Chapter 5. SAFE LEVELS AND SAFE PRACTICES

This chapter was prepared by Ian Falconer, Jamie Bartram, Ingrid Chorus, Tine Kuiper-Goodman, Hans Utkilen, Mike Burch and Geoffrey A. Codd

Throughout their lifespan, humans are exposed to many chemical substances, both beneficial and harmful. It is not possible to eliminate exposure to all toxins in our environment. Of the harmful chemicals, some are anthropogenic and others occur naturally. Although cyanotoxins occur naturally, their presence and abundance are, to a large extent, influenced and increased by human action. The extent to which cyanotoxins pose a human health risk depends on human exposure to these toxins. Reducing human exposure to cyanotoxins may be achieved either through preventing the occurrence of hazardous cell densities of toxic cyanobacteria, or by placing barriers (such as drinking water treatment or bathing restrictions) that would reduce human exposure to the cyanobacterial hazard.

The purpose of this chapter is to describe how "safe" levels, such as guideline values, are derived and how "safe practices" are likely to assist in minimising unsafe human exposures.

Establishing and controlling safe practices and procedures to reduce or prevent microbiological problems are common in the food industry, where the process is known as Hazard Analysis of Critical Control Points (HACCP). There is also a long history of similar activities in relation to drinking water supply, where the process is referred to as "sanitary inspection". This type of approach is also starting to be applied in dealing with some natural toxins, such as fungal toxins (mycotoxins). Safe practice guidelines can assist in reducing exposure, even for substances for which a guideline value cannot be set (for example because of inadequate scientific data), or where implementation of a guideline value is very difficult (for example because of analytical or sampling problems).

Major routes of exposure to cyanotoxins include oral and dermal routes through drinking water and recreational water use. However, the very limited available information suggests that inhalation in aerosols (potentially possible while showering, water-skiing or during certain work practices) may be an equally important route given that the toxicity of microcystin following intranasal instillation approaches that for intraperitoneal (i.p.) injection (Fitzgeorge *et al.,* 1994). In some countries, cyanobacterial dietary supplements may constitute a major route of oral intake for a small sub-population, if the cyanotoxin levels in the supplements are not controlled. A specific route (intravenous) affecting a susceptible sub-population may occur in dialysis clinics (see Box 4.4).

The extent or duration of exposure throughout the year is shorter if water resources are populated by cyanobacteria at specific seasons. In temperate climates, water bodies dominated by the genus *Microcystis* usually exhibit a bloom season of 3-5 months, whereas in milder climates, such as in Australia, some of South America and South Africa, this genus may bloom for 6-10 months. Other taxa, such as *Planktothrix agardhii,* may show perennial mass development even in temperate climates. This is the case in

some lowland regions of north-western Europe, especially in years with mild winters without ice cover (see Figure 2.7).

5.1 Tolerable exposures

5.1.1 Tolerable daily intake

The repair mechanisms of the body are continuously active and ensure that cells and tissues are replaced as they are damaged by normal "wear and tear", as well as by external factors such as toxic chemicals. The tolerable daily intake (TDI) is the amount of a potentially harmful substance that can be consumed daily over a lifetime with negligible risk of adverse health effects (see Chapter 4).

Exposure to several harmful chemicals or conditions simultaneously may lead to potentiation, or to antagonistic interaction. Potentiation results in effects that are larger than the responses caused by the individual chemicals or conditions by themselves and this effect has been observed with cyanotoxins, as noted in section 4.2. However, there is experimental evidence that potentiation is unlikely to occur at low levels of exposure, such as the TDI. For cyanotoxins, the animal and human toxicity data are incomplete for the majority of the compounds. The available data have been reviewed in Chapter 4 and allow only the derivation of a provisional TDI for microcystin-LR (see section 4.2.1). Clearly, this does not imply that microcystin-LR is the only microcystin that is toxic, or that other cyanotoxins are less harmful. It merely reflects the lack of toxicological data.

5.1.2 WHO guideline values for drinking water quality

The World Health Organization (WHO) *Guidelines for Drinking-water Quality* (WHO, 1993, 1996) represent a scientific consensus, based on very broad international participation, of the health risks presented by microbes and chemicals in drinking water. This scientific consensus is used to derive "guideline values" which are associated with guidance on monitoring and management aspects. The guideline values themselves are based upon a number of assumptions that may be amended locally or nationally according to specific circumstances (Box 5.1).

A guideline value for lifetime consumption of a chemical contaminant of drinking water is usually calculated by applying the derived TDI to a typical daily water intake in litres *(L)* by an individual of a given body weight *(bw).* The proportion *(P)* of total daily intake of the contaminant which is ingested from the drinking water needs to be considered, because some intake may come from food or by inhalation from air (WHO, 1996). The guideline value is then calculated as:

$$\text{Guideline value} = \frac{\text{TDI} \times bw \times P}{L}$$

A provisional guideline value of 1.0 µg l^{-1} has been adopted by WHO for microcystin-LR (WHO, 1998). In order to derive this, an average adult body weight of 60 kg and an average water intake for adults of 2 litres per day was used, as is standard practice. The provisional TDI of 0.04 µg kg^{-1} bw per day (section 4.2) was used and the proportion of the TDI allocated to drinking water (*P*) was assumed to be 0.8. The resulting

concentration (0.96 µg l^{-1}) was rounded to 1.0 µg l^{-1}. In water containing cyanobacterial cells, this guideline value should be applied to the total cell-bound and extracellular microcystins.

There were insufficient data to derive a guideline value for cyanotoxins other than microcystin-LR. For saxitoxins (STX), the guideline of 80 µg STX equivalents per 100 g (this is the conventional way of expressing this value) shellfish, used in North America for closing shellfish growing areas for harvesting, may provide preliminary orientation (IPCS, 1984).

Exceeding the provisional guideline value of 1 µg l^{-1} for microcystin-LR can be tolerated (see Box 5.1 for an explanation of handling short-term deviations). This may occur if, for example, discontinuation of exposure is expected in the near future due to implementation of measures to eliminate cyanotoxins from drinking water or cyanobacteria from the water resource. In such instances of guideline exceedances, it may be appropriate that information is communicated to the public, and especially to particularly susceptible sub-populations (such as patients with liver disease, parents of infants, dialysis centres or dialysis patients).

