Chapter 5

Exposure to cyanotoxins

Understanding it and short-term interventions to prevent it

CONTENTS

INTRODUCTION AND GENERAL CONSIDERATIONS

People may be exposed to cyanotoxins through oral, respiratory and dermal routes. Ingestion may occur through drinking-water (see section 5.1) or accidental uptake during water sports, recreational or occupational activity (see section 5.2). In some settings, contaminated food can be a source of dietary exposure, possibly significant (see section 5.3). This includes fish, shellfish and crustaceans collected from bloom-ridden waterbodies as well as leafy vegetable crops spray-irrigated with water containing cyanobacteria. A particularly high risk for specific subpopulations may be caused by exposure through haemodialysis (section 5.4): if dialysis centres do not take appropriate precautions and dialysate is contaminated with cyanotoxins, this can injure patients undergoing renal dialysis, because toxins from a large volume of water (>100 L per treatment) may gain direct access to the bloodstream via the intravenous route several times per week. Cyanobacterial dietary supplements may further be a potentially relevant route of oral intake for a small subpopulation using such products (see section 5.5).

While symptoms from cutaneous exposure to freshwater cyanobacteria have been quite widely reported (see section 5.2), these are usually mild and self-limiting. In contrast, marine cyanobacteria can cause severe skin lesions, for which, however, there is still a lack of dose–response information as a basis for estimating tolerable exposure levels (see section 2.6). Some people may experience allergic reactions to cyanobacteria, whereas others may be unaffected, and by the time of the publication of this book, it remains unclear which constituents of cyanobacterial cells – or associated microorganisms and compounds – actually cause allergic reactions.

The following sections 5.1 – 5.5 outline the specific exposure pathways and health risks through drinking-water, recreation and occupational use of water containing cyanobacteria and/or their toxins dissolved in water, food, renal dialysis and dietary supplements. They summarise available epidemiological knowledge as well as other indications of human exposure and relate these to the information on toxicity as discussed in Chapter 2 for the individual groups of cyanotoxins.

A caveat to keep in mind when assessing reports concerning human exposure to toxic cyanobacteria is that their estimates of exposure are almost always retrospective (it would not be ethically possible to conduct a prospective human study of a toxin at concentrations expected to show effects). That is, they provide information on human symptoms occurring at or just before the time of the study and try to explain these by looking into the past to make an "educated guess" as to what may have caused the observed symptoms. Even cyanotoxins detected in the tissues of people or animals do not solve this problem: while they provide absolute evidence of exposure, they do not necessarily demonstrate cyanotoxins to have been the sole cause of symptoms or elevated serum enzyme levels. Many of the reported symptoms in historical reports are quite general and cannot be considered

in isolation as diagnostic of cyanotoxin poisoning. It is also not possible to know whether all potential causes and their interactions have been considered, nor whether the estimates of exposures are accurate. Thus, this type of study cannot prove that a cause–effect relationship exists, nor can it provide a quantitative dose–response estimate. This is why the guideline values (GVs) for all cyanotoxins except saxitoxins (STX) are based on animal studies, despite these also having many limitations. Saxitoxins are an exception due to the rapid onset of highly specific diagnostic symptoms following the consumption of contaminated seafood.

In spite of these limitations, however, it is highly useful to report incidents of suspected human and/or animal exposure, particularly for enabling direct interventions to prevent further exposure but also, in the longer term, to collate indicative evidence, particularly if reporting includes toxin concentrations observed in the field at the time of exposure or in the serum of those exposed, or cyanobacterial cells observed in stool samples.

Using concentrations of cyanobacterial biomass to trigger cyanotoxin alerts

Sections 5.1 and 5.2 propose Alert Level Frameworks (ALFs) to guide short-term interventions if cyanotoxins or cyanobacterial biomass are present in a waterbody in concentrations that may become or may already be relevant to human health. For triggering alerts, the ALFs offer different points of entry, ranging from visual assessment over microscopy and quantification of cyanobacterial biomass to toxin analysis. This allows the selection of parameters depending on national or local considerations, including the accessibility of analytical methods. Importantly, these ALFs are intended for national or even local adaptation: other parameters may also be used if these are more accessible or appropriate, provided their ratio to toxin concentrations can be determined periodically (see below), for example, cell numbers or turbidity readings in raw water entering a treatment plant. An advantage of defining the Alert Levels with a measure of cyanobacterial biomass (either biovolume or pigment concentrations; see Chapter 13) is that they are thus also protective against further unspecific health effects of blooms not attributable to the cyanotoxins.

The Alert Levels triggering interventions are based on concentrations of cyanobacterial biomass that correspond to the WHO health-based values for cyanotoxins (Table 5.1) – that is, depending on the Alert Level, those for drinking-water (lifetime, short-term or acute) or recreational exposure. Therefore, it is also possible to use the GVs in Table 5.1 directly to trigger alerts. Biomass is measured either as biovolume or as concentration of chlorophyll-*a* (the latter after a brief qualitative check by microscopy of whether chlorophyll-*a* is largely from cyanobacteria), and the Alert Levels for biovolume and chlorophyll-*a* proposed (Table 5.2) are

Table 5.1 Guideline values and health-based reference values for selected cyanotoxins and exposure scenarios (WHO, 2020)

Toxin	*Exposure*[a]	*Value (μg/L)*	*Value type* [b]
Microcystin-LR	Drinking-water, lifetime	1	Provisional guideline value
Microcystin-LR	Drinking-water, short term	12	Provisional guideline value
Microcystin-LR	Recreational	24	Provisional guideline value
Cylindrospermopsin	Drinking-water, lifetime	0.7	Provisional guideline value
Cylindrospermopsin	Drinking-water, short term	3	Provisional guideline value
Cylindrospermopsin	Recreational	6	Provisional guideline value
Anatoxin-a	Drinking-water, acute	30	Health-based reference value
Anatoxin-a	Recreational	60	Health-based reference value
Saxitoxin	Drinking-water, acute	3	Guideline value
Saxitoxin	Recreational	30	Guideline value

For details on derivation of individual values see sections 2.1–2.4.

[a] Note that short-term exposure refers to periods of about two weeks until enhanced drinking-water treatment or other measures can be implemented to achieve concentrations below the lifetime guideline value.

[b] Due to the overall quality of the database for their derivation and since the respective guideline values only cover specific congeners, the guideline values for microcystin-LR and for cylindrospermopsin are considered provisional.

In the absence of oral toxicity data for other congeners, itis recommended that the GVs be applied to total MCs, total CYNs and total STXs as gravimetric or molar equivalents, based on the worst-case assumption of the congeners having similar toxicity. For STX toxicity equivalents, see WHO 2020.

Furthermore, for ATX, the available toxicological information is not sufficient for deriving a formal guideline value (provisional or otherwise) for lifetime exposure, but it does show that health hazards are unlikely at levels above these health-based reference values (see sections 2.1–2.4 for details).

derived on the basis of conservative assumptions on ratios of microcystins (MCs) to either biovolume or chlorophyll-*a* found in publications covering a variety of waterbodies (reviewed in section 2.1 and discussed in section 4.6.2). Thus, if these Alert Levels are not exceeded, the concentrations of MCs are highly unlikely to exceed the respective GVs summarised in Table 5.1.

For the other cyanotoxins, less data are available to determine such ratios. The data available (see sections 2.2–2.4) show that their concentrations in the biomass of the producing cyanobacteria can attain the maximum levels similar to those attained by MCs, although this appears to occur less frequently. Thus, in many cases, the toxin/biomass ratios derived for MCs can be assumed as a conservative approach for these

Table 5.2 Conservative values for parameters of cyanobacterial biomass indicative of possible occurrence of cyanotoxin concentrations reaching guideline values

	Biovolume	*Chlorophyll-a*	
Alert Level	*MC/BV ≤ 3/1 [µg/mm³]*	*MC/ Chl.a ≤ 1:1 [µg/µg]*	*Basis for conservative estimate[a] of toxin/biomass*
Alert Level 1 in drinking-water ALF	0.3 mm³/L	1 µg/L	$GV_{chronic}$ for MCs in drinking-water: 1 µg/L
Alert Level 2 in drinking-water ALF	4 mm³/L	12 µg/L	$GV_{short\text{-}term}$ for MCs in drinking-water: 12 µg/L
Alert Level 2 in recreational ALF	8 mm³/L	24 µg/L	$GV_{recreational}$ for MCs: 24 µg/L

For discussion of the biomass parameters and references, see text above as well as sections 2.1–2.4 and 4.6.2; for specifics of CYN, see Box 5.1.

Examples:

1. Observing 0.3 mm³/L biovolume or 1 µg/L chlorophyll-*a* (with dominance of cyanobacteria seen by brief visual assessment with microscopy) indicates that microcystin or cylindrospermopsin may occur at concentrations reaching the lifetime GV;
2. Observing > 4 mm³/L biovolume or > 12 µg/L chlorophyll-*a* (as above, with dominance of cyanobacteria) indicates that microcystins, cylindrospermopsins or saxitoxins may exceed the short-term GVs for these toxins.

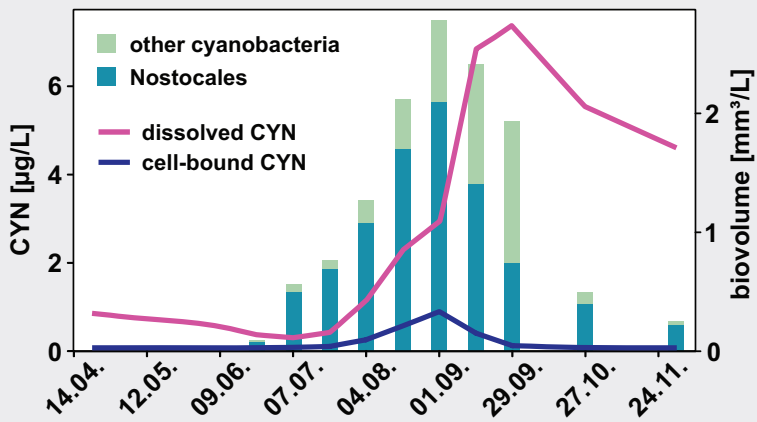
[a] Note that in many cases, the ratio of toxin to either biomass parameter is likely to be substantially lower, often by up to a factor of 10. Periodically (i.e., 2–3 times during a cyanobacterial growing season) "calibrating" them with toxin analysis is likely to enable higher Alert Levels.

toxins as well. This is supported by the concentrations found in water for cylindrospermopsins (CYNs), saxitoxins (STXs) and anatoxins (ATXs), which are typically substantially lower than those of the MCs. For anatoxins, the biomass thresholds proposed in the ALFs are sufficiently protective because their health-based reference values are substantially higher than the GVs for MCs. For lifetime exposure to CYNs, the GV is in the same range as the corresponding GV for MCs, and therefore, if CYNs are monitored as described in Box 5.1, the biomass threshold for Alert Level 1 is considered sufficiently protective. However, for CYNs and STXs, there are some uncertainties about whether the Alert Level 2 biomass thresholds are sufficiently conservative to ensure that toxin levels are below the acute GV for STXs and the short-term GV for CYNs, as these values are fourfold lower than the corresponding value for MCs. For STXs, this applies particularly to *Dolichospermum* spp., for which high STX/biomass ratios have been reported (see Tables 2.6 and 2.7 in Chapter 2). Therefore, toxin concentrations should be determined for blooms when STX- or CYN-producing species are dominant, and there is evidence that Alert Level 1 may be exceeded.

BOX 5.1: CONSIDERATIONS FOR USING CYANOBACTERIAL BIOMASS AS INDICATOR OF CYLINDROSPERMOPSIN CONCENTRATIONS

As discussed in section 2.2, maximum CYN contents per unit biomass of the producing cells are in the same range as for MCs, and thus, the same biomass Alert Levels can be used. However, while MCs largely occur cell-bound, high proportions of CYNs can occur dissolved in water in concentrations exceeding the concentration of cell-bound CYNs and persist even after the producing cyanobacterial cells are no longer present. In consequence, levels of biovolume or chlorophyll-*a* at the time of sampling do not necessarily reliably indicate levels of the total concentration of CYNs.

Integrated samples taken in 2009 in Großer Plessower See illustrate this: Concentrations of cell-bound CYNs (combined cylindrospermopsin and deoxy-cylindrospermopsin) correlate to the biovolume of potentially CYN-producing species, summarized as Nostocales (*Rhaphidiopsis* (*Cylindrospermopsis*), *Aphanizomenon*, *Dolicospermum*, *Chrysosporum*). In contrast, dissolved CYNs reached its maximum concentration only once biovolumes of Nostocales and other cyanobacteria started to decline in September and remained on levels >1 µg/L until December (unpublished data, kindly provided by Karina Preussel, Robert-Koch-Institut, Berlin, and Jutta Fastner, Umweltbundesamt, Berlin).



However, if monitoring on a regular, weekly or at least fortnightly basis has not identified any CYN-producing taxa (i.e., of the genera *Raphidiopsis (Cylindrospermopsis), Aphanizomenon,* or *Chrysosporum*) during the previous

4–6 weeks, the presence of CYNs is unlikely, in particular at concentrations above GVs.

If cyanobacteria of these genera have been found during previous weeks, but not at biovolume or chlorophyll-*a* levels exceeding the Alert Levels, the presence of CYNs exceeding the Alert Levels is also unlikely.

If, however, cyanobacteria of any of these genera have reached biomass levels corresponding to the Alert Levels during the 4–6 previous weeks, monitoring concentrations of dissolved and cell-bound CYNs is advised until concentrations of the sum of cell-bound and dissolved CYNs have declined below the guideline values (GVs).

It is generally useful to adapt the Alert Levels proposed in Table 5.1 to the toxin content of the locally prevalent cyanobacteria by occasional analyses of cyanotoxins together with the parameter used to trigger Alert Levels: periodically "calibrating" the trigger for alerts with cyanotoxin analyses will improve predictive power. As discussed in section 4.6, for any of the cyanotoxins, the ratio of toxin to biovolume or chlorophyll-*a* in a given waterbody may be substantially lower than the generally conservative assumption used in the Alert Level Frameworks, by an order of magnitude or more, and using a locally appropriate toxin/biomass ratio may serve to avoid undue restrictions of waterbody use or to lift restrictions previously implemented.

Moreover, periodic reassessment of the ratio of toxin to the parameter chosen for triggering alerts is recommended because the ratio may vary between seasons and within a season as a bloom develops, as illustrated by the examples in Figure 5.1: the ratio of MCs to cyanobacterial biovolume was fairly constant in the *Microcystis*-dominated waterbodies Müggelsee and Radeburg Reservoirs, Germany, varying only by a factor of three and without seasonal trend, but in contrast in the Weida Reservoir, the ratio of MC to biovolume varied nearly seven fold, with an increasing trend as the season progressed. Yet, in Müggelsee in other years, the MC/biomass ratio declined continuously as the summer progressed (data not shown). These examples illustrate the variability of toxin/biomass ratios not only between waterbodies but also between years for one-and-the-same waterbody. Thus, where resources allow, it is worthwhile to check the toxin/biomass ratio 2–3 times per season until a good understanding of its variability has been established in order to base management actions on the most appropriate information. Where access to capacity for cyanotoxin analysis is not readily possible, an option may be to send samples to regional laboratories or to seek support of research institutions.

For adapting the Alert Level Frameworks (ALFs) to national or local circumstances, the following further considerations are relevant:

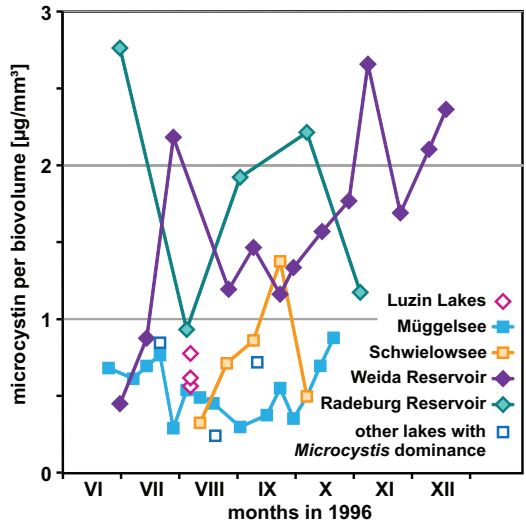


Figure 5.1 Ratios of microcystin (sum of all variants) to cyanobacterial biovolume over time in different lakes and reservoirs in Germany. Diamonds denote dominance of *Planktothrix rubescens* (Weida Reservoir and Luzin Lakes); the other waterbodies were dominated by *Microcystis* spp. (Jutta Fastner and Ingrid Chorus, unpublished data.)

