabitants of the village (as well as some dogs and cats) became ill with vomiting, diarrhoea, muscular cramps and nausea (Anadotter et al., 2001).

ILLNESS ATTRIBUTED TO CYANOTOXINS IN WATER USED FOR HAEMODIALYSIS

1975: **USA:** Endotoxic shock of 23 dialysis patients in Washington, DC, was attributed to a cyanobacterial bloom in a drinking-water reservoir (Hindman et al., 1975).

1996: **Brazil:** In total, 131 dialysis patients were exposed to microcystins from the water used for dialysis; 56 died. At least 44 of these victims showed the typical symptoms associated with microcystin, now referred to as "Caruaru Syndrome", and liver microcystin content corresponded to that of laboratory animals having received a lethal dose of microcystin (Jochimsen et al., 1998).

Most documented cases of human injury through cyanotoxins involved exposure through drinking-water, and they demonstrate that humans have become ill—in some cases seriously—through ingestion or aspiration of toxic cyanobacteria. The low number of reported cases may be due to lack of knowledge about the toxicity of cyanobacteria; neither patients nor doctors associate symptoms with this cause. Symptoms reported include "abdominal pain, nausea, vomiting, diarrhoea, sore throat, dry cough, headache, blistering of the mouth, atypical pneumonia, and elevated liver enzymes in the serum, especially gamma-glutamyl transferase" (Carmichael, 1995, p. 9), as well as hay fever symptoms, dizziness, fatigue, and skin and eye irritations; these symptoms are likely to have diverse causes, with several classes of toxin and genera of cyanobacteria involved.

8.5 Evidence for toxicity of algae

Systematic investigation of the toxicity of freshwater algae is required, particularly for species related to toxic marine taxa (dinoflagellates, diatoms, haptophytes). However, as discussed above, freshwater algae are considerably less likely to pose recreational health hazards comparable to those of scum-forming cyanobacteria, because algae lack similarly effective mechanisms of accumulation.

Oshima et al. (1989) isolated and identified three ichthyotoxins (polonicumtoxins A, B and C) from a dinoflagellate, *Peridinium polonicum*. Toxicity in the mouse bioassay was 1.5–2 mg/kg, i.e., several orders of magnitude lower than the toxicity of microcystin-LR. The Ames test showed no mutagenicity, but the authors emphasized the need for studies on chronic toxicity to evaluate the potential health risk of these toxins.

Allergic reactions have been investigated as outlined in section 8.4.1. Skin reactions in response to a bloom of *Uroglena* spp. were observed in a small number of swimmers. These reactions were especially pronounced under bathing suits, where

cells accumulated and were partially disrupted during swimming (Chorus, 1993). Divers frequently complain of dermal reactions to algal material accumulating under their wet suits, which tend to act as a strainer that lets out water but collects algae between skin and suit. Pressure and friction between fabric and skin lead to cell disruption, liberation of content and intensified dermal exposure, not only to algal cell wall material, but also to substances otherwise largely confined within the cells.

One of the few reports involved the raphidophyte algal species *Gonyostomum semen* (related to *Heterosigma* mentioned in chapter 7), which may develop high population densities in slightly acidic waters and emits a slimy substance causing skin irritation and allergic reactions. In Sweden, occurrence of this species led to closure of a number of freshwater recreational sites (Cronberg et al., 1988).

8.6 Health risk evaluation

Documented evidence of significant human health impairment exists only for cyanobacteria, not for freshwater algae. Data from surveys in a number of countries show that toxicity is to be expected in about 60% of all samples containing cyanobacteria (Table 8.2). Generally, the liver-toxic microcystins appear to be more common than neurotoxins, although the latter have caused severe animal poisonings in North America, Europe and Australia. Blooms containing cylindrospermopsin have been reported from Australia, Hungary, Japan, Israel and Germany.