5.1.3 Assessment of microcystins other than microcystin-LR in relation to the guideline value

There are more than 60 different analogues of microcystin (see section 3.1.1) and, in many regions, microcystin-LR is not the most commonly occurring microcystin. It may not even be amongst the microcystins detected. The expression and interpretation of quantitative results from analysis or assay for these toxins in relation to the WHO guideline value for microcystin-LR may, therefore, be problematic.

Box 5.1 Derivation of WHO guidelines for drinking water quality

The primary aim of the *Guidelines for Drinking-water Quality* (WHO, 1993) is the protection of public health. The Guidelines are intended to be used as the basis for the development of national standards that, if properly implemented, will ensure the safety of drinking water supplies through the elimination, or reduction to a minimum concentration, of constituents of water that are known to be hazardous to health. The guideline values recommended are not mandatory limits, they are intended to be used in the development of risk management strategies which may include national or regional standards in the context of local or national environmental, social, economic and cultural conditions.

The main reason for not promoting the adoption of international standards for drinking water quality is the advantage provided by the use of a risk-benefit approach (qualitative or quantitative) to the establishment of national standards or regulations. This approach should lead to standards and regulations that can be readily implemented and enforced and which ensure the use of available financial, technical and institutional resources for maximum public health benefit.

The judgement of safety, or what is a tolerable level of risk in certain circumstances, is a matter in which society as a whole has a role to play. It should be recalled that water is essential to sustain life and the Guidelines therefore emphasise the importance of securing water supply. They also indicate that protection of supplies from contamination is almost invariably the best method of ensuring safe drinking water and is to be preferred to treating a contaminated water supply to

render it suitable for consumption.

A principle of the Guidelines is that the potential consequences of microbial contamination are such that its control must always be of paramount importance and must never be compromised. The health risk due to toxic chemicals in drinking water differs from that caused by microbiological contaminants. There are few chemical constituents of water that can lead to acute health problems except through massive accidental contamination of a supply. Moreover, experience shows that, in such incidents, the water usually becomes undrinkable because of unacceptable taste, odour and appearance. The fact that chemical contaminants are not normally associated with acute effects places them in a lower priority category than microbial contaminants, the effects of which may be acute and widespread.

It is important that guideline values are both practical and feasible to implement as well as protective of public health. Guideline values are not set at concentrations lower than the detection limits achievable under routine laboratory operating conditions. Moreover, guideline values are recommended only when control techniques are available to remove or reduce the concentration of the contaminant to the desired level. In some instances provisional guideline values are set for constituents for which there is some evidence of a potential hazard but where the available information on health effects is limited. Provisional guideline values are also set for substances for which the calculated guideline value would be: (i) below the practical quantification level; or (ii) below the level that can be achieved through practical treatment methods. Finally, provisional guideline values are set for substances when it is likely that guideline values will be exceeded as a result of disinfection procedures.

The first edition of *Guidelines for Drinking Water Quality was* published by WHO in 1984 and 1985. The second editions of the three volumes of the guidelines were published in 1993, 1996 and 1997; and the Addenda to the second edition were published in 1997 and 1998.

- A guideline value represents the concentration of a constituent that does not result in any significant risk to the health of the consumer over a lifetime of consumption.

- The quality of water defined by the *Guidelines for Drinking-water Quality* is such that it is suitable for human consumption and for all usual domestic purposes, including personal hygiene. However, water of a higher quality may be required for some special purposes, such as renal dialysis.

- When a guideline value is exceeded, this should be a signal: (i) to investigate the cause with a view to taking remedial action; and (ii) to consult with, and seek advice from, the authority responsible for public health.

- Although the guideline values describe a quality of water that is acceptable for lifelong consumption, the establishment of these guideline values should not be regarded as implying that the quality of drinking water may be degraded to the recommended level. Indeed, a continuous effort should be made to maintain drinking water quality at the highest possible level.

- Short-term deviations above the guideline values do not necessarily mean that the water is unsuitable for consumption. The amount by which, and the period for which, any guideline value can be exceeded without affecting public health depends upon the specific substance involved. It is recommended that when a guideline value is exceeded, the surveillance agency (usually the authority responsible for public health) should be consulted for advice on suitable action, taking into account the intake of the substance from sources other than drinking water (for chemical constituents), the toxicity of the substance, the likelihood and nature of any adverse effects, the

practicability of remedial measures, and similar factors.

• In developing national drinking water standards based on these guideline values, it will be necessary to take account of a variety of geographical, socio-economic, dietary and other conditions affecting potential exposure. This may lead to national standards that differ appreciably from the guideline values.

The most widely used analytical technique for the detection and quantification of individual microcystin variants for which quantitative reference materials are available is high pressure liquid chromatography (HPLC) (see section 13.4.1 and Box 13.5). For toxin variants where reference materials are available, HPLC results can be truly quantitative. For HPLC peaks that identify microcystin variants for which no standards are available but the result has been derived from a comparison with the concentration of the standard for microcystin-LR, the estimates can be expressed as "concentration equivalents" (CE) of microcystin-LR. It is therefore possible, using HPLC, to derive an approximate concentration of total microcystins in a sample expressed in terms of microcystin-LR CE. However, some of the observed HPLC peaks may relate to toxicologically inactive or weakly toxic variants. In terms of "toxicity equivalents" (TE) of microcystin-LR the actual toxicity of an unknown sample reported as microcystin-LR equivalents (CE) is likely to be less than the same concentration of pure microcystin-LR, because microcystin-LR is one of the most potent microcystins, at least in acute terms (see Table 3.2). Thus, the microcystin CE approach would result in a "worst case" estimate of toxic microcystin concentration.

A toxin concentration measured from a water or bloom sample by a bioassay (such as the mouse bioassay or the phosphatase bioassay) may give a toxin concentration value which can be expressed as microcystin-LR TE, provided the assay has been calibrated using microcystin-LR as the quantification standard (which is usually the case). This measure of total microcystin concentration expressed as microcystin-LR TE will be closer in actual (acute) toxicity to a solution of the same concentration of pure microcystin-LR. The position of enzyme assays, such as *in vitro* protein phosphatase inhibition assays, in this context is currently unclear. Differences in toxicity of different microcystins include variation in their ability to enter intact cells and in their capacity to inhibit protein phosphatases.