1. If chlorophyll-*a* concentrations, Secchi depth readings or turbidity are used as triggers for alerts, a brief qualitative check by microscopy is important in order to assess whether chlorophyll-*a* or turbidity are largely due to cyanobacteria (and thus serve as effective indicators) or whether other phytoplankton, that is, eukaryotic algae (or in the case of turbidity, other particles), are causing elevated levels.
2. A reason to choose toxin concentrations rather than biomass indicators as parameters to define Alert Levels may be that the target is primarily to protect from cyanotoxins rather than from cyanobacterial cells as such; this may be appropriate particularly where drinking-water treatment reliably removes cells.
3. If cell counts are used to define Alert Levels, it is important to "calibrate" them against occasional toxin analyses because the cell quota data (i.e., toxin per cell) are available in the literature only for some taxa. However, cell sizes vary substantially, and as shown in section 4.6.2, cell size has a substantial impact on toxin quotas: if very small-sized cyanobacteria dominate, cell counts may be high and thus far too conservative, even if the water is very clear and toxin concentrations are negligible. "Calibration" of cell counts with toxin concentrations requires a significantly smaller number of samples over time as compared to regular monitoring.

4. Further parameters may also be used for defining Alert Levels, if locally or nationally more accessible or practical, for example, values for molecular parameters, fluorescence, turbidity readings or signals from remote sensing, provided these also are periodically "calibrated" regarding their ratio to toxin concentrations caused by the ambient cyanobacteria.
5. The GVs for short-term occurrence in drinking-water as well as those for recreational exposure were derived with an allocation factor of one, that is, assuming each of these exposure pathways to be the dominant source of exposure during the short duration of such exposure. The lifetime drinking-water guideline values (GVs) were derived assuming an allocation factor of 0.8, that is, that 80% of the tolerable daily intake (TDI) to be through drinking-water since drinking-water is usually the most likely long-term source of exposure. This implies that other sources such as food and recreational water are less significant (contributing to 20% of the TDI). In practice, the relative importance of each potential exposure route may be different, with food potentially being a particularly high-exposure source in some situations. When adapting an ALF to local circumstances, it is therefore important to assess the likelihood of simultaneous multiple routes of exposure – such as a population using bloom-ridden surface water with insufficient treatment for drinking *and* irrigation, perhaps also with freshwater fish as staple food. In such situations, it may be appropriate to consider reducing the allocation factors used in the derivation of the GVs. However, it is important to balance this with potential other negative consequences for the population's health and/or livelihoods that might result from severe restrictions of water use.

When using the information in this chapter as basis for developing locally appropriate guidance, it is further important to assess the patterns of bloom occurrence over time in the waterbodies of interest (see Chapter 8) and thus the likely duration of potential human exposure. This differs substantially between climates, regions and individual waterbodies: in temperate climates, some waterbodies dominated by surface scum-forming taxa such as *Microcystis* may have a bloom season of 3–5 months, and exposure then is typically seasonal. Other taxa, such as *Planktothrix agardhii*, may show perennial blooms even in cooler temperate climates, although generally with lower abundance during winter. In warmer climates, such as in some regions of Australia, South America, Asia and Africa, cyanobacteria may bloom for 6–10 months, and in relatively stable warm tropical climates, high numbers of cyanobacteria may occur year-round, potentially causing ongoing exposure. Importantly, however, in the same climates, other waterbodies may have no blooms at all or blooms occurring only sporadically and for only a few days or weeks.

5.1 DRINKING-WATER

Andrew Humpage and David Cunliffe

As outlined in the preceding chapters, toxigenic cyanobacteria are encountered in many waterbodies worldwide, including those from which water is abstracted for the production of drinking-water. The concentration of cyanotoxins in lakes and reservoirs can exceed the (provisional) GVs for lifetime daily exposure, as well as for short-term exposure, occasionally by orders of magnitude. To effectively remove cyanotoxins, drinking-water treatment needs to be optimised and validated for this target. Therefore, even when treatment is implemented, the possible breakthrough of cyanotoxins from raw water to the consumed drinking-water needs to be considered as a potential health risk and measures need to be validated to ensure that this risk is effectively controlled, or further measures be put in place to avert it.

A number of studies have concluded that cyanotoxins in drinking-water were the possible cause of documented cases of human illness. Further, even before the toxins were characterised in detail, there was compelling evidence of cyanobacterial toxicity from the deaths of animals following the consumption of water containing cyanobacteria. As discussed in section 5.0, historical literature about human illness after exposure to cyanotoxins must be treated with caution, however, because prior to their chemical characterisation neither the quantification of toxins nor the estimation of doses was possible. Further, other potential causes of the observed illnesses, such as viruses and protozoan pathogens, were not recognised, or could not be tested for, at the time. This does not, however, imply that the cases discussed in the next section are irrelevant with respect to the cyanotoxin risk.

Section 5.1.1 summarises evidence for the occurrence of toxigenic cyanobacteria in drinking-water sources and cyanotoxins in drinking-water distributed to consumers. It also provides data on human drinking-water-related poisoning events that have been documented adequately enough to provide reasonable indication that cyanotoxins were the causative agent of the poisonings. For further overview, readers are referred to the following publications: Harding and Paxton (2001); Chorus (2005); Codd et al. (2005); Falconer (2005); Falconer and Humpage (2005); Funari and Testai (2008); Hudnell (2008); Buratti et al. (2017). Section 5.1.2 gives guidance on assessing the risk of exposure to cyanobacteria or their toxins in drinking-water.

The cases discussed in the following sections demonstrate, firstly, that cyanotoxins in drinking-water sources and/or finished drinking-water are a worldwide phenomenon. Secondly, they also highlight that, depending on the level of contamination and the treatment processes employed, the toxins can contaminate treated drinking-water. Thirdly, they show that where

treatment is insufficient or overwhelmed by a massive bloom, toxin concentrations can significantly exceed guideline values (GVs) in drinking-water.

5.1.1 Evidence of illness from exposure to cyanobacteria in drinking-water

All reports to date reporting symptoms have only been associated with the toxins MC or CYN. However, some of the human effects ascribed to the presence of cyanotoxins in drinking-water, such as gastrointestinal illness and pneumonia, may well be due to other, less well-described, cyanobacterial metabolites (see section 2.10) as well as bloom-associated pathogens or their metabolites. Furthermore, where blooms were treated with copper sulphate, high copper concentrations may be an explanation for symptoms such as diarrhoea, vomiting, stomach cramps and nausea; however, this would require concentrations above the range of 1–2mg/L at which it is used as an algicide (see WHO (2017) for a discussion of copper toxicity).

As discussed above, where cyanobacterial blooms in the source water and illnesses are observed at the same time, the obviousness of the bloom or scums makes it suggestive to presume cyanobacteria as the aetiologic agent. However, substantiating this with data is challenging as it requires analysing water samples taken when patients were exposed, and in the published case studies, this has very rarely been accomplished. Also, even if the cyanobacteria and their metabolites themselves are not the direct cause of the illness, the true aetiology may be closely linked to the bloom, for example, pathogens associated with the bloom (see, e.g., Berg et al., 2009). For effectively targeting measures to ensure or improve water quality, it is important to understand cause–effect relationships, particularly whether or not drinking-water was the actual cause. Illness suspected to be due to drinking-water requires a detailed investigation so that steps can be rationally applied to prevent such occurrences in future. While such follow-up investigations may fail to clearly identify a causative agent for observed illness, they will serve to identify water quality deficits and risks of events causing contamination hazardous to health.

5.1.1.1 Examples of potentially hazardous cyanotoxin concentrations in finished drinking-water

Local knowledge about the hazardous nature of cyanobacterial scums appears to have existed in some regions with eutrophic, bloom-ridden waterbodies for a long time, as discussed in Chapter 1. Scientific screening of occurrence began in the wake of emerging awareness of cyanobacterial toxicity in the 1980s. At this time, screening often followed deaths of farm animals and relied only on mouse bioassays to evaluate toxicity because methods for the chemical analysis of known toxins only became available

from the later 1980s onwards. Since then, surveys have been conducted in many parts of the world, including in drinking-water. A summary of findings from a range of relatively detailed studies is provided below. Less extensive reports on cyanotoxin occurrence have come from Brazil, Europe, New Zealand, China, Thailand and Africa (Chorus, 2005; Codd et al., 2005). For exemplary data on cyanotoxin occurrence in a variety of waterbodies, see Chapter 2.

- One of the first surveys specifically targeting drinking-water sources was conducted in 1991 in the Murray–Darling Basin, Australia, which is a major agricultural region that relies on its rivers for irrigation and drinking-water supply (Baker & Humpage, 1994): Mouse bioassays were performed on 231 cyanobacterial grab samples from sites across the Basin. Approximately 60% of samples were from potential drinking-water sources (rivers, lakes, reservoirs). Mouse bioassays showed that 24% of samples were neurotoxic and a further 18% were hepatotoxic, thus demonstrating a need to ensure sufficiently effective drinking-water treatment.
- Low concentrations of MCs were detected in 15 finished drinking-water samples collected during the fall of 1992 from two Canadian water treatment plants (0.09–0.18 μg/L MC-LR equivalent in a protein phosphatase inhibition assay (Lambert et al., 1994).
- A survey of MCs in drinking-water utilities across the USA and Canada (June 1996 to January 1998; Carmichael, 2001) included over 24 utilities, and 677 samples were screened for MCs by ELISA. The samples were taken from blooms, plant intakes, plant influents (after preoxidation) and finished water. Although 80% of samples contained MC levels above the detection limit of 0.02 μg/L, only two finished water samples showed MC concentrations above 1 μg/L. These occurred in two of the three treatment plants that were facing significant MC challenges at the time of sampling in July 1997: at plant CM-1, MC concentrations at the intake were >1000 μg/L and 8 μg/L in the finished water, respectively. At plant IXC-3, the intake contained just over 2 μg/L MCs and the finished water contained about 1.3 μg/L. Plant CM-1 utilised prechlorination and granular activated carbon, whereas plant IXC-3 only added ammonium and chlorine to otherwise untreated source water (Carmichael, 2001).
- Cyanotoxin surveys in Florida in 1999 and 2000 (Burns, 2008) of surface water sources and finished waters collected 167 samples, of which 88 contained cyanotoxins (MCs, ATX, CYN). MCs were the most commonly found toxins, occurring in both pretreatment and posttreatment waters. Concentrations in the latter ranged from below detection to 12.5 μg/L. Three finished water samples contained ATX up to 8.46 μg/L, whereas nine finished water samples contained CYN at

concentrations of 8.1–97 μg/L. A survey of 52 source and finished water samples from two drinking-water treatment plants in Queensland, Australia, found that only two samples of finished water contained traces (<0.05 μg/L) of STX when the source waters contained up to 17 μg/L STX. The authors concluded that conventional drinking-water treatment (flocculation, sedimentation, PAC during high toxin load, sand filtration and chlorination) removed 99.9% of total STX (free and cell-bound) from water containing a toxic *Anabaena circinalis* bloom (Hoeger et al., 2004).

- During the summer of 2003, MCs were detected at low levels (0.15–0.36 μg/L) in 30 of 77 finished water samples from 33 US drinking-water treatment plants in Northeastern and Midwestern USA. However, only relatively low concentrations (0.15–5.6 μg/L) were detected in 87 of 206 raw water samples from the same plants (Haddix et al., 2007).
- CYN was detected (1.3 and 8.6 μg/L) during March 2007 in finished waters of two conventional treatment plants, as well as throughout the combined distribution system, on Kinmen Island, Taiwan (15 tap samples ranging from 0.7 to 2.2 μg/L), when the plants were challenged by high CYN levels in the raw water (0.7 and 36 μg/L; Yen et al., 2011).
- In another Canadian plant Zamyadi et al. (2012) detected up to 10 μg/L MCs in clarifier supernatants and up to 2.5 μg/L in the finished chlorinated drinking-water during the bloom seasons (June to October) of 2008, 2009 and 2010.
- MCs have also been detected in conventionally treated drinking-water (with flocculation, sedimentation, sand filtration, chlorination) in Saudi Arabia (range 0.33–1.6 μg/L over 8 monthly samples during May to December 2007; Mohamed & Al Shehri, 2009) and Egypt in May 2013 (up to 3.8 μg/L; Mohamed et al., 2015; Mohamed, 2016), and also in Algeria (up to 6.3 μg/L during a bloom in 2013, treatment process not reported; Saoudi et al., 2017).
- In Australia, during the summer of 2013–2014, a bloom of *Raphidiopsis* (*Cylindrospermopsis*) *raciborskii* occurred in the water supply of Mount Isa, Queensland. The water supply was treated by passage through a reed bed filtration lagoon before chlorination. *R. raciborskii* blooms were common in the supply reservoirs (Lake Moondara and Lake Julius), but this was the first time a bloom had occurred in the filtration lagoon. *R. raciborskii* numbers peaked at 425 000 cells/mL in the lagoon and 42 000 cells/mL in the finished water storage reservoir. The maximum toxin levels detected in treated water were 2 μg/L CYN in the storage reservoir and 0.5 μg/L CYN in the town reticulation. Chlorination was increased to maintain a residual and later a mobile ultrafiltration unit was installed. Cell counts and toxins in the treated water returned to safe levels after the ultrafiltration unit was installed (Janet Cumming, Queensland Department of Health, pers. comm., January 2017).

- In August 2014, the city of Toledo, Ohio, total MCs occurred in the city's finished drinking-water at levels up to 2.5 μg/L. A "do not drink or boil advisory" was issued to nearly 500 000 consumers. A cyanobacterial bloom near Toledo's drinking-water intake located on Lake Erie was the source of the MCs. The advisory was lifted 2 days later after optimisation of permanganate and PAC treatments led to the reduction of the MC concentrations to levels below 1 μg/L in all samples (US EPA, 2015).

Options for control of cyanobacterial occurrence and cyanotoxin removal through effective treatment are described in Chapters 7–10.

5.1.1.2 Case reports giving evidence of short-term health risks from acute exposure through drinking-water

Some case studies provide evidence that exposure to cyanobacterial toxins in drinking-water can lead to illness and even death. Due to the inability to identify the toxins at the time, the earliest reported cases offer only circumstantial evidence of a link between exposure to cyanotoxins and human illness.

- Gastroenteritis associated with cyanobacteria was observed in the population of a series of towns along the Ohio River in 1931. Low rainfall had allowed the water of a side branch of the river to develop a cyanobacterial bloom which was then washed into the main river. As this water moved downstream, a series of outbreaks of illness were reported (Tisdale, 1931).
- In Harare, Zimbabwe, children living in an area of the city supplied from a particular water reservoir developed gastroenteritis each year at the time when a natural bloom of *Microcystis* was decaying in the reservoir. Other children in the city with different water supplies were not affected (Zilberg, 1966).
- In an incident in Sewickley, Pennsylvania, 62% of the population connected to a filtered, chlorinated drinking-water supply developed symptoms of gastroenteritis within a period of five days. The water, sourced from groundwater contaminated by an intrusion from the Ohio River, was treated and then held in open holding reservoirs prior to distribution. One reservoir had over 100 000 cells/mL of *Schizothrix calcola*, *Plectonema*, *Phormidium* and *Lyngbya* in the open water. The reservoir had just been treated with copper sulphate when the poisoning event occurred (Lippy & Erb, 1976). Although not known to be toxic at the time, *Schizothrix*, *Phormidium* and *Lyngbya* have all since been shown to be toxin producers elsewhere (Falconer, 2005).

While these reports note that the health effects could not be attributed to infectious agents, a caveat on this conclusion is that many of the aetiologic agents leading to the described symptoms were unknown at the time (e.g., viruses) or not detectable with sufficient sensitivity by a standard laboratory (*Giardia*, *Cryptosporidium*). The following later study addressed many of these issues.

- An outbreak, with a high death rate attributed to cyanobacterial toxins in drinking-water, occurred in the Paulo Alfonso region of Bahia State in Brazil following the flooding of the newly constructed Itaparica Dam reservoir in 1988. Some 2000 gastroenteritis cases were reported over a 42-day period, and 88 deaths, mostly children, occurred (Teixera et al., 1993). Blood and faecal specimens from gastroenteritis patients were subjected to bacteriological, virological and toxicological testing, and drinking-water samples were examined for microorganisms and heavy metals. No infectious agent was identified, and cases occurred in patients who had been drinking only boiled water. The cases were restricted to areas supplied with drinking-water from the dam. Clinical data and water sample tests were reviewed, and it was concluded that the source of the outbreak was water from the dam and that a toxin produced by cyanobacteria (*Anabaena* and *Microcystis* in high densities) was the most likely responsible agent, although the toxin could not be identified.