While a general picture of the frequency of occurrence of cyanotoxins associated with certain cyanobacterial taxa is emerging, it is less clear what cyanotoxin levels may be expected in recreational waters containing cyanobacteria. Very few studies have addressed the variability of toxin content in the course of the development of cyanobacterial populations (Benndorf & Henning, 1989; Jungmann, 1995; Kotak et al., 1995; Fastner et al., 1999), although this knowledge would be important for risk assessment. This is because the cumulative toxicity of microcystins means that hazards are greatest for persons exposed regularly over a number of days or weeks. For management of recreational waters, a few years of regular investigation of the toxin content of prevalent cyanobacterial blooms may provide information on the variability of toxin content in both time and space. If the toxin content proves to show little variation during several weeks or even months of blooming for certain key species, a basis for future predictions of cellular toxin content from frequent cell counts and only occasional toxin analysis may be established.

Most studies have focused on the quantity of toxins contained in the cells of the dominant cyanobacteria. If the cell density is known in addition to the toxin content per cell, toxin concentrations per litre of water can be calculated. A few studies have directly addressed concentrations per litre, and sensitive detection methods now allow direct determination of toxin concentrations per litre rather than requiring enrichment of cell material.

Generally, the cyanotoxin content of cells can reach levels of several milligrams per gram dry weight. This has been established for microcystins, nodularin, cylindrospermopsin, anatoxin-a and saxitoxins, the maximum being found for nodularin:

18 mg/g dry weight (Sivonen & Jones, 1999). If both toxin content and cell density or biomass of cyanobacteria per litre is known for a given water body, maximum toxin concentrations to be expected can be estimated from such data. As the toxic concentrations depend upon cell density, scum formation is critical in determining cell density. In one study, microcystin concentrations ranged from 0.01 to 0.35 mg/litre while the cyanobacteria were evenly dispersed (Fastner et al., 1999). However, sampling of shoreline scums of the same water bodies showed microcystin concentrations of more than 1 mg/litre in 7 of 34 samples, and maxima reached 24 mg/litre (Chorus & Fastner, 2001). Some commonly occurring species, such as *Planktothrix agardhii*, never form scums. The maximum reported microcystin concentration per litre of water for *P. agardhii* is 0.35 mg/litre (Fastner et al., 1999).

TABLE 8.2. FREQUENCIES OF MASS OCCURRENCES OF TOXIC CYANOBACTERIA IN FRESH WATERS[a]

Country	No. of samples tested	% of toxic samples
Australia	231	42
Australia	31	84[b]
Brazil	16	75
Canada, Alberta	24	66
Canada, Alberta	39	95
Canada, Alberta (three lakes)	226	74[b]
Canada, Saskatchewan	50	10
China	26	73
Czech Republic and Slovakia	63	82
Finland	215	44
France, Brittany	22	73[b]
Germany	533	72[b]
Germany	393	22
Former German Democratic Republic	10	70
Greece	18	?
Hungary	50	66
Japan	23	39
Netherlands	10	90
Portugal	30	60
Scandinavia	81	60
Denmark	296	82
Norway	64	92
Sweden	331	47
United Kingdom	50	48
United Kingdom	50	28[b]
USA, Minnesota	92	53
USA, Wisconsin	102	25
Mean		**59**

[a] From Sivonen & Jones (1999).

[b] High-performance liquid chromatography was used to determine the toxin content of the samples.

For practical purposes, the present state of knowledge implies that health authorities should regard any mass development of cyanobacteria as a potential health hazard.

8.7 Guideline values

As discussed above, approaches to recreational water safety should address the occurrence of cyanobacteria as such, because it is as yet unclear whether all important cyanotoxins have been identified, and the health outcomes observed after recreational exposure—particularly irritation of the skin and mucous membranes—are probably related to cyanobacterial substances other than the well known toxins listed in Table 8.1. Additionally, the particular hazard of liver damage by microcystins should be considered. In face of the difficulty of representative quantitative sampling due to the heterogeneous distribution of cyanobacteria in time and space, particularly with respect to scum formation and scum location, approaches should further include addressing the capacity of a water body to sustain large cyanobacterial populations.

Health impairments from cyanobacteria in recreational waters must be differentiated between the chiefly irritative symptoms caused by unknown cyanobacterial substances and the potentially more severe hazard of exposure to high concentrations of known cyanotoxins, particularly microcystins. A single guideline value therefore is not appropriate. Rather, a series of guideline values associated with incremental severity and probability of health effects is defined at three levels (Table 8.3).