In practice, it is important to report quantitative analytical results for samples containing several microcystins with the above qualifications in mind and to indicate the method and assumptions used for deriving the quantitative value. If it is necessary to calculate quantitative values for total microcystins in a sample (and it is certainly important not to ignore microcystins other than microcystin-LR), then the value should be qualified as either microcystin-LR CE or TE. This information can be used (at best) as a preliminary orientation of the hazard presented by the sample in relation to the guideline value (see section 5.1.2).

Box 5.2 Epidemiological evidence for low-level cyanobacterial hazard

The epidemiological data of Pilotto *et al.* (1997) can be used as a basis for guideline derivation for acute, non-cumulative health effects which are more likely to result in discomfort rather than serious health outcomes. These data encompass the health effects on humans of intact cyanobacterial cells and colonies and thus include effects of currently unknown substances and bacteria associated with cyanobacterial colonies. The effects measured were eye irritation, ear irritation, skin rash, as well as vomiting, diarrhoea, cold/flu symptoms, mouth ulcers and fever. An elevated "Odds Ratio" for symptoms (3.44) was shown by the people who were in water contact for more than one hour, at above 5,000 cyanobacterial cells per ml. Similar Odds Ratios were seen for symptoms in people bathing in water with 5,000-20,000 cells per ml (2.71) and above 80,000 cells per ml (2.90).

5.1.4 Recreational water exposure

Three potential routes of exposure to cyanotoxins can be distinguished: 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 (Pilotto *et al.,* 1997); accidental swallowing (Turner *et al.,* 1990); and inhalation of water. Cases of illness from accidental swallowing and inhalation of *Microcystis* have been reported (see section 4.1) and provide direct evidence of harm to recreational water users from cyanobacterial blooms in the recreational water bodies.

Health effects observed in the prospective epidemiological study of Pilotto *et al.* (1997) occurred at low cyanobacterial cell densities. These related clearly to the cyanobacterial cell population, but not to the concentration of microcystins (see Box 5.2). Thus, this hazard appears to be due to additional, or other unidentified, cyanobacterial metabolites or compounds from associated bacteria, even at moderate levels of exposure.

Intake through oral ingestion or inhalation

Incorporation of toxins through swallowing, contact with nasal mucosa, or by inhalation are likely to be important routes of exposure to cyanotoxins during water-contact sports. Well-documented evidence from one animal experiment (Fitzgeorge *et al.,* 1994) and one case of multiple human illness (Turner *et al.,* 1990) indicates that inhalation and resorption through nasal and pharyngeal mucous membranes may present a high risk in water sports involving intensive submersion of the head (jumping from diving boards, sailboarding, canoe capsizing, competitive swimming) and inhalation of aerosols (water skiing).

Experimental results indicate a hazard of cumulative liver damage by repeated microcystin intake (Fitzgeorge *et al.,* 1994, see section 4.2.1), as can occur during a holiday with daily bathing at a recreational site with a high density of microcystin-containing cyanobacteria. Sub-acute liver injury is likely to go unnoticed, because signs of liver injury are only apparent after severe injury. In addition, the dose-response curve for liver injury from microcystins is relatively steep. There may be little evidence of acute liver damage when levels are close to those that could lead to severe acute toxicity, and

thus exposure at such levels is likely to be continued by people if they are uninformed of the risk (e.g. for consecutive days of a holiday or hot spell), thereby increasing the risk of cumulative liver damage.

Risks of ingestion are particularly high for children playing in shallow near-shore water where scums tend to accumulate. Because the hazard of microcystin uptake is directly related to the levels of toxins in the water (cell-bound as well as dissolved) and the volume of water ingested or inhaled, the range in these levels needs to be recognised in deriving guidelines for recreational water safety.

Direct contact

Allergic and toxic dermal reactions of varying severity are known from cyanobacteria as well as from freshwater algae, but have not been documented extensively. Bathing suits, and particularly diving suits, tend to aggravate such effects by accumulating cyanobacterial cells, thereby enhancing the disruption of cells and hence the liberation of cell contents onto the wearer's skin. Reports from the USA have recorded allergic reactions from recreational exposure, and the cyanobacterial pigment phycocyanin was shown to be responsible in one case (Cohen and Reif, 1953). Severe dermatitis, resembling skin bums, has been reported from marine bathing in the presence of cyanobacteria dislodged from rocks, particularly after storms in tropical seas (see section 4.2.8).

5.2 Safe practices

The placing of barriers that reduce exposure to a cyanotoxin hazard is an important measure and involves identifying "critical control points" and implementing measures for their monitoring and control. In the case of cyanobacteria, critical control points might include, for example, noting the tendency of a water body to develop blooms, scums or mats. Monitoring schemes need to be developed that are capable of detecting proliferation of cyanobacteria (linked to a programme of appropriate actions) and drinking water treatment technology needs to be in existence that is capable of preventing human exposure if cyanobacteria occur in source waters.

5.2.1 Drinking water

A drinking water supply safe from cyanotoxins will either draw upon a resource which does not harbour cyanotoxins (e.g. groundwater or surface water which does not support cyanobacterial growth), or have treatment in place that is likely to remove cyanobacterial cells (without causing their disrupture) as well as removing cyanotoxins. However, in many circumstances a potential cyanotoxin hazard can be managed effectively without the necessity of advanced treatment processes, through water resource management techniques (see Chapter 8) and removal of intact cells (see Chapter 9). The critical control points for safe practices are indicated in Table 5.1.

Most of the reported incidents of human injury that have raised awareness of the importance of cyanotoxins in drinking water have involved the inappropriate treatment of water supplies, such as the use of copper sulphate in dealing with an established bloom of cyanobacteria.