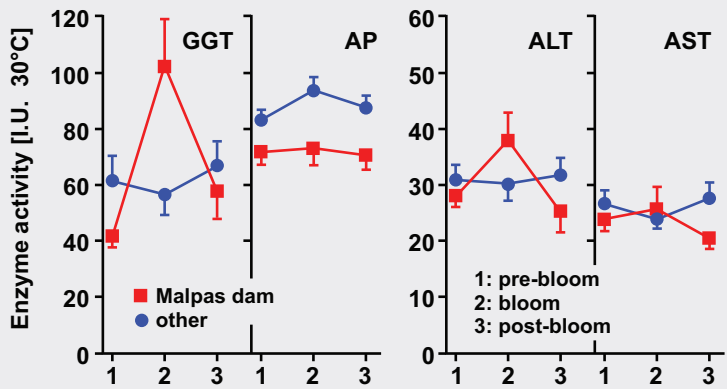
A closer association between human illness and exposure to cyanotoxins is demonstrated when the cyanobacteria were shown to be toxin producers, as illustrated in the following examples:

- In Armidale, Australia, the water supply reservoir had been monitored for blooms of toxic *Microcystis* for several years, and MC-YM had been identified in these blooms. When a particularly dense bloom occurred, the water supply authority treated the reservoir with 1 mg/L of copper sulphate, which lysed the bloom, possibly causing a pulse of toxin release from the cells. An epidemiological study of the local population indicated subclinical liver damage occurring simultaneously with this treatment of the bloom (see Box 5.2).
- A more severe outbreak of cyanobacterial toxicity in a human population occurred on Palm Island, off the north-eastern coast of Australia in 1979. Complaints of bad taste and odour in the water supply were attributed to a cyanobacterial bloom, and the authorities therefore treated the reservoir with copper sulphate. Within a week, numerous children developed severe hepatoenteritis, and a total of 140 children and 10 adults required hospital treatment (Byth, 1980). A CYN-producing strain of *Raphidiopsis raciborskii* was later identified as the agent most likely to be responsible for this episode (see Box 5.3).

BOX 5.2: TOXIC *MICROCYSTIS* IN THE ARMIDALE WATER SUPPLY RESERVOIR AND PUBLIC HEALTH

At the time of this study, the city of Armidale, New South Wales, Australia, had a drinking-water supply from a eutrophic reservoir which had been experiencing repeated blooms of cyanobacteria since the early 1970s.

In 1981, a particularly extensive toxic bloom of *Microcystis aeruginosa* was monitored during its development. During the bloom, complaints of bad taste and odour in the drinking-water were received, leading to copper sulphate treatment of the reservoir. The toxicity of the bloom was monitored by mouse bioassay. A toxin had previously been isolated from Malpas Dam and partially described, which was later characterised as MC-YM (Botes et al., 1985). This event was used as the basis for a retrospective epidemiological study of liver function in the population consuming the water, compared with a population in the same region supplied from other reservoirs. The data for the activity of plasma enzymes reflecting liver function were obtained for patients having blood samples examined at the Regional Pathology Laboratory for the 5 weeks prior to the bloom, the 5 weeks of peak bloom and its termination and for 5 weeks after that. The data were then separated into those from patients having used the Malpas drinking-water supply and those using other supplies.



Serum enzymes reflecting liver function in patients consuming drinking-water from Malpas Dam or from other supplies included GGT = γ-glutamyl transferase; ALT = alanine aminotransferase; AST = aspartate aminotransferase and AP = alkaline phosphatase (Falconer et al., 1983). As shown in the figure above (redrawn from Falconer et al., 1983), γ-glutamyl transferase in the

blood of the group using the Malpas Dam water supply during the peak of the bloom and its lysis with copper sulphate was significantly higher than that in the same population before and after the bloom, and higher than that in the other population served by different water supplies. The clinical record gave no evidence of an infectious hepatitis outbreak or disproportionate alcoholism (Falconer et al., 1983). While the mean increase in γ-glutamyl transferase activity was indicative of minor liver toxicity, some individuals within the population studied showed highly elevated enzyme activity, indicating substantial liver damage. This enzyme has also been shown to be elevated as a result of *Microcystis* toxicity in experimental studies with pigs and rodents, where it is used as an effective marker for liver injury (Fawell et al., 1993; Falconer et al., 1994).

BOX 5.3: PALM ISLAND MYSTERY DISEASE

In 1979, there was a major outbreak of hepatoenteritis among the children of an Aboriginal community living on a tropical island off the coast of Queensland, Australia. Altogether 140 children and 10 adults required treatment, which was provided by the local hospital for less severe cases and by the regional hospital on the mainland for severe cases possibly requiring intensive care. Diagnostic information included a detailed clinical examination showing malaise, anorexia, vomiting, headache, painful liver enlargement, initial constipation followed by bloody diarrhoea and varying levels of severity of dehydration. Urine analysis showed electrolyte loss together with glucose, ketones, protein and blood in the urine, demonstrating extensive kidney damage. This was the major life-threatening element of the poisoning. Blood analysis showed elevated serum liver enzymes in some children, indicating liver damage. Sixty-nine percent of patients required intravenous electrolyte therapy and, in the more severe cases, the individuals went into hypovolaemic/acidotic shock. After appropriate treatment, all the patients recovered (Byth, 1980).

Examination of faecal samples and foods eliminated a range of infectious organisms and toxins as possible causes for the outbreak and failed to identify the cause, hence the name "Palm Island Mystery Disease". The affected population, however, all received their drinking-water supply from one source, Solomon Dam. Families on alternative water supplies on the island were not affected by the disease. Prior to the outbreak of the illness, a bloom of cyanobacteria occurred in Solomon Dam. The bloom

discoloured the water and gave it a disagreeable odour and taste. When the bloom became dense, the dam reservoir was treated with 1 ppm of copper sulphate (Bourke et al., 1983). Clinical injury among consumers on that water supply was reported the following week. In subsequent investigations, the organisms from the dam were cultured and administered to mice. Mice treated with *Raphidiopsis* (*Cylindrospermopsis*) *raciborskii* culture slowly developed (over several days) widespread tissue injury involving the gastrointestinal tract, the kidney and the liver (Hawkins et al., 1985). The widespread tissue damage and delayed effects are quite different to those following *Microcystis aeruginosa* administration (Falconer et al., 1981). Subsequent monitoring of the blooms in the dam – well after the outbreak – identified *R. raciborskii* as the cause of the blooms, with seasonal cell concentrations of up to 300 000 cells/mL of water. This organism did not form scums and has the highest cell concentrations well below the water surface. In order to reduce bloom formation, the responsible authorities later introduced destratification of the reservoir (Hawkins & Griffiths, 1993). Subsequent research on toxins produced by *R. raciborskii* has identified the cytotoxic alkaloid cylindrospermopsin.

5.1.1.3 Epidemiological studies addressing health risks from chronic, low-dose exposure through drinking-water

While a number of epidemiological studies of the possible association of MC exposure with cancer incidence are available, all of them have used retrospective estimates of MC exposure. However, as discussed at the beginning of this chapter, such retrospective approaches face pronounced uncertainty regarding both the concentrations of cyanotoxins and those of any other pollutants to which the population was exposed during the formative stages of their cancer. In fact, the occurrence of other pollutants in surface waterbodies with heavy cyanobacterial blooms is quite likely, as blooms are caused by heavy nutrient loads and these are often associated with substantial loads of pesticides and/or other contaminants from agriculture and/or poorly treated wastewater. In addition, demographic information was usually not provided so it is not clear whether dietary, genetic and/or lifestyle factors associated with cancer were adequately controlled in the analyses. It is therefore important that where an observed health impairment is connected to cyanobacterial blooms (as the most prominent and visible phenomenon), health authorities also look for other potential causative agents. In consequence, it is currently not possible to show causation or to derive concentration–response data from the epidemiological studies available to date. While for this reason they cannot serve as basis for

deriving guideline values (GVs) (see above and Chapter 2), they are of some indicative value and are therefore summarised as follows:

- The possible link between chronic exposure to cyanotoxins and the incidence of human cancer has been studied in China and the USA. The incidence of hepatocellular carcinoma (HCC) in China has historically been one of the highest in the world, at least in part due to two proven risk factors: infection with hepatitis B virus (HBV), which increases the risk almost 10-fold (Yu et al., 2002), and intake of aflatoxin B1 from foods infected with moulds, which increases the risk in HBV-positive individuals by a further threefold (Lian et al., 2006). However, the uneven geographic distribution of HCC incidence in China could not be entirely explained by these factors and so other environmental factors were investigated (Yu, 1989; Yu, 1995; Yu et al., 2001). The source of a person's drinking-water was also found to be a significant risk factor with people drinking pond or ditch water having about 10-fold higher incidence of HCC when compared to those drinking deep well water. MCs were found to occur seasonally in water sources of Haimen city, China, with a summer survey detecting MCs in 17% of pond/ditch water samples, 32% of river water samples, 4% of shallow well and 0% of deep well water samples, with averages of 0.10, 0.16 and 0.07 μg/L for the first three, respectively (Ueno et al., 1996). Similar concentrations were found in a parallel study using different analytical methods (Harada et al., 1996). These concentrations seem quite low for untreated raw waters and are more similar to concentrations observed elsewhere in the world in finished waters (see examples given above). Nevertheless, based on the average MC contents of river and pond/ditch samples, Ueno et al. (1996) provide limited data that would lead to an estimated average daily exposure in the range of 0.2 μg/person during the summer months (note that the authors report 0.2 pg/person, but this is clearly a typographical error). Later studies from China have associated slightly higher exposure rates from food and water combined (0.36 to 2.03 μg/person per day) with detectable concentrations of serum MCs and increased levels of liver enzymes in the serum (Chen et al., 2009; Li et al., 2011), see below.
- A later case control study in Haimen city, China, did not find an association with drinking-water sources (Yu et al., 2002). However, this study did not analyse for the prevalence of aflatoxin-B1 antigens in the study population. There is evidence from animal studies that MC acts synergistically with aflatoxin tumour initiation to increase rates of liver cancer (Sekijima et al., 1999; Lian et al., 2006), whereas this may not be the case for HBV-related HCC (Lian et al., 2006).
- An increase in serum markers for hepatotoxicity (AST, ALP, ALT and lactate dehydrogenase, LDH) was observed in a cohort study of

Chinese fishermen exposed to MC-RR, MC-YR and MC-LR in Lake Chaohu through the consumption of contaminated water and food (Chen et al., 2009). The fishermen had a median serum MC concentration of about 0.2 ng/mL and an estimated daily intake of MC of 2.2–3.9 μg MC-LR equivalents (Chen et al., 2009). The relative proportion of the three variants in the fishermen's blood were similar to those in the carp and duck tissues used as typical food.

- Li et al. (2011) conducted a cross-sectional study assessing the relationship between liver damage in children ($n > 1000$) and MC levels in drinking-water and aquatic food (carp and duck) in China. MC levels measured in three local sources of drinking-water were classified in three groups, as negative controls, low and high exposure, with children in the low-exposure group consuming an estimated 0.36 μg/day and high-exposure children consuming 2.03 μg/day. Mean serum levels of MC-LR equivalents in the groups were below the detection limit in the negative control, 0.4 in the low-exposure and 1.3 μg/L in the high-exposure groups, with mean detection rates of 1.9%, 84.2% and 91.9%, respectively (1.9% in the control group caused by 1 MC-positive among 54 serum samples). MC was associated with increases in aspartate aminotransferase (AST) and alkaline phosphatase (ALP), but not ALT or γ-glutamyl transferase (GGT). The odds ratio (OR) for liver damage associated with MC was 1.72 (95% CI: 1.05–2.76), after adjustment for HBV infection and use of hepatotoxic medicines as confounding factors. HBV infection was a greater risk for liver damage in children.

  Although these findings suggest a potential role of MCs in the high HCC incidences, they cannot be used, as was proposed by Ueno et al. (1996), to derive a guideline for MCs in drinking-water because (i) although the authors demonstrated an association between the type of water consumed by people living in high HCC areas and the presence of MCs in that water, they derive no quantitative relationship between MC exposure and cancer incidence; (ii) MC concentrations in similar waters in low HCC areas were not determined, so the association remains only suggestive; and (iii) as noted above, the high incidence of HCC in certain regions of China has also been linked to high hepatitis B infection rates and exposures to aflatoxin B1, so it would not be correct to extrapolate data from this population to other populations not exposed to these additional risk factors. These results about the possible, although not proven, higher HCC incidence are consistent with the activity of MC-LR as a tumour promoter, increasing the potency of known tumour initiators such as aflatoxin B1 (see section 2.1 and below).
- Another Chinese study has looked at the association between the incidence of colorectal cancer and drinking-water source (Zhou et al., 2002). In this case, 408 cases of colon or rectal cancer were retrospectively

categorised by the source of drinking-water consumed by the patients (well, tap, river, pond). The relative risk of developing colorectal cancer was almost fourfold higher in consumers of pond or river water. Average and maximal concentrations of MCs were reported as follows: river waters (average 0.141 μg/L, maximum 1.083 μg/L, $n=69$), pond waters (0.106, 1.937 and 35), well waters (0.004, 0.009 and 12) and tap waters (0.005, 0.011 and 17). A positive association was found between MC concentration and colorectal cancer incidence, although as with the other studies, this association remains only suggestive.

- Svirčev et al. (2009; 2013) report an observational study that found an elevated incidence of primary liver cancer in regions served by drinking-water reservoirs that are subject to frequent summer blooms of cyanobacteria. However, no information on cyanotoxin exposures was presented.
- In the USA, the incidences of primary hepatocellular carcinoma (HCC) and colorectal cancer have been evaluated in relation to the study population's likely water source – surface water or ground water (Fleming et al., 2002). Only weak (HCC) or no (colorectal cancer) associations were found in these pilot studies.

As discussed above, such studies cannot be used for the derivation of GVs for safe levels in drinking-water. Because of the limitations of the human epidemiology studies, the best available animal studies have been used to derive the lowest, most protective GVs that are scientifically supported by robust quantitative evidence (see Chapter 2).

5.1.2 Assessing the risk of exposure to cyanotoxins through drinking-water and short-term responses to occurrence

A modern water treatment plant equipped with an effective filtration system for physical removal of cells as well as the removal of dissolved toxins should remove cyanotoxins to below hazardous levels, provided it is operated with attention to avoid disruption of cyanobacterial cells and release of dissolved toxin (see Chapter 10). However, this requires it to be validated for meeting this target. Also, many of the world's drinking-water supply systems and treatment plants are more rudimentary, and large populations may depend upon such vulnerable water supplies or on untreated surface waters for drinking and preparing food.