8.7.1 Relatively low probability of adverse health effects

For protection from health outcomes not due to cyanotoxin toxicity, but rather to the irritative or allergenic effects of other cyanobacterial compounds, a guideline level of 20 000 cyanobacterial cells/ml (corresponding to 10 µg chlorophyll-a/litre under conditions of cyanobacterial dominance) can be derived from the prospective epidemiological study by Pilotto et al. (1997). Whereas the health outcomes reported in this study were related to cyanobacterial density and duration of exposure, they affected less than 30% of the individuals exposed. At this cyanobacterial density, 2–4 µg microcystin/litre may be expected if microcystin-producing cyanobacteria are dominant, with 10 µg/litre being possible with highly toxic blooms. This level is close to the WHO provisional drinking-water guideline value of 1 µg/litre for microcystin-LR (WHO, 1998), which is intended to be safe for lifelong consumption. Thus, health outcomes due to microcystin are unlikely, and providing information for visitors to swimming areas with this low-level risk is considered to be sufficient. Additionally, it is recommended that the authorities be informed in order to initiate further surveillance of the site. The results of the epidemiological study (Pilotto et al., 1997) reported some mild irritative effects at 5000 cells but the level of health effect and the small number of people affected were not considered to be a basis to justify action.

8.7.2 Moderate probability of adverse health effects

At higher concentrations of cyanobacterial cells, the probability of irritative symptoms is elevated. Additionally, cyanotoxins (usually cell-bound) may reach concentrations with potential health impact. To assess risk under these circumstances, the data used for the drinking-water provisional guideline value for microcystin-LR

TABLE 8.3. GUIDELINES FOR SAFE PRACTICE IN MANAGING RECREATIONAL WATERS[a]

Guidance level or situation	How guidance level derived	Health risks	Typical actions[b]
Relatively low probability of adverse health effects			
20 000 cyanobacterial cells/ml *or* 10 µg chlorophyll-a/litre with dominance of cyanobacteria	• From human bathing epidemiological study	• Short-term adverse health outcomes, e.g., skin irritations, gastrointestinal illness	• Post on-site risk advisory signs • Inform relevant authorities
Moderate probability of adverse health effects			
100 000 cyanobacterial cells/ml *or* 50 µg chlorophyll-a/litre with dominance of cyanobacteria	• From provisional drinking-water guideline value for microcystin-LR[c] and data concerning other cyanotoxins	• Potential for long-term illness with some cyanobacterial species • Short-term adverse health outcomes, e.g., skin irritations, gastrointestinal illness	• Watch for scums or conditions conducive to scums • Discourage swimming and further investigate hazard • Post on-site risk advisory signs • Inform relevant authorities
High probability of adverse health effects			
Cyanobacterial scum formation in areas where whole-body contact and/or risk of ingestion/aspiration occur	• Inference from oral animal lethal poisonings • Actual human illness case histories	• Potential for acute poisoning • Potential for long-term illness with some cyanobacterial species • Short-term adverse health outcomes, e.g., skin irritations, gastrointestinal illness	• Immediate action to control contact with scums; possible prohibition of swimming and other water contact activities • Public health follow-up investigation • Inform public and relevant authorities

[a] Derived from Chorus & Bartram, 1999.

[b] Actual action taken should be determined in light of extent of use and public health assessment of hazard.

[c] The provisional drinking-water guideline value for microcystin-LR is 1 µg/litre (WHO, 1998).

(WHO, 1998) may be applied. Swimmers involuntarily swallow some water while swimming, and the harm from ingestion of recreational water will be comparable to the harm from ingestion of water from a drinking-water supply with the same toxin content. For recreational water users with whole-body contact (see chapter 1), a swimmer can expect to ingest 100–200 ml of water in one session, sailboard riders and waterskiers probably more.

A level of 100 000 cyanobacterial cells/ml (which is equivalent to approximately 50 µg chlorophyll-a/litre if cyanobacteria dominate) represents a guideline value for a moderate health alert in recreational waters. At this level, a concentration of 20 µg microcystin/litre is likely if the bloom consists of *Microcystis* and has an average toxin

content of 0.2 pg/cell, or 0.4 μg microcystin/μg chlorophyll-a. Levels may be approximately double if *Planktothrix agardhii* dominates. With very high cellular microcystin content, 50–100 μg microcystin/litre would be possible.