A very effective approach to safe practices may involve changing the drinking water source. In a number of regions, surface waters are used for reasons of easy access and tradition, although groundwater of high quality is available. Exploring options of improving practices of drinking water abstraction with low technological input (such as drilling wells, or using bankside filtration) may lead to health benefits. In China, a high prevalence of endemic primary liver cancer was related to several factors: hepatitis B, aflatoxins in the diet, and drinking surface water polluted with cyanobacteria likely to contain microcystins. Changing the drinking water source from shallow, eutrophic ponds and ditches to groundwater was a major element of a package of measures which showed some success in improving health (Box 5.3).

5.2.2 Recreational waters

Recreational water use is likely to be a major route of exposure to cyanotoxins in some parts of the world. Whereas similar approaches to resource protection apply as for drinking water, there are very few further management options available once cyanobacteria proliferate or accumulate in a recreational water. Because adequate surveillance is sometimes difficult and management options, except precluding or discouraging use, may be scarce, a large share of the responsibility for safe practices lies with the users of a bathing site. The provision of adequate information to the public becomes, therefore, a major responsibility of public authorities.

The growth of cyanobacteria in lakes and rivers used for recreational purposes has been well recognised as a public nuisance. Water blooms of cyanobacteria may be associated with unpleasant odours and the offensive appearance of lake shores, especially when scums of the organisms accumulate and decay. Areas with extensive cyanobacterial scums or accumulated detached mats on bathing beaches may be avoided by swimmers and other water users because of the obviously unpleasant environment, particularly if locally anaerobic water conditions or cyanobacterial toxins cause fish-kills, further emphasising the unattractiveness of water contact. In temperate climates, cyanobacterial dominance is most pronounced during the summer months, when the demand for recreational water is highest. In some regions, cyanobacteria have been abundant for more than a generation and visitors have accepted this water quality as "normal" for their region. Multiple anecdotal observations of children playing with scum material have been reported.

Table 5.1 Critical control points for assessing the intrinsic safety of a drinking water supply which may contain cyanobacterial cells and/or toxins

Control point/issue	Comments
Source water type	The health risk associated with cyanobacterial contamination of groundwaters is generally negligible. An exception may occur where infiltration galleries are strongly influenced by eutrophic surface waters
Occurrence of cyanobacteria in source water and tendency for bloom formation	Many surface water sources do not support cyanobacterial growth. In others, cyanobacteria may occur occasionally at low population densities. In reservoirs and lakes with very low nutrient concentrations (total phosphorus < 10 µg P l^{-1}) or rivers and reservoirs with a hydrodynamic regime unfavourable for cyanobacteria (continuous high flows especially during summer, or deep vertical mixing), other phytoplankton species may regularly out-compete cyanobacteria. A water source which does not have a history of cyanobacterial growth or bloom formation is generally considered to present a low cyanotoxin risk, regardless of treatment type. Where bloom formation is well characterised in terms of annual cycles, the health risk may similarly be low if control measures are in place for times of bloom formation. If regular monitoring of source phytoplankton is in place, waters presenting no significant cyanotoxin risk are easily identified (see Table 6.2)
Likelihood of cell lysis in transport or treatment	Throughout cyanobacterial growth, most cyanotoxins are cell-bound. Removal of intact cyanobacterial cells therefore largely removes cyanotoxins (see section 3.4). Neurotoxins may be an exception under some circumstances. When cyanobacterial cells die and decay (lyse), toxins are released. Lysis can occur naturally or be caused by chemical treatment, hydraulic and pumping regimes in different treatment steps, and by long transport pipes for raw water. Thus, abstraction and treatment systems which lead to cell lysis present an increased risk of cyanotoxin release.
Treatment systems capable of toxin removal	Methods, such as adsorption to some types of granular activated carbon, and oxidation, can be effective in cyanotoxin removal. However, conditions of operation are critical for success. If processes are operated only periodically during cyanobacterial growth or reservoir treatment, monitoring of plant functioning must be adequate to ensure cyanotoxin removal. Substantially less is known about removal of neurotoxins and cylindrospermopsin than about microcystins, thus toxin monitoring of treatment steps and finished water is especially important if potentially neurotoxic or cylindrospermopsin-producing cyanobacteria proliferate

Box 5.3 Primary liver cancer and cyanotoxins in China

Primary Liver Cancer is one of the most common cancers in China. In 1994 and 1995, it accounted for 24 mortalities per 100,000 population in some rural counties and cities; in these areas it was ranked with stomach cancer as the two most important causes of cancer death.

The uneven geographic distribution of liver cancer was conspicuous, and "hot spots" could be related to drinking water supply, e.g. in some clearly delineated areas of Nandong District, in Jiangsu Province (particularly in Rudong, but also in Haimen and Qidong), in Nanhui (suburb of Shanghai) and Fusui (Guangxi).

- In Rudong, Nanhui and Fusui people had blocked the drainage system, causing stagnation of the water used for the drinking supply.

- In areas of Qidong-Haimen, with mortality rates 20 per 100,000, people drank water from the Yangtze River, but in areas with mortalities of 100 per 100 000, pond and ditch water was used.

- Primary liver cancer mortalities 10 per 100,000 were found in areas where water from deep wells were used for drinking.

Epidemiological study of the mortality showed strongest correlation with hepatitis B incidence, a lesser correlation with aflatoxins in the diet, and a third correlation with drinking of pond and ditch water. No correlations were found with insecticides. Samples of pond and ditch water showed microcystin present in both endemic liver cancer areas and in areas with lower liver cancer rates. Children in some endemic areas were fed corn paste and drank pond or ditch water from infancy. Further, up to 43 per cent carry the hepatitis-B virus from infection by their HBsAg positive mothers. The evidence suggests that aflatoxins from corn and microcystins from drinking water act together with the hepatitis B virus in causing and promoting primary liver cancer.

In order to alleviate this situation, attempts have been launched over the past 20 years to change the staple food and drinking habits of the people. Efforts began with the methods of harvest, following the motto *"quick to reap, quick to store, at a moisture content 12.5 per cent",* aimed at the reduction of fungal contamination. For some time, the government bought corn and exchanged it for rice to reduce aflatoxin exposure, but this function has now been transferred to a private initiative in the market economy. Recently, it has been estimated that more than 95 per cent of the population eats rice rather than corn.