For exposure assessment, particularly for MCs and CYNs, it is important to differentiate between daily exposure for significant parts of a lifetime and short-term episodic exposure. If concentrations exceed the values intended for lifetime daily consumption of drinking-water, but are below the short-term guideline values (GVs) given in Table 5.1 (or nationally derived

standards; Table 5.3), use of the water supply for drinking may continue, and action may first focus on assessing which measures are locally most appropriate to ensure better control of the cyanotoxin concentrations. These might include addressing the cause for waterbody conditions leading to

Table 5.3 Standards, guideline values, maximum acceptable concentrations or maximum values set by a number of countries for cyanotoxins in drinking-water

Cyanotoxin	*Type of value*	*Numerical value*	*Country*
Microcystins	Guideline value	1.3 μg/L MC-LR toxicity equivalents	Australia
	Standard	1 μg/L MCs	Brazil
	Maximum acceptable concentration	1.5 μg/L MC-LR	Canada
	Standard	1 μg/L MC-LR	Czech Republic
	Standard	1 μg/L sum of MCs	France
	Provisional maximum value	1.3 μg/L MC-LR equiv.	New Zealand
	Restrictions on water use	>1.0 μg/L sum of MCs	Finland
	Ban on water use	>10.0 μg/L sum of MCs	Finland
	Standard	1 μg/L MC-LR	Singapore
	Standard	1 μg/L sum of MCs	Spain
	Standard	1 μg/L MC-LR	Uruguay
	Standard	1 μg/L sum of MCs	Turkey
	Guideline value	1 μg/L MC-LR	South Africa
	Provisional maximum value	1 μg/L	New Zealand
Nodularin	Health Alert Level	1 μg/L	Australia
Cylindrospermopsin	Guideline value	1 μg/L	Brazil
	Provisional maximum value	1 μg/L	New Zealand
	Health Alert Level	3 μg/L	Australia
Saxitoxins (as saxitoxin toxicity equivalents)	Guideline value	3 μg/L	Brazil
	Provisional maximum value	3 μg/L	New Zealand
	Provisional maximum acceptable concentration	3.7 μg/L	Canada
Anatoxin-a	Provisional maximum value (valid also for homoanatoxin-a)	1 μg/L	New Zealand
	Provisional maximum value	1 μg/L	New Zealand

Source: Data from Ibelings et al. (2014).

blooms (which may or may not be feasible in the short term; see Chapters 7 and 8), shifting the raw water offtake to avoid blooms (Chapter 9) or implementing additional treatment steps (Chapter 10). Allowing such flexibility for the locally most effective response if a GV intended for lifetime daily exposure is exceeded is particularly pertinent to short-lived bloom situations if past experience shows that they are likely to disperse within a few days, thus no longer causing elevated cyanotoxin concentrations. The short-term GVs are intended for periods of about 2 weeks and are not intended to endorse repeated seasonal exceedances of the lifetime GV. Where water with concentrations ranging up to these values is distributed, it is important to inform the population about this situation so that specifically vulnerable groups may take specific measures, such as using bottled water. This may be relevant, for example, for hepatitis patients in the case of hepatotoxins and is particularly important for those responsible for bottle-fed infants because the short-term drinking-water GV is based on exposure of adults. Since infants and children can ingest a significantly larger volume of water per body weight (e.g., up to 5 times more drinking-water/kg bw for bottle-fed infants compared to an adult), as a precautionary measure WHO recommends that alternative water sources such as bottled water are provided for bottle-fed infants and small children when MC concentrations are greater than 3 μg/L for short periods (WHO, 2020).

5.1.2.1 Defining national or regional cyanotoxin levels requiring action

As discussed at the beginning of this chapter, when setting national standards or defining threshold concentrations that should trigger specific action, it is important to consider whether the WHO GVs given in Table 5.1 and used in the Alert Levels Framework (ALF) below are locally or nationally appropriate, or whether they would better be adapted to local or national circumstances. Besides differences in the ratios between toxin concentration and the indicators used to trigger the alert, such circumstances may include the amount of drinking-water consumed and the fraction of cyanotoxin allocated to uptake through drinking-water in relation to other exposure pathways (see sections 5.2–5.5). Further considerations include the extent and duration of cyanotoxin exposure in relation to other hazards: where public health impacts from exposure to other hazards (in particular pathogens) are substantial and toxic cyanobacterial blooms are short-lived events, a decision might be to tolerate somewhat higher concentrations (possibly only as an interim solution) in order to focus available capacity and resources on controlling exposure first to those hazards which are causing the highest risks for health. Such considerations are particularly important when setting national or local water quality regulations, because where other quality issues are likely to have a higher public health

impact, enforcing a low cyanotoxin standard may distract funding from investments needed to remediate the more pressing public health problems.

A number of countries have implemented concentrations triggering action for a range of cyanotoxins (see examples in Table 5.3). Particularly for cyanotoxins other than MCs, they have typically not been set as standards in the legal sense of values that all water suppliers in the country need to meet in order to be in compliance with regulations but rather guideline values (GVs) or "Health Alert Levels" that are used to trigger a notification to the health authority, further assessment of the situation and/or other management responses.

5.1.2.2 Alert Levels for short-term responses to toxic cyanobacteria in drinking-water supplies

An Alert Levels Framework (ALF) is a monitoring and management action sequence, presented as a "decision tree" in Figure 5.2, which water treatment plant operators and managers can use to provide an immediate, graduated response to the onset and progress of a cyanobacterial bloom. An ALF was first developed in Australia in the 1990s and then introduced in the first edition of "Toxic Cyanobacteria in Water" in 1999 (Chorus & Bartram, 1999). Since then, this approach has been widely used, typically with some adaptation to local or national conditions (Ibelings et al., 2014). Circumstances and operational alternatives may vary depending upon the source of the water supply, as well as the analytical and water treatment facilities available. The ALF presented here is therefore intended as a general framework, recognising that it may be appropriate to adapt specific Alert Levels and actions to suit local conditions. This includes the choice of parameters used to trigger alerts: as discussed at the beginning of this chapter and in more detail below, other parameters such as cell numbers or turbidity readings may be used if they are periodically "calibrated" against toxin concentrations.

One important aspect of an ALF for potentially toxic cyanobacteria is that this specific hazard often occurs with some predictability. In many surface waters, cyanobacterial blooms (and phytoplankton blooms in general) follow a seasonal pattern, or they occur following distinct events such as drought or heavy rainfall (highly dependent on local circumstances). It is therefore important to keep any records that are taken when following the ALF. These data can serve to significantly refine the ALF for individual water supplies (see also Chapter 10). This applies equally to patterns of spatial heterogeneity (see Chapter 3) of cyanobacterial blooms in individual waterbodies. The formation and location of surface scum can potentially be anticipated, although with some uncertainty, for a given waterbody. Since accumulations of cyanobacteria next to sensitive sites, such as raw water offtakes, are highly relevant, these sites need to be included in the ALF.

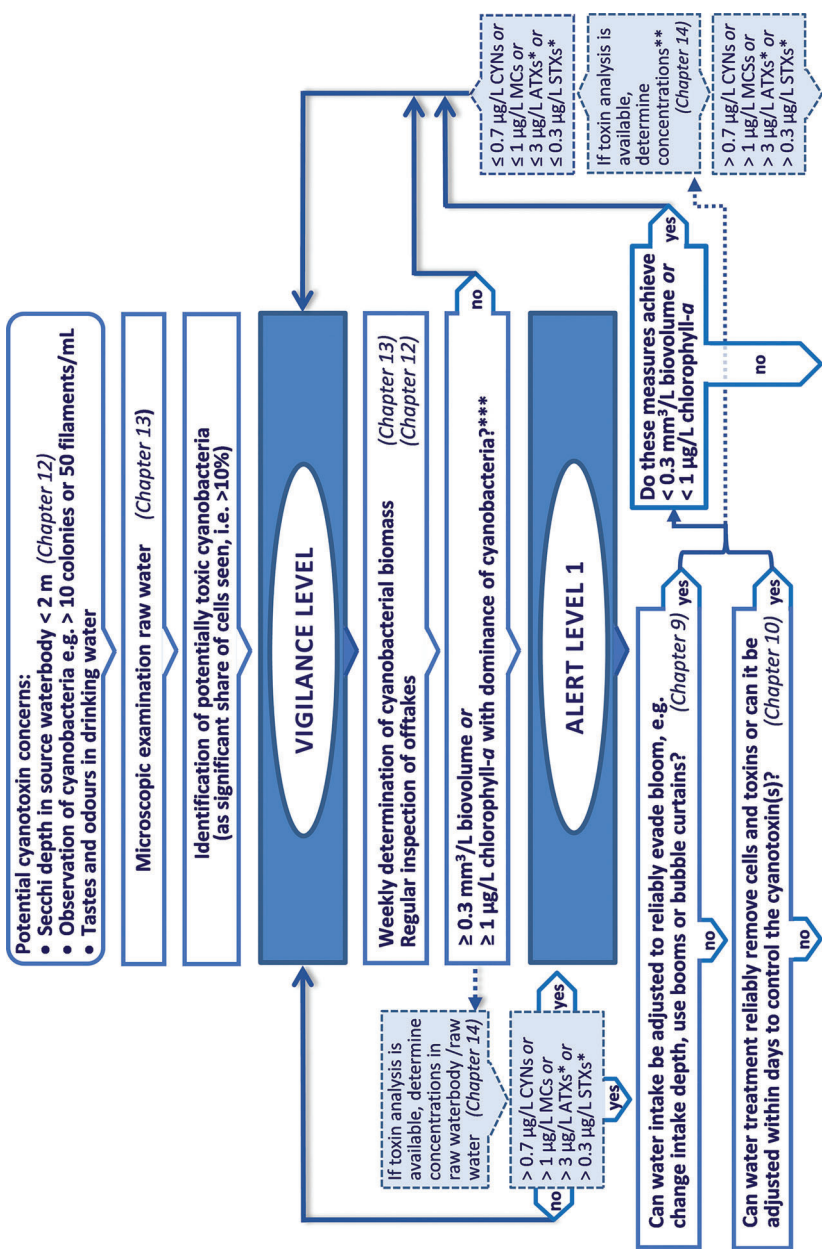
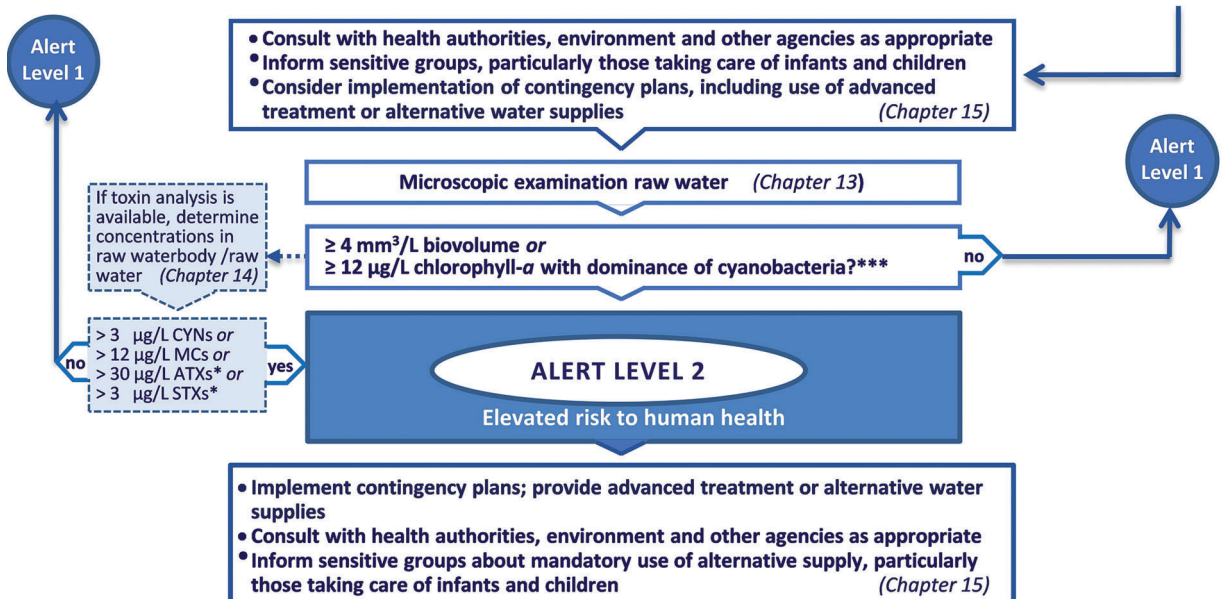


Figure 5.2 Alert Levels Framework (ALF): Decision tree for monitoring and managing cyanobacteria in drinking-water supplies (as template to be adapted to local conditions).

(*Continued*)



Note: As dissolved CYNs may be persistent, regular microscopy is particularly important to detect possible producer organisms and then trigger chemical analysis of CYNs (see Box 5.1); alternatively, monitor CYNs

* these values for STXs and ATXs are NOT Guideline values for lifetime exposure, but merely concentrations 10-fold below those for acute exposure (see text).

**analyse in raw water for checking effect of measures to avoid bloom intake and in treated water for checking the efficacy of treatment

*** if CYN- or STX-producers are dominant, analysing CYN/STX is recommended in order to determine whether Alert Levels for biovolume/chlorophyll *a* are sufficiently protective

Figure 5.2 (Continued) Alert Levels Framework (ALF): Decision tree for monitoring and managing cyanobacteria in drinking-water supplies (as template to be adapted to local conditions).

The ALF decision tree uses three "threshold" levels to guide the assessment of a potentially toxic cyanobacterial bloom, with appropriate actions and responses. The sequence of response levels is based upon the initial detection of cyanobacteria at the Vigilance Level, progressing to moderate to high cyanobacterial biovolumes and possible detection of toxins above lifetime GV concentrations at Alert Level 1. Alert Level 1 conditions require decisions to be made about the suitability of treated drinking-water, based on the efficacy of water treatment and – if access to toxin analysis is available – the concentrations of toxins detected.

An important issue regarding the parameters triggering immediate responses is confidence in the reliability of the data, particularly for toxin analyses. This is supported by quality assurance of the laboratories providing the data, for example, through accreditation or certification (see Chapters 11–14). At very high cyanobacterial biomass levels in raw water, the potential health risks associated with treatment system failure, or the inability to implement effective treatment systems at all, are significantly increased. This justifies progression to a situation of elevated risk, denoted by Alert Level 2 conditions. The framework has been developed largely from the perspective of the drinking-water supply operator, but is also important for the manager of the raw water supply. The actions accompanying each level cover different types of responses, such as additional sampling and testing, operational options, consultation with health authorities and informing the public through media releases. An important part of the framework at various stages is consultation with other agencies, particularly health authorities that generally have responsibility to oversee the safety of drinking-water.

The Vigilance Level encompasses the possible early stages of bloom development, when cyanobacteria are first detected in samples of the waterbody or raw water intake. Thresholds that may be used to trigger the Vigilance Level include elevated turbidity (e.g., Secchi depth readings of less than 2 m), detection of cyanobacteria by microscopy, particularly of potentially toxic species and, in some cases, musty tastes and odours. If the Vigilance Level is exceeded, it is appropriate to increase the sampling frequency of the raw water to at least once a week, so that potentially rapid changes in cyanobacterial biomass can be detected. In contrast, visible scums, particularly if associated with health complaints or animal deaths, immediately trigger Alert Level 1.

Elevated turbidity, with Secchi depth readings below 2 m due to greenish discoloration, or a correspondingly high online turbidity reading (e.g., at the raw water intake), serves as a first indication of bloom development, provided microscopic examination confirms this to be – at least partially – caused by cyanobacteria. Reduced water transparency can be seasonally caused by other phytoplankton, such as diatoms, green algae or euglenophytes. Therefore, for efficient management, microscopy skills and some taxonomic expertise (sufficient to recognise cyanobacteria on the genus

level; see Chapter 13) are highly valuable. The detection of more than 10 colonies, or more than 50 filaments, of a cyanobacterium per 1 mL water sample is suggested as the trigger value for the Vigilance Level, although this threshold may be adapted according to local knowledge and prior history of occurrence. Taste and odour may become noticeable in the supply as the cyanobacterial population develops above the Vigilance Level and thus serve as a warning signal if they do occur, but their absence does not indicate the absence of toxic cyanobacteria (see section 2.8).

Alert Level 1 thresholds are defined in terms of cyanobacterial biomass, estimated as a biovolume of 0.3 mm^3/L or alternatively as a concentration of chlorophyll-*a* in the range of 1.0 µg/L, provided this chlorophyll is largely from cyanobacteria (for details see below). This can be ascertained by using probes which also detect phycocyanin – a pigment only found in cyanobacteria – or by qualitatively checking with microscopy. Qualitative microscopy is recommended in either case for obtaining visual information about the phytoplankton composition and the genera of cyanobacteria present.

These biomass indicators correspond to cyanotoxin concentrations possibly above the lifetime GVs but most likely well below the short-term GVs (i.e., for ATX the health-based reference value and for STX the acute GV). Biomass levels up to those corresponding to these short-term values may be tolerated in drinking-water for up to 2 weeks, provided the situation assessment and remediation steps taken show that the situation will not last longer, the public is informed and remediation measures are initiated. As discussed above, this approach provides important leeway for effective management: provided cyanotoxin concentrations stay below the Alert Level 2 thresholds, funds available may thus be focused on establishing remediation measures that avoid blooms or on bringing concentrations in finished waters back to below the lifetime guideline value (GV), rather than investing into short-term measures such as the provision of bottled water for the general population or expensive temporary technical remediation measures. Note that, as mentioned above, information to sensitive groups and those taking care of bottle-fed infants is important under Alert Level 1 conditions.

For CYN concentrations, cyanobacterial biomass can be a poor indicator, as (in contrast to MCs, ATXs and STXs) a large fraction of this toxin often occurs extracellularly and (in contrast to, e.g., ATXs) degradation in water may be slow, particularly at low temperatures (Chapter 2). Therefore, if CYN producers (e.g., *Raphidiopsis raciborskii* in the Americas and Australia and *Aphanizomenon* spp. in Europe) are, or have been, present, analysis of CYNs is recommended (see Box 5.1). Regular phytoplankton monitoring (visual, via qualitative microscopy) is important for identifying such situations.