The level of 20 μg microcystin/litre is equivalent to 20 times the WHO provisional guideline value concentration for microcystin-LR in drinking-water (WHO, 1998) and would result in consumption of an amount close to the tolerable daily intake (TDI) for a 60-kg adult consuming 100 ml of water while swimming (rather than 2 litres of drinking-water). However, a 15-kg child consuming 250 ml of water during extensive playing could be exposed to 10 times the TDI. The health risk will be increased if the person exposed is particularly susceptible because of, for example, chronic hepatitis B. Therefore, cyanobacterial levels likely to cause microcystin concentrations of 20 μg/litre should trigger further action.

Non-scum-forming species of cyanobacteria such as *Planktothrix agardhii* have been observed to reach cell densities corresponding to 250 μg chlorophyll-a/litre or even more in shallow water bodies. Transparency in such situations will be less than 0.5 m measured with a Secchi disc. *Planktothrix agardhii* has been shown to contain very high cell levels of microcystin (1–2 μg microcystin/μg chlorophyll-a), and therefore toxin concentrations of 200–400 μg/litre can occur without scum formation.

An additional reason for increased alert at 100 000 cells/ml is the potential for some frequently occurring cyanobacterial species (particularly *Microcystis* spp. and *Anabaena* spp.) to form scums. These scums may increase local cell density and thus toxin concentration by a factor of 1000 or more in a few hours (as illustrated in Figure 8.1), thus rapidly changing the risk from moderate to high for bathers and others involved in body-contact water sports. Cyanobacterial scum formation presents a unique problem for routine monitoring at the usual time intervals (e.g., 1 or 2 weeks) because such monitoring intervals are unlikely to pick up hazardous maximum levels. Because of the potential for rapid scum formation at a cyanobacterial density of 100 000 cells/ml or 50 μg chlorophyll-a/litre (from scum-forming cyanobacterial taxa), intensification of surveillance and protective measures are appropriate at these levels. Daily inspection for scum formation (if scum-forming taxa are present) and measures to prevent exposures in areas prone to scum formation are the two principal actions important in these situations.

Intervention is recommended to trigger effective public information campaigns to educate people on avoidance of scum contact. Furthermore, in some cases (e.g., areas with frequent scum formation), restriction of water contact activities may be judged to be appropriate. An intensified monitoring programme should be implemented, particularly looking for scum accumulations. Health authorities should be notified immediately.

8.7.3 High probability of adverse health effects

Abundant evidence exists for potentially severe health outcomes associated with scums caused by toxic cyanobacteria. No human fatalities have been unequivocally associated with cyanotoxin ingestion during recreational water activities, although

numerous animals have been killed by consuming water with cyanobacterial scum material. This discrepancy can be explained by the fact that animals will drink greater volumes of scum-containing water in relation to their body weight, whereas accidental ingestion of scums by humans during swimming will typically result in a lower dose.

Cyanobacterial scums can represent thousand-fold to million-fold concentrations of cyanobacterial cell populations. Calculations suggest that a child playing in *Microcystis* scums for a protracted period and ingesting a significant volume could receive a lethal dose, although no reports indicate that this has occurred. Based on evidence that a lethal oral dose of microcystin-LR in mice is 5000–11 600 µg/kg body weight and sensitivity between individuals may vary approximately 10-fold, the ingestion of 5–50 mg of microcystin could be expected to cause acute liver injury in a 10-kg child. Concentrations of up to 24 mg microcystin/litre from scum material have been published (Chorus & Fastner, 2001). Substantially higher enrichment of scums—up to gelatinous consistency—is occasionally observed, of which accidental ingestion of smaller volumes could cause serious harm. Anecdotal evidence indicates that children, and even adults, may be attracted to play in scums. The presence of scums caused by cyanobacteria is thus a readily detected indicator of a risk of potentially severe adverse health effects for those who come into contact with the scums. Immediate action to control scum contact is recommended for such situations.

8.7.4 *Conclusions*

The approach outlined in this section does not cover all conceivable situations. Swimmers may be in contact with benthic cyanobacteria after a storm breaks off clumps of filaments or cyanobacterial mats naturally detach from the sediment and are accumulated on shorelines (Edwards et al., 1992). Measures of cyanobacterial cell density will not detect these hazards. Instead, this cyanotoxin hazard calls for critical and well informed observation of swimming areas, coupled with a flexible response.