Even prior to the recognition of microcystins as possible promoters of endemic primary liver cancer, the connection to poor quality surface water for drinking was observed and programmes for construction of deep wells were begun. At present, 80 per cent of the population in some of the afflicted regions have changed their water source to deep well water, and the incidence of liver cancer has dropped consistently.

The mottoes for prevention of primary liver cancer now are:

"control of water - control of crops - prevention of hepatitis"

For additional discussion, see section 4.1.2.

Health impairments from cyanobacteria in recreational waters must be differentiated between the chiefly irritative symptoms caused by unknown cyanobacterial substances (as described in Box 5.2), and the more severe hazard of exposure to high concentrations of known cyanotoxins, particularly microcystins. A single guideline therefore, is not appropriate. Rather, a series of guidelines associated with incremental severity and probability of adverse effects has been defined at three levels as described below.

1. Relatively mild and/or low probabilities of adverse health effects

For protection from health outcomes not due to cyanotoxin toxicity, but due to the irritative or allergenic effects of other cyanobacterial compounds, a guideline level of 20,000 cyanobacterial cells per ml (corresponding to 10 µg l^{-1} of chlorophyll *a* under conditions of cyanobacterial dominance) can be derived from the prospective epidemiological study by Pilotto *et al.* (1997) (see Box 5.2). Whereas the health outcomes reported in this study were related to cyanobacterial density and duration of exposure, they affected less than 30 per cent of the individuals exposed. At this cyanobacterial density, 2-4 µg l^{-1} of microcystins may be expected if microcystin-producing cyanobacteria are dominant, with 10 µg l^{-1} being possible with highly toxic blooms (regional differences in microcystin content of the cells may be substantial). This level is close to the WHO provisional drinking water guideline value of 1 µg l^{-1} for microcystin-LR (WHO, 1998) which is intended to be safe for life-long consumption. Thus, health outcomes due to microcystin are unlikely and providing information for visitors to bathing sites with this low-level risk is considered to be sufficient. Additionally, it is recommended that the authorities are informed in order to initiate further surveillance of the site.

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 may be applied. Swimmers involuntarily swallow some water while bathing and the harm from ingestion of bathing water will be comparable with that from a drinking water supply with the same toxin content. A swimmer can expect to ingest up to 100-200 ml of water in one session, sail-board riders and water skiers would probably ingest more.

A density of 100,000 cyanobacterial cells per ml (which is equivalent to approximately 50 µg l^{-1} of chlorophyll *a* if cyanobacteria dominate) is a guideline for a moderate health alert in recreational waters. At this density, 20 µg l^{-1} of microcystins are likely, if the bloom consists of *Microcystis* and has an average toxin content per cell of 0.2 pg, or 0.4 µg microcystin per µg chlorophyll *a* (up to 50 µg l^{-1} of microcystin are possible) but toxin levels may approximately double if *Planktothrix agardhii* is dominant. This toxin concentration is equivalent to 20 times the WHO provisional guideline value for microcystin-LR in drinking water, but would result in consumption of an amount close to the TDI for an adult of 60 kg consuming 100 ml of water while swimming (rather than 2 litres of drinking water). However, a child of 15 kg 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 (e.g. because of chronic hepatitis B). Therefore, cyanobacterial densities likely to cause microcystin concentrations of 20 µg l^{-1} should trigger further action.

Non-scum-forming species of cyanobacteria, such as *Planktothrix agardhii,* have been observed to reach cell densities corresponding to 200 µg l^{-1} of chlorophyll *a* or even more in shallow water bodies. Transparency in such situations will be less than 0.5 m when measured with a Secchi disk (see Chapter 11). *Planktothrix agardhii* has been shown to contain a very high cell content of microcystin (1-2 µg per µg chlorophyll *a)* (see Figure 3.5) and therefore toxin concentrations of 200-400 µg l^{-1} can occur without scum formation.

An additional reason for increased alert at 100,000 cells per ml is the potential of some frequently occurring cyanobacterial species (particularly *Microcystis* spp. and *Anabaena* spp.) to form scums (see Figure 5.1). These scums may increase local cell density and thus toxin concentration by a factor of 1,000 or more in a few hours, thus rapidly changing the risk from moderate to high (see next subsection) for bathers and others involved in body-contact water sports.

Cyanobacterial scum formation presents a unique problem for routine monitoring carried out at the usual time intervals of one or two weeks, because such monitoring intervals are unlikely to detect hazardous maxima. Because of the potential for rapid scum formation at a cyanobacterial density of 100,000 cells per ml or 50 µg l^{-1} chlorophyll *a* (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 exposure in areas prone to scum formation are the two main options.

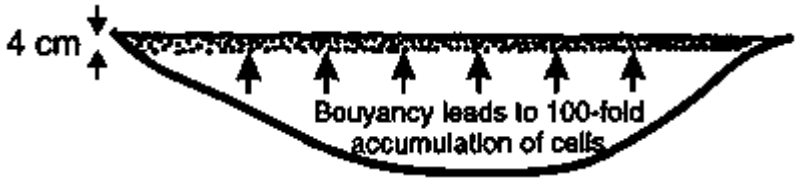
Intervention is recommended to trigger effective public information campaigns educating people on avoidance of scum contact. Furthermore, in some cases (e.g. with frequent scum formation), restriction of bathing may be judged to be appropriate. An intensified monitoring programme should be implemented,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

Figure 5.1 Schematic illustration of scum-forming potential changing the cyanotoxin risk from moderate to very high

Lake profile

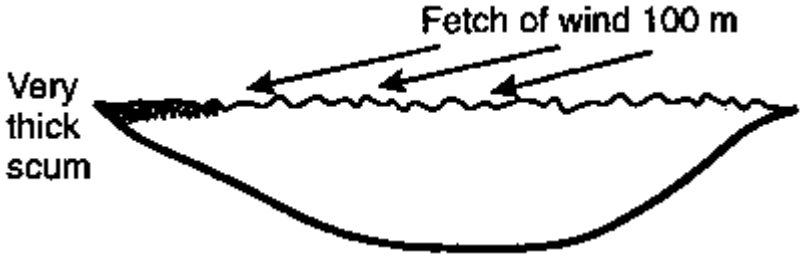
Moderate risk level:

- 50 µg l^{-1} chlorophyll *a*
- or 100,000 cells l^{-1}
- possibly 20 µg l^{-1} of microcystin in top 4 m of water body



100-fold accumulation to high risk level scum:

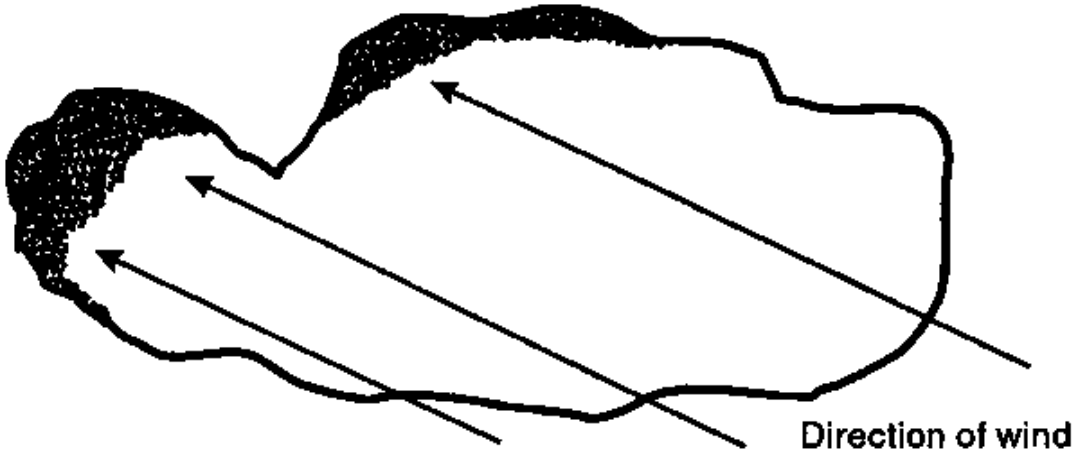
- 5,000 µg l^{-1} chlorophyll *a*
- or 10,000,000 cells l^{-1}
- possibly 2,000 µg l^{-1} of microcystin in top 4 cm of water body

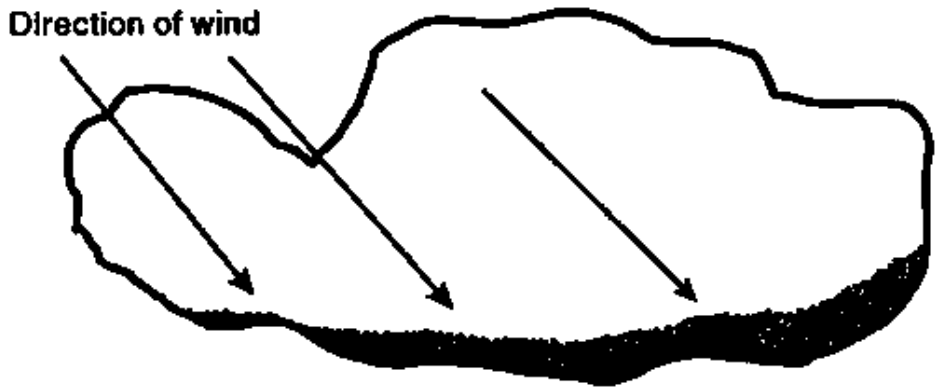


1,000-fold accumulation to very high risk level shore scum if wind sweeps scums from 100 m into 10 m:

- 50,000 µg l^{-1} chlorophyll *a*
- or 100,000,000 cells l^{-1}
- possibly 20,000 µg l^{-1} of microcystin concentrated in one bay of the water body

Lake plan





3. High risk of adverse health effects

Abundant evidence exists for potentially severe health hazards associated with scums caused by toxic cyanobacteria (see section 4.1). No human fatalities have been unequivocally associated with oral ingestion of scum, even though numerous animals have been killed by consuming water containing cyanobacterial scum material (see section 4.1). This discrepancy can be explained by the fact that animals would drink higher volumes of scum-containing water, compared with the small amounts of scum accidentally ingested by humans during bathing (resulting in a lower dose).

Cyanobacterial scums can represent a thousand-fold to million-fold concentration of cyanobacterial cell populations. It has been calculated that a child playing in a *Microcystis* scum for a protracted period and ingesting a significant volume could receive a lethal exposure, although there are no reports that this has actually occurred. Based on evidence that the oral LD_{50} of microcystin-LR in mice is 5,000-11,600 µg kg^{-1} bw (see section 4.2), for a child of 10 kg the ingestion of 2 mg of microcystin or less could be expected to cause liver injury, because concentrations of up to 24 mg l^{-1} of microcystins have been published from scum material (see section 3.2). Substantially higher enrichment of scums (up to gelatinous consistency) is occasionally observed, and accidental ingestion of smaller volumes of these 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 a readily detected indicator of a high risk of adverse health effects for those bathers who come into contact with the scum. Immediate action to control scum contact is recommended for such situations.

The approach outlined in this section, however, 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 the shore (Edwards *et al.,* 1992). Some marine beaches have been reported to have widespread problems due to a benthic marine cyanobacterium, *Lyngbya majuscula,* growing on rocks in tropical seas and causing severe blistering when trapped under the bathing suits of people swimming following a storm (Grauer, 1961). This response may be due to acute toxicity; *Lyngbya* can produce irritant toxins. Measures of cyanobacterial population cell density as outlined in Table 5.2, will not detect these hazards. Instead, this type of hazard calls for critical and well-informed observation of bathing sites, coupled with a flexible response.

It is difficult to define "safe" concentrations of cyanobacteria in recreational water in relation to allergenic effects or skin reactions, because 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 all densities below the guidelines described above. Further information related to monitoring and management of recreational waters is available in Bartram and Rees (1999).

5.3 Other exposure routes

5.3.1 Renal dialysis

Renal dialysis patients are at great risk when water used for dialysis contains contaminants such as cyanotoxins. For these patients large volumes of water (120 litres) are used and the route of exposure is similar to the i.v. route, which allows for a much greater uptake of toxin than following oral ingestion. One serious incident, including a number of deaths arising from exposure through this route, has already been described in section 4.1.