Actions to take under Alert Level 1 include an assessment as to whether water treatment plant intakes can be adjusted or other physical actions can be implemented to reduce the cyanobacterial challenge; whether the water treatment system(s) available are effective in reducing toxin concentrations

to acceptable levels (see Chapter 10) and whether waterbody conditions render a prolonged bloom likely or it is rather expected to be an occasional, short-lived event (Chapters 7 and 8). Cyanotoxin analysis of the raw and treated water (see Chapter 14) will allow a better assessment of the situation, potentially including adapting the biomass indicator values to the toxin content of the local bloom (see below). Alert Level 1 should further trigger an assessment of longer-term options to reduce the concentration of potentially toxic cyanobacteria in the raw water supply by measures in the catchment (see Chapter 7), in the waterbody (see Chapter 8) or in offtake management (Chapter 9).

Alert Level 1 conditions further require consultation with health authorities for ongoing assessment of the status of the bloom and of the suitability of treated water for human consumption. This consultation is best initiated early and should continue after the results of toxin analysis on drinking-water become available. Clearly, as the biomass of potentially toxic cyanobacteria increases in the raw water, so does the risk of adverse human health effects, particularly if water treatment systems are insufficient or other physical measures such as water treatment plant intake adjustments are not available or sufficiently effective. Therefore, ongoing monitoring for cyanobacterial biomass and, where possible, of toxin concentrations is important. It may also be appropriate to extend the monitoring programme, which should be at least weekly in frequency (in hot climates possibly more). Monitoring should be designed to establish the spatial variability of the cyanobacterial population and of toxin concentration (see Chapters 4 and 11).

An Alert Level 1 situation requires extensive public communication, particularly about the rationale for transiently tolerating levels above the lifetime GVs. Easing possible concerns of the public may be very important during phases with cyanobacterial biomass or toxin concentrations between the lifetime and short-term GVs. Media releases and even direct contact with consumers via letterbox delivery of leaflets with appropriate advice to householders may be appropriate (see Chapter 15 for further guidance). It may also be important to explicitly inform government departments, authorities and stakeholders with possible interests or legal responsibilities (beyond informing the health authority directly responsible for the surveillance of the water supply). Stakeholders may range from farmers needing information about possible impacts on livestock potentially exposed to blooms to organisations or facilities that treat or care for special "at-risk" members of the public (such as kidney dialysis patients, see section 5.4 or paediatricians and other health organisations advising parents of bottle-fed infants). Chapter 15 gives guidance on public communication.

If Alert Level 1 conditions continue, but toxins or toxicity are not detected in cyanobacterial or raw water samples, regular monitoring should nonetheless continue to ensure that toxic strains or species do not develop over ensuing weeks or months.

Alert Level 2 thresholds are defined as cyanobacterial biomass levels at ≥ 4 mm^3/L biovolume, or ≥ 12 µg/L chlorophyll-*a* (preferably with the presence of toxins confirmed by toxin analysis), and describe an established toxic bloom with rather high biomass and an elevated probability of scums. For CYNs, the caveat is – as for the Alert Level 1 threshold – the persistence of dissolved toxin, and regular microscopy is important to ensure that occurrence of possible producer organisms is detected on time to trigger chemical analysis of CYNs (see Box 5.1); alternatively, CYNs may be regularly included in the sampling programme.

In the Alert Level 2 situation, the sampling programme will have indicated that the bloom is widespread. Conditions in Alert level 2 correspond to cyanotoxin concentrations that may exceed even the short-term guidance values given in Table 5.1 and thus indicate an increased risk of adverse human health effects. Once the Alert Level 2 threshold is exceeded, an alternative water supply or effective water treatment system becomes urgent, as does ongoing monitoring of the performance of the system in place to control toxin concentrations.

Filtration systems (possibly combined with flocculation–coagulation) may remove cell-bound toxins, whereas dissolved toxin is likely to break through and require advanced treatment (see Chapter 10). If advanced treatment is not available or not sufficiently effective, Alert Level 2 conditions should result in the activation of a contingency water supply plan which is appropriate for the operator and the users or community. This may involve switching to an alternative supply for human consumption, the implementation of contingent treatment systems or, in some circumstances, the delivery of safe drinking-water to consumers by tanker or in bottles. While hydrophysical measures to reduce cyanobacterial growth or intake into the drinking-water system may still be attempted in this phase, application of algicides runs the risk of exacerbating the problem by causing high concentrations of dissolved toxins as a consequence of cell lysis (see Chapter 8).

Where advice is provided to the public not to drink water because of a cyanobacterial hazard to human health, it will usually emphasise that the water is still suitable for purposes such as washing, laundry and toilet flushing. Complete withdrawal of a piped drinking-water supply because of a cyanobacterial toxin hazard is not an option because the adverse health effects resulting from the disruption of supply (e.g., lack of water for toilet flushing, personal and household hygiene and in some situations also for firefighting) are likely to substantially outweigh the likely impact of the cyanobacterial toxin risk itself.

Monitoring of the bloom should continue in order to determine when the bloom starts to decline and normal supply can be resumed. The sequence at Alert Level 2 may follow through to deactivation of Alert Level conditions with media releases as well as advice to government departments and health authorities to confirm this. The collapse of a bloom, or a management

action such as the flushing or mixing of a reservoir (Chapter 8), may lead to a rapid decline from Alert Level 2 back to Alert Level 1 or below.

Likewise, the sequence might escalate rapidly, bypassing Alert Level 1 to Alert Level 2, particularly if adequate monitoring and early warning information are not available. Cyanobacterial populations in natural waterbodies may increase by two- to threefold within 2 days (growth rate, $\mu = 0.3$/d; see Figure 5.3), especially in hot climates. Monitoring frequency needs to take such potentially rapid population growth rates into account.

The basis for deactivating Alert Level 2 and reverting back to Alert Level 1 or the Vigilance Level will depend on how it was triggered. If it was triggered by biovolumes or chlorophyll-*a* without cyanotoxin analyses, then deactivation can be based on biovolumes or chlorophyll-*a*. If cyanotoxin concentrations have been determined, these take precedence and Alert Level 2 should only be deactivated once the cyanotoxin concentrations have declined below the short-term guideline values (GVs).

5.1.2.3 *Considerations for choosing parameters to trigger Alert Levels when adapting the Framework to local circumstances*

The Alert Level Framework (ALF) proposed here focuses on indicators for which analytical methods are likely to be more readily accessible than for toxin analyses, that is, visual inspection (Secchi depth reading; scums) and cyanobacterial biomass. Their choice can be adapted as is nationally or locally practical: for example, measuring turbidity in the raw water entering

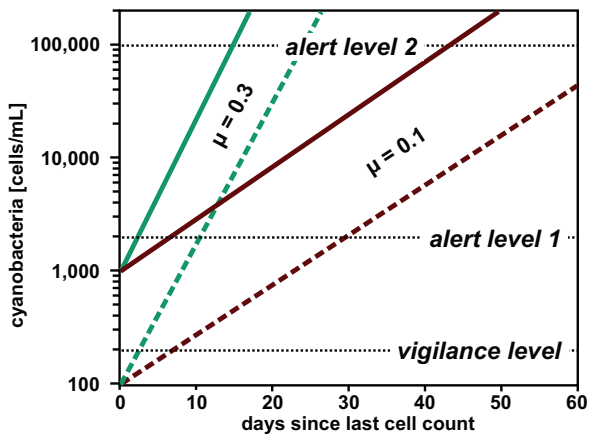


Figure 5.3 Predicted development of cyanobacterial population from initial concentration of 100 (dotted dashed lines) or 1000 (solid lines) cells per mL at exponential growth rates (μ) of 0.1 (dark lines) and 0.3 (light lines) per day. (Modified from Jones, 1997.)

a drinking-water treatment system (e.g., online) can replace measuring transparency in the waterbody with a Secchi disc. Cyanobacterial biomass is best determined as biovolume or, alternatively, as chlorophyll-*a* (in the latter case combined with qualitative microscopy); analysing both is not necessary and which of the two to choose will depend on locally available expertise and instrumentation. Also, techniques such as fluorescence probes (online or handheld), remote sensing (via satellite images, airplanes or drones), cell counts or molecular analyses for toxin-production genes may be used in a local adaptation of the ALF, provided the signals are "calibrated" with data from local sampling programmes and they depict local cyanobacterial biomass sufficiently well to be used as triggers in an ALF (see Chapter 13 for methods).

Alternatively, it is possible to analyse cyanotoxins directly if methods are accessible (see Chapter 14 for methods). However, including a biomass parameter to trigger action in the ALF offers the further advantage of encompassing any hazard caused by a cyanobacterial bloom: as discussed in section 2.10, while cyanotoxin concentrations below the trigger values given in the ALF imply a low health risk from exposure to cyanotoxins, blooms may contain further, yet unknown substances and/or organisms that may be hazardous. It is therefore prudent to avoid exposure to high concentrations of cyanobacterial biomass even if concentrations of the known cyanotoxins are low.

However, for any parameter used to trigger Alert Levels – including cyanotoxins – other than cyanobacterial biovolume, it is strongly recommended to include qualitative or semiquantitative microscopy in order to collect information on the dominant cyanobacterial genera in the waterbody. This is particularly important for timely recognition of possible CYN occurrence, as concentrations of dissolved CYNs do not relate to biovolume or chlorophyll-*a* (as measures of biomass) as immediately as do other cyanotoxins (see Box 5.1), but observing substantial amounts of potential CYN producers should trigger targeted analysis of CYNs. While identifying cyanobacterial species is often described as intimidating, as discussed in Chapter 12, identification on the genus level already provides highly valuable information, often quite sufficient for assessing the situation, and this is readily learnt by staff with some experience in microscopy. An understanding of the dominant cyanobacterial genera is also important for estimating their distribution in the waterbody as well as their likely responses to measures for control and remediation discussed in Chapters 7–9.

5.1.2.4 Considerations for setting the ALF thresholds and adjusting them to local circumstances

The value for chlorophyll-a at Alert Level 2 given in Figure 5.2 is now substantially lower than the values given for Alert Level 2 in the 1999 edition of this book. This is because the GVs for short-term exposure (Table 5.1) are now available (WHO, 2020), and Alert Level 2 should reflect the risk

of exceeding these: at biomass concentrations up to Alert Level 2, that is, 4 mm^3/L biovolume or 12 µg/L chlorophyll-*a*, it is highly unlikely that concentrations of MCs can significantly exceed the provisional short-term guideline value (GV). Nor is it likely that concentrations of STXs can exceed the acute GV for STXs, or concentrations of ATXs exceed the health-based reference value for ATXs (see Chapter 2 for an explanation of these values). The same applies to the Alert Level 1 values of 0.3 mm^3/L biovolume or 1 µg/L chlorophyll-*a*: these are sufficiently conservative to maintain concentrations of MCs below the provisional lifetime GV, and the same applies to CYNs if monitored as described in Box 5.1.

For STXs and ATXs, the rationale for Alert Level 1 is different as no GVs for lifetime exposure are available. The Alert Level 1 value of 0.3 µg/L STX is merely 10-fold lower than the acute GV with the function of serving as a trigger for increased vigilance to avoid reaching the acute GV. This applies equally to the value of 3 µg/L proposed for ATX as a trigger in Alert Level 1: this is also not a toxicologically derived lifetime GV but merely a value set to be 10-fold lower than the proposed Health-based Reference Value as a trigger for increased vigilance (Table 5.1).

This is relevant because a further rationale for the thresholds proposed for Alert Level 1 is the potential for rapid exponential increase once cyanobacteria have been detected at this threshold level: even if the toxin content of the cells is substantially lower, concentrations in the water can increase exponentially as cells divide exponentially and thus reach levels exceeding Alert Level 1 within a few days: Figure 5.2 gives an indication of the rate of change of an exponentially dividing population at two growth rates typically observed in field studies of cyanobacteria (in the field, growth rates rarely exceed 0.3 per day).

Furthermore, as discussed at the beginning of this chapter, the Alert Levels proposed for biovolume and concentrations of chlorophyll-*a* are based on the upper range of cyanotoxin content typically found in cyanobacterial cells in the field (discussed in section 4.6.2), that is, on worst-case assumptions for the ratio of toxins to biovolume or chlorophyll-*a*. In many field situations, cyanotoxin concentrations will be lower, possibly by a factor of 10. It is therefore useful to support the assessment by analysing for the presence of cyanotoxins, and if their concentrations prove lower than the Alert Level values, this may revert the situation back to a lower level. Also, if the toxin content of the local cyanobacterial population is well understood, other, often higher Alert Levels may be set for biovolume or chlorophyll-*a*. In that case, checking the cyanotoxin content of the cyanobacterial population would remain necessary at larger intervals, for example, 2–3 times per season or monthly; however, for the more frequent monitoring between those occasions (e.g., weekly, daily or – with probes – continuously) biovolume or chlorophyll-*a* is likely to be sufficient.

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5.2 RECREATION AND OCCUPATIONAL ACTIVITIES

Ingrid Chorus and Emanuela Testai

Recreational activities may be a significant route of exposure to cyanotoxins. Throughout the world, the range and scope of recreational water activities vary as widely as does access to recreational waterbodies and their propensity to be impacted by cyanobacteria blooms. Where cyanobacterial blooms are pronounced and water sports are nonetheless popular, recreational activities are likely to be a major route of exposure to cyanotoxins. Occupational activities using cyanobacteria-affected waters may lead to similar patterns of cutaneous and inhalational exposures to cyanotoxins, though opportunities are available to reduce exposure through the use of personal protective equipment and other occupational management strategies. Understanding the usage patterns of untreated surface water is therefore fundamental for assessing exposure.

Scums of cyanobacteria in lakes and rivers used for recreational purposes have been well recognised as a public nuisance. Moreover, deaths of livestock, wild animals or pets have been observed after exposure to cyanobacteria. Such incidents raise the question whether affected waterbodies are safe for recreational use. Sometimes blooms are associated with unpleasant odours and a degraded appearance of lake shores, especially when scums aggregate and decay. Swimmers and other water users may avoid areas with extensive cyanobacterial scums or accumulated detached mats because of the obviously unpleasant environment, particularly when associated with related fish-kills.

However, sensory responses and reactions to cyanobacteria blooms vary. The smell of some blooms is not necessarily unpleasant, but more like freshly-mown grass, and some observers have described waters vividly coloured by blue-green cyanobacterial blooms as looking beautiful. Multiple anecdotal observations of children and adults playing with scum material have been reported (Figure 5.4). Where alternative recreational sites without cyanobacterial blooms are lacking and the demand for recreational water access is high, visual and olfactory amenity tend to be of lower priority, and people may tolerate water quality conditions that might otherwise discourage them from using the site. This has been observed in numerous countries, for example, in many parts of inland Australia that are subject to water scarcity, in arid regions of Hungary where few waterbodies are available for recreation, and in north-western Germany where for decades the majority of waterbodies were heavily eutrophic. In some regions in which cyanobacterial blooms have become a widespread phenomenon for more than a generation, site visitors have come to accept the degraded water quality as “natural” or “normal” for the region. In temperate climates, cyanobacterial dominance is most pronounced during the summer months, when the demand for recreational water is highest.

Figure 5.4 Playing children are particularly at risk to be exposed to critical quantities of cyanotoxins. (Kindly provided by Yora Tolman.)

Various enterprises may use untreated water from cyanobacteria-affected surface waters for a wide range of processes that can result in occupational exposure to cyanotoxins: for example, cooling in production processes, dust suppression by spraying, spray irrigation, workers exposed to raw water spray in waterworks or cooling of enclosed or semienclosed workspaces. Cell lysis and, in consequence, liberation of cell-bound toxins may be caused by pressure and shear stress during pumping. Occupational exposure may also occur through work directly in or on waterbodies affected by scums. Marine blooms of filamentous *Moorea* species (previously known as *Lyngbya majuscula*) can dry on fishing nets, and contact with fresh and dried material has caused severe skin reactions as well as breathing difficulties for workers in the fishing industry (Grauer & Arnold, 1961; Osborne et al., 2001).