It is difficult to define "safe" concentrations of cyanobacteria in recreational water for allergenic effects or skin reactions, as individual sensitivities vary greatly. Aggravation of dermal reactions due to accumulation of cyanobacterial material and enhanced disruption of cells under bathing suits and wet suits may be a problem even at densities below the guideline levels described above.

8.8 Management options

For purposes of management, it is important to understand that cyanotoxins are chiefly found within cyanobacterial cells. Liberation into the surrounding water is possible, particularly when cells die and lyse, and differences may occur between toxins and species regarding "leakage" from intact cells. However, toxin dissolved in water is rapidly diluted and probably also degraded, whereas hazardously high toxin concentrations usually result from the accumulation of cell material as scums.

Because adequate surveillance is difficult and few immediate management options are available (other than precluding or discouraging use or cancelling water sports

activities such as competitions), provision of adequate public information is a key short-term measure. Medium- to long-term measures are identification of the sources of nutrient (in many ecosystems phosphorus, sometimes nitrogen) pollution and significant reduction of nutrient input in order to effectively reduce proliferation not only of cyanobacteria, but of potentially harmful algae as well.

8.8.1 Short-term measures

Providing adequate information to the public on the cyanobacterial risk associated with using a particular recreational water area is important not only for avoiding this hazard, but also for understanding symptoms potentially caused by exposure and identifying their cause. Communication of warnings to the public may occur through local news media, by posting warning notices and through other means. They may accompany information on other recreational water quality parameters regularly monitored by the authorities and/or some further information on cyanobacteria.

Differentiation between the degree of water contact in different types of water sports should be included in warning notices. Information on the frequently transient nature and very variable local distribution of scums is important to convey the message that recreational activities are restricted only temporarily and often only very locally, and that in such cases acceptable water quality may be found nearby, e.g., at another site of the same lake.

As a precaution, the following guidance is recommended for all freshwater-based recreation and should be included in public information:

- Avoid areas with visible cyanobacterial or algal concentrations and/or scums in the water as well as on the shore. Direct contact and swallowing appreciable amounts are associated with the greatest health risk.
- Where no scums are visible, but the water shows strong greenish discoloration and turbidity, test if you can still see your feet when standing knee-deep in the water (after wading in without stirring up sediment). If not, avoid bathing—or at least avoid ingestion of water, i.e., submersion of your head.
- In such situations, avoid water-skiing because of potentially substantial exposure to aerosol.
- If sailing, sailboarding or undertaking any other activity likely to involve accidental water immersion in the presence of cyanobacterial or algal blooms, wear clothing that is close fitting in the openings. The use of wet suits for water sports may result in a greater risk of rashes, because cyanobacterial or algal material in the water trapped inside the wet suit will be in contact with the skin for long periods of time.
- After coming ashore, shower or wash yourself down to remove cyanobacterial or algal material.

- Wash and dry all clothing and equipment after contact with cyanobacterial or algal blooms and scum.

8.8.2 Long-term measures

The aim of long-term measures to minimize health risks due to toxic algae and cyanobacteria is to prevent or reduce the formation of cyanobacterial blooms in water used for recreational water activities. This can be achieved by keeping total phosphorus concentrations below the "carrying capacity," which sustains substantial population densities. Experience from numerous water bodies shows that this can be achieved if total phosphorus concentrations are 0.01–0.03 µg/litre (depending somewhat on the size and mixing regime of the water body).

This threshold may be difficult to reach in water bodies with multiple sources of nutrient pollution. However, nutrient sources are locally very variable. Therefore, identifying the chief sources and developing strategies for preventing the formation of cyanobacterial blooms are recommended and may in many cases prove to be more feasible than initially assumed (Chorus & Mur, 1999). In particular, nutrient input from agricultural runoff may in many cases be reduced by decreasing the application of fertilizers to match the actual demand of the crop or by protecting the shoreline from erosion by planting shrubs along a buffer strip about 20 m wide along the shoreline, rather than ploughing and fertilizing to the very edge of the water.

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