The WHO *Guidelines for Drinking-water Quality* (WHO, 1993) do not consider the especially high quality of water needed for dialysis treatment, intravenous therapy or other clinical uses. The treatment processes used at conventional surface water treatment plants (such as coagulation, clarification and sand filtration) are normally effective in removing cyanobacterial cells, but are not effective in removing or destroying dissolved cyanotoxins, especially from water supplies with a high organic content and cyanobacterial dominance (see Chapter 9). Consequently, clinics and hospitals with special water needs, such as for dialysis treatment or for transfusions (intravenous administration), may need to provide additional water treatment to remove the cyanotoxins. Such treatment ranges from granular activated carbon filtration, followed by reverse osmosis, to more elaborate treatment including membrane filtration (e.g. 25 µm pore size filter). The extent of treatment necessary depends on the quality of the municipal water supply.

Continuous monitoring of performance and equipment is essential to ensure adequate quality of the water. On-site water treatment systems in clinics and hospitals require rigorous monitoring and regular maintenance, including back-flushing of filters and recharge of activated carbon, according to manufacturers' specifications. It is important that manufacturers' specifications should be assessed for their adequacy for maintaining performance under local conditions. Activated carbon, for example, may be exhausted for its ability to remove cyanotoxins long before it reaches saturation for removal of other organic compounds, and some manufacturers may be unaware of this.

Table 5.2 Guidelines for safe practice in managing bathing waters which may produce or contain cyanobacterial cells and/or toxins

Guidance level or situation	How guidance level derived	Health risks	Recommended action
Cyanobacterial scum formation in bathing areas	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 prevent contact with scums; possible prohibition of swimming and other water-contact activities Public health follow-up investigation Inform relevant authorities
100,000 cells cyanobacteria per ml *or* 50 µg chlorophyll *a* per litre with dominance of cyanobacteria	From provisional drinking water guideline for microcystin-LR, 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 Restrict bathing and further investigate hazard Post on-site risk advisory signs Inform relevant health authorities
20,000 cells cyanobacteria per ml *or* 10 µg chlorophyll *a* per litre with dominance of cyanobacteria	From human bathing epidemiological study	Short-term adverse health outcomes, e.g. skin irritations, gastrointestinal illness, probably at low frequency	Post on-site risk advisory signs Inform relevant authorities

5.3.2 Irrigation water

The use of water from sources containing cyanobacterial blooms and toxins for spray irrigation of crops presents potential health hazards through several exposure routes, including uptake into the food chain. Workers or passers-by may inhale toxins with spray drift, and skin contact with cyanobacteria and dissolved toxins may also occur. Questions therefore arise about the health significance of spray irrigation with water containing cyanobacterial toxins. As shown in section 4.2, animal experimentation has indicated that microcystin uptake through nasal mucosa may be considerable. When considered together with the skin irritations, respiratory distress and nasal mucosal irritations observed after recreational exposure (see sections 4.1 and 5.2.2), the indicators are that occupational exposure to spray irrigation water should be avoided (by appropriate work practices) if the water contains cyanobacterial toxins. Incidental exposure of humans and animals to such spray irrigation water, for example by downwind drift, should also be avoided.

There are several indications that terrestrial plants, including food crop plants, can take up microcystins. Mustard seedling development is inhibited if microcystin-LR is presented to the roots in aqueous solution (Kos *et al.,* 1995). Microcystins have several perturbatory effects on plant physiology and metabolism, when sufficient levels of toxin enter the plant cells. Plant protein phosphatases show high susceptibility to inhibition by microcystin-LR *in vitro* (MacKintosh *et al.,* 1990). Plant sucrose metabolism is inhibited if microcystin-LR is administered in solution by injection into the transpiration stream (Siegl *et al.,* 1990). Inhibition of whole leaf photosynthesis by French Bean plants occurred after topical exposure of the leaves to an aqueous solution of microcystin-LR during greenhouse studies (Abe *et al.,* 1996). The degree of whole leaf photosynthesis inhibition increased with subsequent brief exposures to the toxin in solution at 48-hour intervals, eventually becoming irreversible. These results were observed at dissolved microcystin-LR concentrations which can be found in untreated waters containing cyanobacterial blooms (20 μg l^{-1} of toxin); leaf necrosis occurred at higher exposure levels (Abe *et al.,* 1996). The relevance of these findings for field situations is currently unclear.

In addition to the possibility of internal accumulation of microcystins, irrigation may lead to accumulation of toxins on the external surfaces of edible plant material. The toxins are deposited when the water dries on the plant surface between irrigation periods or when the water becomes trapped in the centres of, for example, salad plants. Further research is needed into the uptake and fate of microcystins and other cyanobacterial toxins by food plants and the persistence of the toxins on plant surfaces.

5.3.3 Cyanobacteria sold as dietary supplements

In some countries cyanobacteria are sold as dietary supplements, with the number of users of these products estimated to be well over a million in North America alone. Large-scale production of cyanobacteria and microalgae started some 50 years ago. Much of the early research work dealt with the basic photosynthetic properties of microalgae, their possible therapeutic, antibiotic and toxicological properties and their potential as an agricultural commodity. The microalgae biomass industry now provides significant biomass for pigments and speciality chemicals used primarily in the food industry. The bulk of this microalgal biomass comes from two filamentous genera of cyanobacteria: *Spirulina* including *S. platensis* and *S*. *maxima* (Belay *et al.,* 1994) and *Aphanizomenon flos-aquae.* While *Spirulina* is grown in artificial outdoor ponds, mainly in southern California, Hawaii, Thailand, Taiwan and Japan, *Aphanizomenon* is at present harvested from a natural lake. Production of food-grade *Spirulina* exceeds 1×10^6 kg a^{-1} (Belay *et al.,* 1993). *Aphanizomenon* production is also substantial.

As a consequence of the consumption of these products many quality control issues arise. One such issue concerns the possible production of cyanotoxins by cyanobacterial genera used for dietary supplements. In particular, *Aphanizomenon flos-aquae* has been shown to be capable of producing saxitoxins (Mahmood and Carmichael, 1986) and the neurotoxin anatoxin-a (Bumke-Vogt *et al.,* 1999). While no saxitoxins have been detected in *Aphanizomenon flos-aquae* marketed as a dietary supplement, it is appropriate to monitor all supplements in order to ensure safety. In natural lakes, mixtures of species often occur in cyanobacterial blooms. In particular *Microcystis* and *Anabaena,* which usually contain microcystins, can both occur in association with *Aphanizomenon.* The microcystins may then become part of the biomass harvested for

human consumption. Failure to monitor and regulate these toxins in cyanobacterial biomass used as part of a human diet could lead to an increased risk for the consumer (see Box 5.4).