Potential routes of occupational exposure to cyanotoxins include direct contact via exposed parts of the body and cell material trapped under clothing, accidental swallowing of contaminated water and inhalation. While some exposure pathways at workplaces are similar to those experienced during recreation, a difference may be longer and more frequently repeated exposure periods in occupational settings. Uncharacterised water supplies may contain further hazardous agents, and skin abrasion by protective clothing, potentially augmented in heat and by moist skin, may increase exposure.

Occupational settings may also involve a risk of exposure via drinking-water through cross-contamination of the potable water supply if this is not effectively separated from the process water or subject to poor labelling of pipework and fittings or poor process design and control. Where temperatures are high (e.g., >35 °C in some agricultural situations or in open-cast mines), poor access to potable water in sufficient quantity and proximity to the workplace may increase the risk of untreated water – potentially containing not only cyanotoxins but also pathogens and other hazards – being used for drinking.

Recreational and occupational exposure may be to whole cyanobacterial cells, lysates, dried cells or mixtures of these forms. Where blooms are of concern, water containing them may well contain further hazards, particularly microbial pathogens.

5.2.1 Evidence of health effects associated with exposure to cyanobacteria in water used for recreation or at workplaces

While reported concentrations of cyanotoxins in drinking-water are rarely found above the low microgram per litre range (see section 5.1), contact with scums through recreational activities more frequently results in exposure to cyanotoxin concentrations in a range of up to milligrams per litre (see Chapter 2), and acutely hazardous exposure is a realistic scenario if site users ingest scum. Evidence of health effects from recreational exposure has been published mainly as anecdotal reports, case studies and from epidemiological studies.

5.2.1.1 Case reports of short-term health effects from acute exposure

A number of published case reports of illness after exposure to cyanobacteria during recreation have been widely quoted to illustrate the relevance of this pathway. As discussed at the beginning of this chapter, in most of the published cases, the presence of infectious pathogens cannot be unambiguously excluded, and it is typically unclear whether the symptoms reported were caused by the known cyanotoxins or by other components of the bloom, including the possibility of yet unknown cyanobacterial metabolites. For example, enteritic viral or parasite pathogens may well have been present even where bacterial indicators were reported to have been absent. The case in Box 5.4 shows that later availability of new analytical methods can support or exclude cyanotoxins as cause if sample material is still available.

BOX 5.4: HUMAN MORTALITY FROM ACCIDENTAL INGESTION OF TOXIC CYANOBACTERIA – A CASE RE-EXAMINED

Wayne W. Carmichael

In July 2002, a 17 year-old male was taken to a local hospital emergency department in full cardiopulmonary arrest following an episode of vomiting and diarrhoea followed by seizure at his home. The patient, an athletic otherwise healthy individual, had no previous history of seizures, syncope

or diarrhoeal illness. Extensive resuscitation efforts failed and the patient expired in the emergency department. An autopsy was performed the following day to determine the cause of death. After ruling out several possible aetiologies for death, including toxic chemicals and pathogenic microbes, the possible role of cyanotoxins was pursued since the youth was reported to have accidentally ingested water while swimming in a local golf course pond, about 2 h prior to symptoms, that was described as "dirty and scummy". Unfortunately, because cyanotoxins were considered as possible cause only late in the course of the investigation, no samples were taken from the pond. Samples of the youth's blood, liver and vitreous fluid were tested for MCs, STX, ATX and CYN. In addition, stool collected from autopsy was examined for the presence of cyanobacterial cells. ELISA was negative for microcystins and LC/MS analyses was negative for STX and CYN. ESI LC/MS did reveal a strong peak with *m*/*z* 166 with a retention time of 9.08 min, "similar"' to that of anatoxin-a, 8.51 min. This evidence allowed an initial listing of this cyanotoxin as a possible cause of death. Further analyses showed, however, that this peak with *m*/*z* 166 is not anatoxin-a but the ubiquitous amino acid phenylalanine.

In consequence, this example of a false-positive investigation of mortality from anatoxin-a should now be considered one of unknown cause.

A number of reports contain substantial evidence of the uptake of cyanobacteria and a likely connection to the symptoms observed:

- Dillenberg and Dehnel (1960) reported a case series of illness in 13 persons after swimming at various bloom-affected Canadian lakes (despite warnings posted following animal deaths); symptoms included headache, nausea, vomiting, painful diarrhoea, arthralgia and myalgia (i.e., pain in joints and muscles). Stool samples from two of the more severely affected individuals, one of whom was hospitalised overnight, were sent to the Saskatchewan public health laboratories, where *Microcystis* cells were identified in the specimens.
- Turner et al. (1990) reported that 10 out of 18 army recruits fell ill after training exercises involving canoeing – including practicing Eskimo rolls – in a waterbody affected by a *Microcystis* bloom, with two soldiers needing hospitalisation for a week because of severe atypical pneumonia and generalised illnesses. The authors suggested that inhalational exposure to cyanotoxins, especially to microcystin, may have been the probable cause, although that assertion has been challenged by others. This was the incident that first triggered wider attention to cyanobacterial toxicity in humans.

- In Argentina, a teenage jet-ski rider was hospitalised for several weeks, including an 8-day period in an intensive care unit during which time he required artificial ventilation. Acute respiratory symptoms were followed by hepatic insufficiency, which was essentially self-limiting. The presumed aetiologic agent was a microcystin-producing bloom of *Microcystis*, which was present as heavy scum in the dam at the time the young man spent several hours on and in the water (Giannuzzi et al., 2011).
- In Uruguay, a 20-month-old child suffered acute liver failure after repeated recreational activity at a beach of the Rio de La Plata River (Vidal et al., 2017) in January 2015. During this month, the river had a pronounced bloom of *Microcystis* sp. and microcystin concentrations up to 25 700 µg/L were reported in scum material. The child and her family first showed gastrointestinal symptoms a few hours after the final exposure, but she also developed jaundice and increased serum levels of liver enzymes as well as a need for mechanical respiratory support. A liver transplant was performed after 20 days, and microcystins were detected in the removed liver in concentrations up to 78 ng/g of tissue, which is in the range of the concentrations found in livers of the Caruaru victims (discussed in section 5.4). While the authors explicitly do not exclude other factors, for example, autoimmune hepatitis type II as cause (possibly triggered by the exposure to microcystins), they identify a high plausibility of direct damage through the repeated exposure to an estimated total of at least 1.78 L of microcystin containing water over a few days.
- In a review of CDC's Waterborne Disease and Outbreak Surveillance System in the USA in 2009–2010, 11 outbreaks were associated with cyanobacteria. In 70% of cases, health effects were associated with the major exposure route: rash, irritation, swelling or sores were reported in those outbreaks where exposure occurred mainly through dermal contact while gastrointestinal symptoms were reported after water ingestion. The outbreak with the more severe gastrointestinal and neurologic symptoms (one of the two hospitalisation cases) was characterised by the highest levels of MCs (>2000 µg MC-LR eq/L) and 9, 15 and 0.09 µg/L of CYN, ATX and STX. In the three cases in which ATX and STX were present, neurologic symptoms or confusion/visual disturbance were reported in addition to fever, headache and eye irritation. However, in all three cases, microcystins were also detected at often substantially higher concentrations (0.3–>2000 µg/L), and in one of them, CYN and STX were also present (Hilborn et al., 2014).

For assessing cases such as these, it is important that mere co-occurrence of cyanotoxins and unspecific symptoms (skin irritation, gastrointestinal,

etc., see above) is not indicative of the known cyanotoxins having caused the symptoms; more likely the cyanobacterial biomass contains both toxins and other, yet unknown agents causing such general symptoms. In contrast, cause–effect relationships are likely if symptoms or analytical results are toxin-specific (e.g., for hepatotoxins elevated serum enzyme levels such as gamma glutamyl transferase; for neurotoxins respiratory difficulties, tingling of extremities, confusion or visual disturbance). While finding cyanotoxins in body fluids of patients and/or cyanobacterial cells in their stool confirms exposure, even this does not allow the conclusion that these were the cause of symptoms, as it is currently unknown how concentrations in serum relate to damage in the liver, for example.

Regarding occupational exposure, two studies have been undertaken by the mining industry in Australia. The Australian Coal Association Research Programme projects (Fabbro et al., 2008; Fabbro et al., 2010) investigated cyanobacteria and their toxicity in various waterbodies available to industry in Central Queensland, Australia, a semiarid region with a history of cyanobacterial blooms. Of the 180 samples tested for toxin, 17% contained CYN and 3% contained microcystin. Total CYN concentrations (CYN plus deoxycylindrospermopsin) ranged from 0.2 to 22.1 μg/L. Microcystin concentrations ranged from 1.7 to 3200 μg/L. Concentrations of toxin-producing cyanobacteria (*Dolichospermum circinale*) as high as 500 000 cells/mL were recorded from pit water (Fabbro et al., 2008). Workers can potentially have direct contact with pit water when installing pump facilities or when it is used for dust suppression, cooling or wash down. This research also provided the initial identification of novel toxicity associated with *Limnothrix/Geitlerinema* (Fabbro et al., 2010; Bernard et al., 2011; Humpage et al., 2012).

Other anecdotal and case reports of varying reliability describe acute gastrointestinal and respiratory illnesses associated with activities such as waterskiing (likely forming aerosols and spray) in recreational waters contaminated by cyanobacteria (reviewed in Stewart et al., 2006d), including a report of a windsurfer in the UK with hepatic dysfunction diagnosed by liver function tests and liver biopsy (Probert et al., 1995). In only a small proportion of such anecdotal reports documented in the biomedical literature were the subjects examined by medical practitioners. Anecdotal reports of illness are occasionally reported in local broadcast or print media, and some descriptions of the number and type of complaints received by public health authorities can be found in overview publications (see, e.g., Backer et al., 2015). A report from the US State of Nebraska recorded more than 50 complaints of skin eruptions, vomiting, diarrhoea and headache after swimming or waterskiing at a cyanobacteria-affected lake over a single summer weekend (Walker et al., 2008).

Severe skin reactions have been reported from contact with marine cyanobacteria, particularly with *Lyngbya majuscula* (now termed *Moorea*

producens), which causes deep blistering particularly when trapped under bathing suits and where blooms have contained the toxins lyngbyatoxin A and debromoaplysiatoxin (see section 2.6). Severe dermatitis, resembling skin burns, has been reported from marine bathing in the presence of cyanobacteria dislodged from rocks, particularly after storms in tropical seas (Hashimoto et al., 1976; Moore et al., 1993). *Lyngbya/Moorea* has been recorded in many marine ecosystems worldwide, but is most common in tropical/subtropical locations. Intoxication events have been reported primarily in midsummer when both numbers of people engaged in recreational activities and the potential for bloom formation are high. Reports are chiefly from economically more developed countries, potentially due to a recording bias, and often include multiple morbidities.

Complaints of acute skin reactions have been associated with exposure to freshwater cyanobacteria as well as with eukaryotic microalgae; however, cyanobacteria are the focus of the majority of these reports (Stewart et al., 2006c) with clinical investigations suggesting allergic responses (Cohen & Reif, 1953; Stewart et al., 2006a; Stewart et al., 2006b; Geh et al., 2016). Two reports focus on the pigment phycocyanin as a suspect allergen (Cohen & Reif, 1953), and indeed a case investigation of anaphylaxis following consumption of *Spirulina* in tablet form (Petrus et al., 2009) and clinical laboratory allergy studies identified phycocyanin as an allergen (Geh et al., 2015; Lang-Yona et al., 2018). However, this requires further clarification as it would contradict other reports assigning antiallergic, anti-inflammatory and antioxidant properties to phycocyanins (Strasky et al., 2013; Liu et al., 2015; Wu et al., 2016). Investigators conducting epidemiological fieldwork at cyanobacteria-affected waters have received a small number of anecdotal reports from individuals with a history of allergy, though the association between anticipated symptom occurrence and cyanobacteria in such cases remains speculative. The possibility of serious anaphylactic reactions has been raised for some benthic cyanobacteria (Stewart et al., 2011). Thus, while allergic responses to some cyanobacteria are discussed in the literature, their relevance remains unclear.

A widespread problem that case studies, such as those discussed above, face is that in the course of steps taken to elucidate the possible cause of the observed symptoms, cyanobacteria are typically considered only rather late. If many days pass between symptom observation and sampling the water to which patients were exposed, a bloom may already have disappeared and the chance for establishing a causal connection is missed. This is true in particular for surface blooms or scums which can disperse within a few hours, for example, due to increased wind. Informing the medical community about toxic cyanobacteria may help to reduce the time between exposure and water sampling as well as to document the situation at the time of possible exposure, for example, with images taken with mobile phones.

5.2.1.2 Epidemiological studies of acute health risks from short-term recreational exposure

Several epidemiological studies investigating acute illness following recreational exposure to freshwater cyanobacteria have been conducted between 1990 and 2011. These studies utilised various retrospective and prospective designs capable of detecting relative differences in commonly reported symptoms between exposed and unexposed groups; however, levels of exposure were usually poorly characterised, and hence, these studies are inadequate for risk assessment purposes. Symptoms assessed included both cutaneous and systemic reactions – the statistical analyses of the studies do not differentiate between both.

- Philipp and coworkers conducted the first three formal epidemiological investigations into recreational exposure to cyanobacteria: These comprised a series of cross-sectional studies conducted in 1990 at inland waters in the UK, affected some weeks earlier by cyanobacteria blooms. They found only minor illnesses, with no statistically significant differences between symptoms reported by exposed and unexposed groups (Philipp, 1992; Philipp & Bates, 1992; Philipp et al., 1992).
- A retrospective study conducted in Australia in response to an extensive bloom of *Anabaena circinalis* in the River Murray in South Australia also did not detect any statistically significant increase in symptoms between those exposed to river water during recreational activities and nonexposed controls (El Saadi et al., 1995).
- Pilotto et al. (1997) conducted a prospective cohort study in 1995 at recreational waters in southern and south-east Australia and reported a statistically increased likelihood of symptom reporting compared to unexposed controls after 7 days (but not after 2 days) following exposure to low levels of cyanobacteria (5000 cells/mL) for more than 1 h. The cohort size for the statistically significant finding was small, comprising 93 exposed and 43 unexposed subjects.
- Stewart et al. (2006c) conducted a larger prospective cohort study in Australia and the USA and detected a statistically significant increase in symptom reporting, particularly respiratory symptoms, three days following exposure. These authors used multivariable analysis after adjusting for confounding variables such as age, smoking, geographic region and a prior history of allergic disease. Increased symptom reporting rates were seen only at higher cyanobacterial densities, using a biomass estimate of exposure, and symptom severity was rated as mild by most study subjects. These associations were linked to cyanobacterial cell densities higher than 100 000 cells/mL

- Two prospective cohort studies conducted in the USA by Backer et al. (2008; 2010) found no relationship to symptom reporting and exposure to microcystins, as measured by ELISA and LC-MS in lake water, aerosols and blood.
- Lévesque et al. (2014) conducted a prospective cohort study of residents living near three lakes in Quebec, Canada, which had a history of being impacted by cyanobacteria, one of which is also used as source for drinking-water. Exposure to cyanobacteria included a range of recreational water activities, drinking-water (for residents living near the lake with drinking-water abstraction from the lake) and consumption of fish from study lakes. Recreational exposure to cyanobacteria was associated with increased reporting of gastrointestinal symptoms; 466 individuals were enrolled in the study, although the number of subjects that engaged in recreational activities was not reported. The authors reported a strong statistically significant relationship between gastrointestinal illness and exposure to cyanobacterial cells above 100 000 cells/mL.

Most of the symptoms reported in these studies are mild and self-limiting. In contrast, the toxicological considerations discussed in section 5.2.3 show that serious morbidity or death through oral uptake of toxin is a realistic scenario in recreational water settings, if larger amounts of a highly toxic bloom are ingested. While the case study from Uruguay (Vidal et al., 2017) provides supporting evidence that they may occur, such events are, however, probably rare, and with the possible exception of the case–control design adopted by El Saadi et al. (1995), the prospective and retrospective epidemiological studies discussed above were not designed to detect the impact of massive oral exposure to high toxin concentrations.

The "gold standard" epidemiological design, a randomised controlled trial, could in theory be employed to investigate exposures and outcomes from oral consumption of cyanotoxin-contaminated recreational water, but this could not be done in practice on ethical grounds and would be logistically challenging. Future epidemiological investigations that seek to document events of severe acute illness following oral ingestion of cyanotoxin-contaminated waters would probably need to employ a case–control design. An advantage of these studies is that outcome data is ascertained by medical practitioners; however, disadvantages include exposure recall bias and recruitment of appropriate control groups (Stewart et al., 2006c). El Saadi et al. (1995) also alluded to difficulties in gaining cooperation of diagnosing practitioners.

In contrast to the limitations of field epidemiology, clinical studies overcome the reliance on self-reporting of symptom occurrence, severity

and duration. The diagnosis and history of acute intoxication or allergic response to cyanobacteria and/or cyanotoxins is likely to be more reliable when conducted by expert clinicians, particularly when clinical histories and examinations can be supported by confirmatory or complementary diagnostic tests. Early clinical investigations, and in some cases desensitisation treatments, were concerned with allergic reactions to cyanobacteria in recreational waters (reviewed in Stewart et al., 2006c), and more recent clinical studies have addressed the topic of cutaneous and respiratory reactivity to cyanobacteria (Pilotto et al., 2004; Stewart et al., 2006a; Bernstein et al., 2011). The results of these clinical investigations confirm the case study reports discussed above that certain freshwater cyanobacteria can elicit hypersensitivity reactions in some individuals.

5.2.1.3 Responses to presumed cyanotoxin-related acute illness following exposure

With increasing public information and awareness of cyanotoxin occurrence, it is possible that more individuals will consult medical services if they develop symptoms after exposure – symptoms which not necessarily are caused by cyanobacteria and their toxins. However, particularly where symptoms set in rapidly, that is, within only a few hours after exposure, intoxication should be a diagnostic consideration. Medical consultation will primarily serve to clarify and treat symptoms. Although very few cases are known to date, patients may present with concerns of intoxication after exposure to scums or high concentrations of suspended cyanobacterial cells. For neurotoxins, these would be associated with symptoms of respiratory distress, and urgent respiratory support, including supplementary oxygen therapy, would be the appropriate response. Concerns about possible liver damage from microcystins or cylindrospermopsin after exposure can be met by surveillance of serum parameters reflecting liver function, particularly markers of acute injury such as hepatic transaminases.

Beyond this primary function, however, reporting such cases to public health authorities is helpful for promoting the understanding of the public health impact of recreational exposure to (toxic) cyanobacteria. As discussed above, analysis of water samples for cyanobacteria and cyanotoxins very soon after exposure would be most useful, and to make this happen, it is important that medical services or public health authorities trigger such action. Specific biomarkers of exposure to cyanotoxins are not routinely available, but a range of diagnostic criteria may be applied to support the identification of possible cyanobacterial intoxication (Box 5.5).

BOX 5.5: DIAGNOSTIC CRITERIA TO SUPPORT THE IDENTIFICATION OF POSSIBLE CYANOBACTERIAL INTOXICATION

- Routine diagnostic tests used by clinicians in fields such as clinical microbiology and clinical biochemistry, to investigate whether other causes may explain presenting signs and symptoms;
- a recent history of engaging in recreational water activity, with ingestion of water at a site contaminated by a planktonic bloom, scum material or detached benthic mats of cyanobacteria;
- the confirmation of cyanotoxins and/or cyanotoxin-producing cyanobacteria in water samples or benthic mats collected at or close to the time and location of exposure;
- signs and symptoms of acute hepatic toxicity, supported by findings of hepatic impairment at clinical examination and abnormal liver function tests;
- signs and symptoms of motor nerve deficit, which may or may not manifest in acute respiratory insufficiency, seen at clinical examination where the clinical history indicates recent exposure to cyanobacteria;
- cyanobacterial cells and trichomes in vomitus and stool samples identified by microscopy; although this procedure is a simple, low-tech method for identifying a biomarker of exposure to cyanobacteria, it seems to have been scarcely reported in human case investigations since the 1960s (Dillenberg & Dehnel, 1960; Schwimmer & Schwimmer, 1964).

When allocating symptoms to cyanotoxins, it is important to realise that mere co-occurrence is insufficient for establishing a causal connection: even if cyanotoxins are found in patients' serum, it remains possible that other components of the bloom caused the symptoms, particularly if symptoms are unspecific. If, however, they relate to the mode of action and exposure to high toxin concentrations, this is indicative of the respective toxin to be a likely cause. To support diagnosis, awareness and networking of laboratories involved in microbiological and chemical analyses is important so that they too can trigger a timely sampling campaign at the site where patients were exposed – within a short reaction time to capture the situation *in situ* as close to the potential exposure event as possible.

5.2.2 Pathways for exposure through recreational or occupational water activities

With the exception of the toxins from marine cyanobacteria (see below), the water-soluble cyanotoxins known to date are highly unlikely to be able to disrupt the normal protective barrier function of the skin. Thus, cutaneous exposure will not cause access to the bloodstream in concentrations sufficient to cause generalised organ system dysfunction. Activities involving full immersion (e.g., jumping from diving boards, sailboarding, canoe capsizing, competitive swimming) or potential exposure to spray and aerosols (e.g., jet skiing, spray irrigation, cooling of mining drills) may facilitate the entry of cyanotoxins into the systemic circulation, both through ingestion and through inhalation (these are sometimes termed "primary exposure"). Powered watercraft activities such as tube skiing and wakeboarding are likely to cause more frequent and forceful immersions than, for example, sailing or fishing from a dinghy. Other recreational or occupational activities present low risks of ingesting cyanotoxin-contaminated water, for example, shoreline or jetty fishing, wading, low-speed boating, operating irrigation channels. Exposure to cyanotoxins is potentially through the following routes:

- unintentional ingestion of water through reflex swallowing, or in the case of infants "intentionally" during playing;
- water entering the nasopharynx which is subsequently swallowed;
- inhalation when respirable aerosol or spray is formed and droplets/particles enter the nasopharynx and are subsequently swallowed or when dried scums present on the shore are raised as respirable dust;
- for marine cyanotoxins skin and mucous membrane contact.

Of these exposure routes, the one understood best from numerous animal studies is ingestion (see Chapter 2), and dose–effect relationships will follow the patterns assumed for other oral exposure routes, for example, through drinking-water or food. Moreover, toxin concentrations in water can be measured and amounts ingested be estimated from this.

In contrast, while inhalation has frequently been flagged as a concern, quantitative information on exposure is scant: while the formation of spray through fast power boats, jet skis and water skiing appears likely and exposure may well be enhanced by wind, the dynamics of spray formation are poorly understood and the amount of water to which a person is thus exposed is difficult to quantify. Data on toxin concentrations in spray are limited to microcystins for which concentrations were mostly in the low pg/m^3 range but occasionally up to a few 2.89 ng/m^3 when the toxin concentration in water was high (Backer et al., 2010; Wood & Dietrich, 2011; Gambaro et al., 2012). The particle size of the contaminated aerosols or spray droplets will determine their ability to reach the alveoli, but

information on cyanotoxin uptake through the respiratory tract is limited. The following information is available:

- Benson et al. (2005) exposed male BALB/c mice with the nose-only modality to purified MC-LR and described slight to moderate multifocal degeneration and necrosis in the respiratory epithelium and atrophy of the olfactory epithelium at doses up to 265 μg/m³ after 7 days of daily exposure up to 180 min/d. The authors identified a no observed adverse effect level (NOAEL) for nasal lesions after inhalation of 3 μg/kg bw or 20 ng/cm² of nasal epithelium.
- Fitzgeorge et al. (1994) performed an acute study with MC-LR administered via intratracheal instillation to guinea pigs and determined an LD_{50} of 250 μg/kg (similar to the i.p. lethal dose), with necrosis starting in the high airways, progressing to alveoli and resulting in liver damage, but this route of exposure with purified toxin is poorly representative of human exposure via inhalation (Buratti et al., 2017).
- Backer et al. (2008; 2010) detected microcystins in environmental air samples (0.052–2.89 ng MC/m³ in aerosol with MC-LA as the dominant variant in water at 15–350 μg/L) and at lower levels in nasal swabs (from below the limit of detection to 5 ng) of 81 individuals practising recreational activities in lakes during cyanobacterial blooms. However, MCs were undetectable (<1 μg/L) in the blood of those exposed. This can suggest that the aerosol had a limited systemic bioavailability after inhalation, but no conclusion can be drawn due to the small size of control group ($n=7$), the variability of aerosol particle size and some analytical problems with the detection of microcystins (matrix effects with ELISA detection in blood; Buratti et al. (2017)).
- Wood and Dietrich (2011) give theoretical considerations for protection from systemic effects of microcystins in spray: from the tolerable daily intake (TDI) of 0.04 μg/kg bw per day and considering an average ventilation volume of 30.3 L/min, typical of sustained activity, and a high bioavailability of inhaled toxin (similar to that after i.p. administration, based on the similar lethal dose as proposed by Fitzgeorge et al., 1994), they estimate that people should not be exposed to more than 4.58 ng/m³ of air. This is higher than the levels so far detected in air or in spray.

In consequence, available data are not sufficient to derive cell densities specifically associated with local or systemic symptoms due to inhalation of contaminated water (Funari et al., 2017). A further possible effect is local irritation of the upper airway mucosa through other substances in cyanobacteria. Also, in many recreational activities, multiple exposure scenarios will occur simultaneously, rendering discrimination between them difficult.

With respect to cutaneous, ocular and respiratory tract symptoms, as discussed above, there is strong evidence, both experimentally and from field observations, of marine toxic *Moorea* species (*Lyngbya majuscula*) containing lyngbyatoxins and/or debromoaplysiatoxin causing such symptoms in a high proportion of exposed individuals. However, no comparable body of evidence exists to support a similar clinical profile and symptomatology for exposure to freshwater planktonic cyanobacteria. Cutaneous exposure may be aggravated by bathing and diving suits, as these may trap and accumulate cyanobacterial cells, enhance their disruption and hence the liberation of cell contents onto the wearer's skin. Disruption by bathing costumes of *L. majuscula* filaments has been reported (Osborne et al., 2001).

5.2.3 Assessing the risk of exposure to planktonic cyanotoxins through recreational or occupational activities and short-term responses to occurrence

In contrast to dogs or to livestock lacking access to scum-free water, humans will rarely swallow a cupful of thick scum intentionally, but bolus-type exposure can occur, for example, in the context of accidents such as capsizing boats or sailboards. Exposure scenario estimates from scum concentrations of cyanotoxins in the range of mg/L show that an acutely hazardous cyanotoxin dose is rarely likely, but cannot be dismissed as a possibility if fairly large water volumes containing highly toxic scum are ingested. If a toddler of 10 kg body weight swallows 100–200 ml of scum containing 25 mg/L, it would reach an exposure of 2.5–5 mg for microcystin-LR, sufficient to cause liver damage, and the case report from Uruguay mentioned above (Vidal et al., 2017) highlights that such scenarios may be realistic. Thus, even a life-threatening dose cannot be totally excluded, particularly for sensitive individuals, if scums are thick and highly toxic. A possibly more relevant concern, however, is injury through frequently repeated exposure to a subacute dose, most likely for microcystins in face of their high concentrations in surface scums.

The extent to which public authorities are able to conduct surveillance and to respond to blooms with temporary warnings or bans may be limited by the number of sites to monitor in relation to their institutional capacity. For example, north-western Germany faces the challenge of a high number of eutrophic, frequently cyanobacteria-ridden lakes used with varying intensity for recreation by the local population, regardless as to whether or not sites are officially designated as recreational sites and are accordingly monitored. Another common scenario is that of densely populated lowland regions with slowly flowing, nutrient-rich and bloom-affected rivers which are nonetheless intensively used for sailboarding, swimming and other water sports even

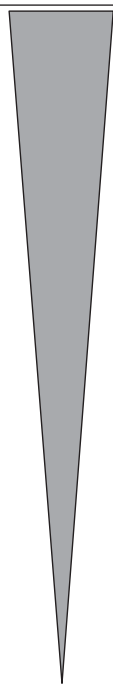
though sites are not explicitly designated for recreational use and are monitored accordingly. In Australia, blooms may affect over 1000 km of continuous stretches of inland river systems (Al-Tebrineh et al., 2012). Unless regular monitoring is in place for other reasons such as drinking-water abstraction, such situations may pose considerable challenges for the monitoring of recreational water and for interventions to protect public health.

Where people use waterbodies for recreation or irrigation, technical barriers against exposure to water potentially containing toxic cyanobacteria are typically lacking. They may also be lacking for other occupational uses of surface water, even where, in principle, some treatment would be possible, for example, for water used to cool drills in mining. Control options to avoid exposure to cyanobacteria and cyanotoxins include catchment and waterbody management geared towards reducing the potential for blooms, as discussed in Chapters 7 and 8. They also include the considerations discussed in section 9.1 for the assessment and choice of drinking-water offtake sites to avoid scums: where the shoreline geography of a given waterbody and/or other already established usages allow a choice, similar considerations may serve to optimise the choice of sites for recreational use. Where none of these management approaches are successful, the option that remains is to guide and influence the behaviour of site users. Options for this range from informing users, that is, creating awareness and enabling individual responses to bloom situations, to temporarily banning waterbody use for the duration of the bloom.

Site users differ in their risk perception, in how receptive they are to information and how willing they are to adapt their behaviour in order to avoid contact. As discussed above, areas with extensive cyanobacterial scums or accumulated detached mats on bathing beaches may be avoided by swimmers and other water users, but as cyanobacterial dominance is typically most pronounced in climates and seasons in which the demand for recreational water is high, scums may also be ignored. Differences in usage patterns, user perception and willingness to engage are depicted in Table 5.4. It is valuable to consider likely behaviour of site users when deciding how intensive monitoring should be at a specific site and whether temporary usage bans are necessary or whether information and warning are sufficient.

Understanding the potential for blooms in a waterbody is a further important basis for prioritising monitoring. It depends on a few key conditions, in particular the concentration of total phosphorus, turbidity, water exchange rate and for lakes or reservoirs also on thermal stratification. For example, if total phosphorus concentrations in a waterbody do not exceed 20 µg/L and the water is clear, with Secchi depths above 2–3 m, blooms are very unlikely. Waterbody conditions that render blooms likely are discussed in detail in Chapters 7 and 8, which also give checklists for assessing the risk of bloom occurrence. The advantage of understanding the potential for blooms is that usually this potential does not change quickly in a given

Table 5.4 Usage patterns of waterbodies prone to blooms as criteria for monitoring and intervention

Appropriate intensity of monitoring and intervention	*Waterbody usage pattern*	
	Almost daily exposure during the bloom season, for example, at lakeside holiday homes and campsites or at a workplace	Recreational sites used by a high number of people Occupational exposure to aerosol likely for a high number of workers and/or regularly over several weeks
	Water sports with high probability of immersion of the head and/or oral uptake of bloom material; lakeshore bathing sites with diving boards or rafts, water slides or other attractions likely to increase the probability of incidental oral uptake	
	Sites used only by a small number of people and only occasionally, discontinuously Occupational exposure only occasionally, intermittedly and/or to a small number of workers	
	Site users/workers receptive to information on blooms, how to recognise them and how to respond to them	Site users/workers willing to engage in initiatives to assist surveillance, for example, by scum scouting and checking turbidity, reporting observations to the responsible authority and thus triggering targeted surveillance

waterbody, and after once having assessed such baseline data throughout one to three bloom seasons and their patterns over time, such analyses may not need to be repeated frequently; occasional checking whether the situation has substantially changed may be sufficient.

Longer-term data on cyanobacteria and toxin concentrations help to understand their variability in the given waterbody and are therefore highly valuable for prioritising waterbodies of concern: for example, if data covering 2–3 years or seasons of cyanobacterial dominance regularly showed high amounts of toxic cyanobacteria occurrence, this would indicate a high priority for the monitoring of cyanobacteria at recreational sites or in water used at a workplace. By contrast, if data with sufficient resolution over time (i.e., at least monthly, preferably fortnightly sampling) show that over a period of 2–3 years, cyanobacteria were never dominant or exceeding the biovolumes given in the Alert Levels Framework (ALF; see Figure 5.5), monitoring of such a waterbody could be a lower priority (see also Chapter 11).

Checklist 5.1 summarises aspects to consider when designing the overall approach to assessing exposure risks through direct contact to cyanobacteria in recreational or occupational settings.

CHECKLIST 5.1 FOR ASSESSING THE LIKELIHOOD OF EXPOSURE TO CYANOTOXINS THROUGH RECREATIONAL AND OCCUPATIONAL USE OF A WATERBODY

- Is information available to indicate the likelihood of bloom occurrence, that is, from catchment characteristics and land use governing nutrient loads (see checklists in Chapter 7) or from direct observations of cyanobacteria and/or waterbody characteristics (see checklists in Chapter 8)?
- If scums occur, are there bays and shorelines where they chiefly tend to accumulate (see section 4.1.2, Figure 8.1 and the checklist in section 9.1.5), and if so, how does the location of the site used (e.g., for a beach or for the offtake of water for production purposes) relate to these?
- How intensively is the site used (see Table 5.4)? Is individual use occasional, or are the same people exposed frequently, for example, almost daily?
- Are the majority of users receptive to information and likely to adapt their behaviour accordingly?
- Are site operators or users potentially willing to engage in initiatives to assist surveillance, for example, by scum scouting and/or checking turbidity and reporting observations?