5.3.4 Cyanobacteria and *Vibrio cholerae*

Islam (1991) described detection of *Vibrio cholerae* inside the mucilaginous sheath of *Anabaena variabilis* for up to 15 months after artificial exposure and also noted that *V. cholerae* 01 did not lose toxigenicity during the association (Islam, 1991). Field studies have also detected *V. cholerae* in the mucilaginous sheath of *Anabaena* sp. from a pond in Dhaka when it could not be detected in association with microalgae collected from the same environment *(Euglena* sp. and *Phacus* sp. (Islam *et al.,* 1994)).

The association between *V. cholerae* and cyanobacteria remains poorly understood, but it has been noted that vibrios may produce mucinase (Schneider and Parker, 1982) and it has been suggested that exchange of oxygen (from photosynthesis for aerobic respiration) and carbon dioxide may permit a symbiotic relationship (Islam, 1987; Islam *et al.,* 1994). Other workers have noted that motile bacteria can easily discriminate heterocysts from vegetative cells and attach to the heterocyst vegetative cell junction, following which both host and epiphytes start growing (Paerl and Gallucci, 1985; Islam *et al.,* 1990). The bacteria rarely penetrate cyanobacterial cell walls (Islam *et al.,* 1990). Evidence is accumulating that association with mucilaginous cyanobacteria may be an important factor in inter-epidemic survival of *V. cholerae.* The implications of this for the control of cholera in humans remain unclear and it should be noted that the evidence relates to mucilaginous cyanobacteria, and *Anabaena* in particular. No studies have suggested or apparently investigated a relationship with cyanobacterial toxigenicity.

Box 5.4 Calculation of risk associated with consumption of cyanobacterial products contaminated with microcystins

The State of Oregon, USA, has adopted 1 µg g^{-1} (1 ppm) of microcystins as a standard for cyanobacterial products. The consumption of 2 g (as suggested by some producers) by a 60 kg person of product containing 1 µg g^{-1} would result in a microcystin intake of 0.033 µg kg^{-1} bw. This intake is slightly below the tolerable daily intake (TDI) of 0.04 µg kg^{-1} bw per day used for derivation of the WHO guideline for microcystin-LR in drinking water (WHO, 1998) (see section 4.2). However, an intake exceeding 2 g per day of a product containing microcystins at a concentration near the State of Oregon standard may exceed the TDI, and a consumption of 2 g per day by children may also exceed the TDI because of their lower body weight. In deriving its drinking water guideline value, WHO apportioned 20 per cent of intake to other sources. For persons consuming cyanobacterial products, this apportionment may be inappropriate.

5.4 Tastes and odours

Cyanobacteria have, for a long time, been recognised as a nuisance in the drinking water industry because of the ability of several taxa to produce earthy and musty smelling compounds, notably geosmin and 2-methyl isoborneol (2-MIB), for which the odour detection thresholds of less than 10 ng l^{-1} are remarkably low among sensitive individuals.

The cyanobacterial genera that are known to produce geosmin are *Anabaena, Aphanizomenon, Lyngbya, Microcystis, Oscillatoria, Phormidium, Schizothrix* and *Symploca* (Perrson, 1983). All these (except *Symploca)* are also known to include toxin-forming species and strains.

Because of this, the possibility of using odour compounds as an early warning for the development of toxin-producing cyanobacteria blooms has been considered. However, there is no evidence of a correlation between toxin production and the production of taste- and odour-producing compounds that would provide a warning of toxicity. Evidence from the literature on the capability of various cyanobacterial species to produce both toxins and taste and odour compounds has been summarised by Kenefick *et al.* (1992) and does not indicate that cyanobacterial species which produce toxins invariably also produce taste and odour. Nevertheless, some characteristic tastes and odours may indicate the presence of cyanobacteria, and toxic cyanobacteria frequently occur without noticeable tastes or odours. In Alberta, Canada, 89 bloom samples from 10 lakes were analysed for the presence of microcystin-LR, and the taste and odour compounds geosmin, 2-MIB and β-cyclocitral. The latter compound, which is only mildly odorous compared with geosmin and 2-MIB, was reported to be produced in large quantity by *Microcystis* spp. (Jüttner, 1988). The results showed that all but three of the bloom samples had detectable levels of microcystin while none had detectable levels of 2-MIB. Several samples had detectable levels of geosmin, but there was no clear relationship between the presence of geosmin and the presence of microcystin-LR. In the case of β-cyclocitral, there was a significant correlation, at the 1 per cent level. However, this relationship is of no practical use for providing early warning of the presence of microcystin-LR, because the relationship is not consistent and β-cyclocitral is not odorous enough to act as a sensitive surrogate for microcystin-LR (Hrudey *et al.,* 1993). Although there have been some rare occasions when cyanobacterial isolates have been found to produce geosmin, microcystin and anatoxin-a simultaneously, as for *Anabaena lemmermannii* from Lake Hallevann in Norway (Haneberg *et al.,* 1994), such reports are exceptions. The biochemical pathways to the biosynthesis of microcystin (Dittmann *et al.,* 1996), anatoxin (Gallon *et al.,* 1989) and saxitoxin (Skimizu *et al.,* 1984) are becoming understood. These show no connection between toxin production and the production of the alcohols geosmin and 2-MIB. It is therefore very unlikely that the production of the taste and odour compounds are biochemically connected to the production of the cyanotoxins.

Just as the presence of earthy or musty odours in water indicates the presence of cyanobacteria and/or actinomycetes, taste and odour problems can be used as a warning of the need for further investigation in the event that the occurrence of cyanobacteria could result in the presence of cyanotoxins. It is, however, important to recognise that the converse does not apply: lack of taste and odour by no means implies the absence of cyanobacteria.

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