5.2.3.1 Defining national cyanotoxin levels that trigger action

The edition of "Toxic Cyanobacteria in Water" published in 1999 proposed two points of entry for assessing the "guidance level or situation" – either the concentration of chlorophyll-*a* (with dominance of cyanobacteria) as measure for biomass, or cell numbers, with Table 5.2 and Figure 6.5 in that edition differentiating three "Guidance levels" for recreational exposure.

A number of countries have since used this guidance as basis for implementing guidelines or action levels for assessing health risks from cyanobacteria through recreational usage of waterbodies (see Chorus, 2012; Ibelings et al., 2014; Funari et al., 2017 for overviews). While the actions taken in these countries at each of the three levels are similar (ranging from information and the issuing of warnings to temporary site closure), they vary considerably in the cell count levels triggering them and in their assessments of

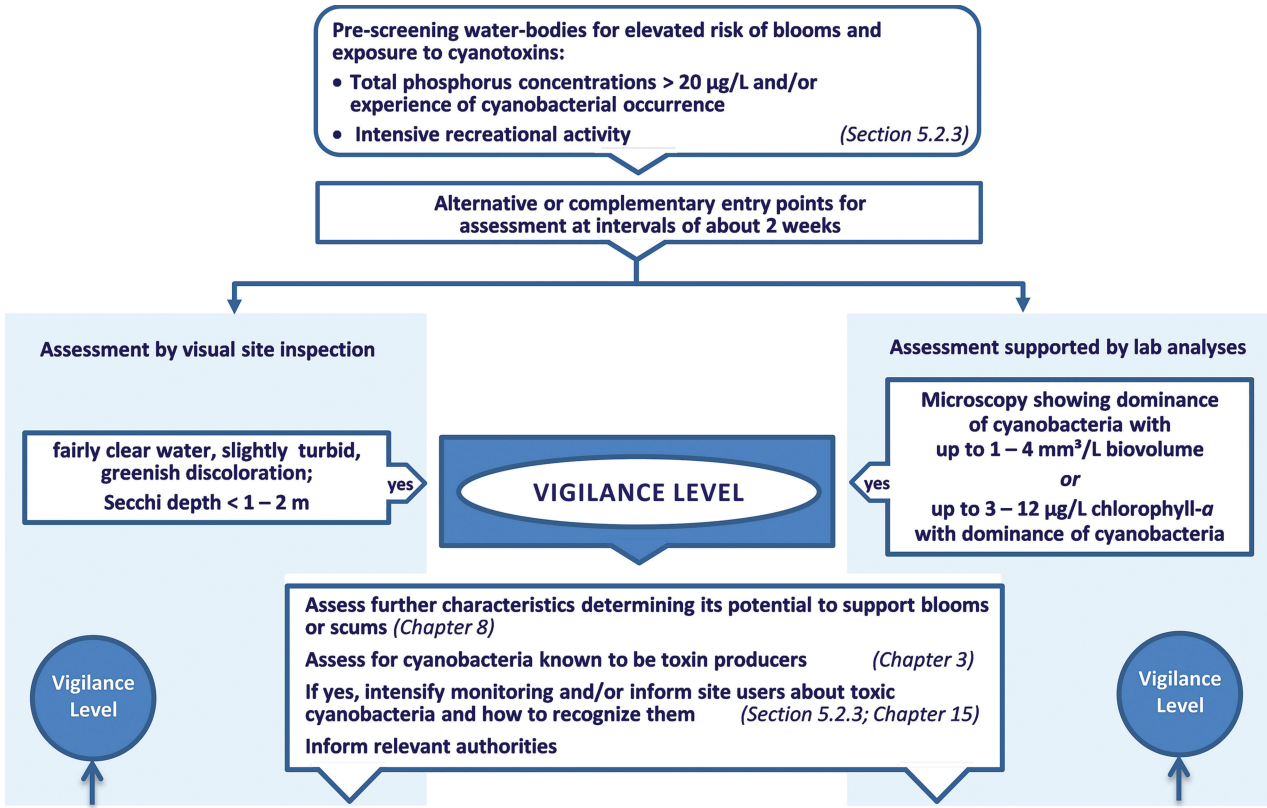


Figure 5.5 Alert Levels Framework (ALF): Decision tree for monitoring and managing cyanobacteria in waterbodies used for recreation.

(*Continued*)

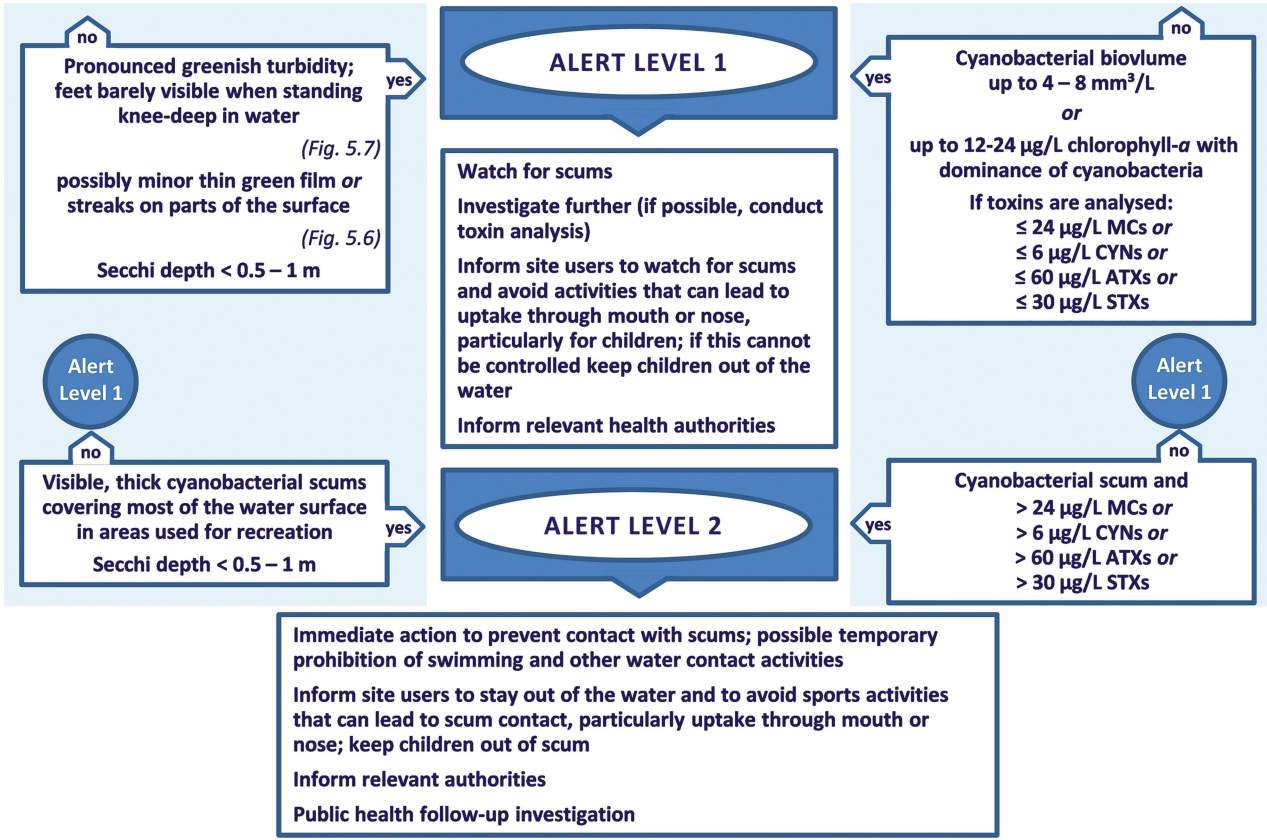


Figure 5.5 (Continued) Alert Levels Framework (ALF): Decision tree for monitoring and managing cyanobacteria in waterbodies used for recreation.

the health risk arising from exposure: the distinction between risks categorised as "low" and those categorised as "moderate" varies 20-fold including one extreme case even 200-fold, that is, from 500 cells/mL in New Zealand to 100 000 cells/mL in Canada (with intermediate values such as 5000 cells/mL in Australia and 20 000 cells/mL in the Czech Republic, Italy and France).

In contrast, where countries base their Alert Levels on cyanobacterial biovolumes, differences between levels triggering alerts are less pronounced, varying only by a factor of less than 10 and ranging from 1.8 mm^3/L in New Zealand to 15 mm^3/L in the Netherlands, and almost all countries place the presence of scums in the high-risk category. The range of variation is similar where countries include microcystins in their risk assessment: levels considered as high risk range from 10 to 20 μg/L.

Such variation reflects differences not only in the assessment of the uncertainty of the toxicological data used but also in the estimates of exposure, particularly regarding the water volumes assumed for oral uptake and the duration of exposure. The background of local experience with the recreational use of waterbodies affected by blooms is also relevant: in countries with a long history of recreational use, despite waterbodies frequently suffering visible discoloration, high turbidity and blooms (such as the Netherlands and Germany), authorities tend to set triggers for warning and for closure at higher levels. For example, in two provinces of the Netherlands warnings, discouraging of bathing and even prohibition occurred in numerous waterbodies even though the thresholds triggering these actions are set quite high, and Ibelings et al. (2014) analyse the situation as follows:

> *"Setting the alert levels in the Netherlands is the outcome of intensive discussions between scientists, lake managers and policy makers, in a country known for the highly eutrophic state of its lakes (despite successful restoration efforts …), where stricter alert levels might result in extended closure of many lakes. Safety clearly must come first, but the protocol used in the Netherlands – in addition to health risks – takes into account the promotion of outdoor activities, feasibility, complexity and costs of monitoring or risk control of cyanobacteria, as well as the ease of communication to the public. Given the large uncertainty in the derivation of TDI for cyanotoxins it is not possible to say whether the higher alert levels in the Netherlands truly result in decreased protection. We merely know it is uncertain." (p. 68)*

The WHO guidance level of 100 000 cells/mL potentially triggering restrictions of site use, published in 1999, was based on the potential for health impacts of cyanotoxins through ingestion and systemic intoxication inferred from toxicological considerations, using the provisional WHO TDI for microcystin-LR. The lower guidance level of 20 000 cell/mL triggering

information to site users was also based on the complementary criterion of a potential for irritant and/or allergic reactions, inferred as outcomes of the epidemiological study on health effects from recreational exposure to cyanobacteria conducted by Pilotto et al. (1997). The subsequent studies by Stewart et al. and Backer et al. discussed above did not find increased symptoms at the low levels of cyanobacteria that Pilotto et al. (1997) concluded to be associated with illness, thus casting doubt about the validity of this complementary criterion. In addition, Pilotto et al. (2004) in a subsequent study found no direct dose–response to a wide range of cell densities during a study with skin patches to assess skin effects after exposure to cyanobacteria. These new results question the basis for the criterion of 20 000 cells/mL.

Meanwhile, WHO has derived guideline values (GVs) for recreational exposure to microcystins, cylindrospemopsin and saxitoxins as well as a health-based reference value for anatoxin-a (Table 5.1). This has opened a new rationale for setting guidance or Alert Levels for recreational exposure. The values for the Alert Levels are now based on the minimum amount of biomass that is likely to contain the toxin concentrations amounting to the recreational GVs. Also, an Alert Levels Framework (ALF) for recreational exposure (Figure 5.5) now replaces the guidance levels described in 1999, while maintaining the differentiation of three levels. A further change reflects experience with cell numbers leading to undue restrictions of recreational use if the dominant cyanobacteria are species with very small cells: as toxin concentrations relate to biomass rather than numbers, even at high cell numbers of very small cells water is clear and toxin concentrations are negligible (see discussion in section 4.6). Therefore, while the ALF continues the use of concentrations of chlorophyll-*a* as indicator for possible cyanotoxin occurrence, it now uses biovolumes instead of cell numbers as a further parameter for defining Alert Levels.

Importantly, as for the drinking-water ALF described in section 5.1.2.2, this ALF for recreational water use is intended as template for adaptation to national circumstances or even to local conditions: where appropriate, regulators can modify both the choice of parameters as indicators of possible cyanotoxin concentrations and the levels at which alerts are set. The Alert Levels on the basis of biomass are defined from experience regarding the maximum ratio between microcystins and cyanobacterial biovolume or chlorophyll-*a* typically found in the field, based on the assumption that maximum ratios of toxin to biomass will not be higher for the other cyanotoxins (see section 4.6). Note that for CYNs, due to the high share of this toxin potentially dissolved in water, the biomass at the time of sampling does not necessarily indicate occurrence of dissolved CYNs; this needs to be inferred from the biomass of CYN producers observed in the 4–6 preceding weeks (see Box 5.1 for details).

As discussed at the beginning of this chapter, the ratio used is in the upper range of ratios from field data reported in the literature and thus

is highly conservative: if these Alert Levels are met, it is highly likely that the WHO GVs for recreational exposure to cyanotoxins summarised in Table 5.1 are not exceeded. However, the cyanobacteria in a given waterbody may well contain far less toxin per unit biovolume or chlorophyll-*a*, and determining such ratios specifically for the waterbody may well lead to setting different Alert Levels, that is, tolerating higher amounts of cyanobacteria before moving on to the next Alert Level. Monitoring can then continue based on cyanobacteria; however, during the course of a bloom or if its composition changes, it may be important to repeat toxin analysis to check whether the toxin/biomass ratio is still appropriate, as this may change as a bloom develops.

If other parameters that serve as indicators of toxin concentrations are chosen to define the Alert Levels (e.g., cell numbers, pigment concentration measured by hand-held probes, remote sensing signals or molecular tools), it is important to periodically "calibrate" these locally against toxin concentrations in order to ensure that they adequately reflect these. When using such parameters or when using data from toxin analyses to define Alert Levels, qualitative microscopy is recommended in order to assess which cyanobacterial genera dominate, as this information is important both for understanding scum behaviour and for waterbody management. An advantage of using cyanobacterial biomass for defining Alert Levels is that this encompasses any further hazards potentially associated with cyanobacteria, including those from yet unknown substances they may contain (section 2.10) or pathogenic organisms associated with their mucilage. Although health risks from such poorly understood agents cannot be quantified and thus no health-based limits for cyanobacteria can be derived, meeting the biomass-based Alert Levels is likely to provide level of protection from such agents as well.

Furthermore, water-use patterns may determine the ALF thresholds used locally. The WHO recreational guideline values (GVs) are calculated on the basis of a 15 kg child ingesting 250 mL of water, and an adult swimmer, sailboard rider or water skier would need to ingest 1 L to reach the WHO GV.

5.2.3.2 Alert Levels for short-term responses to toxic cyanobacteria in waterbodies used for recreation

As for drinking-water in section 5.1, Figure 5.5 provides an Alert Levels Framework (ALF), that is, monitoring and management action decision tree as for planning immediate short-term responses to cyanobacterial occurrence in waterbodies used for recreation (for longer-term measures addressing the causes of cyanobacterial proliferation, see Chapters 6–9). Depending on local circumstances, this ALF may be adapted for water used at workplaces as well.

As for drinking-water, the basis for this ALF is an assessment of the likelihood for the waterbody to harbour health-relevant amounts of cyanobacteria. While for drinking-water supplies such an assessment may be driven by the water supplier for the specific waterbody used as raw water, for recreational sites, in some cases, a private operator may be responsible for a site, but often it is a public authority. Sometimes these carry the responsibility for assessing the safety of a larger number of sites. Prescreening waterbodies to set priorities for the surveillance of bathing sites may then be important, and criteria include data from previous monitoring (if available) as discussed above in section 5.2.3 as well as the

- likelihood of cyanotoxin risks to occur, to be assessed from characteristics of the waterbody and/or previous observations of their occurrence as described in Chapter 6;
- the potential public health impact which is influenced by the intensity of site use, as depicted in Table 5.4.

Furthermore, in many settings, waterbodies for recreational use are not monitored as intensively as drinking-water, and in consequence, monitoring is less likely to follow the onset and progress of a cyanobacterial bloom. Inspection conducted only occasionally may well find a pronounced bloom, and the outcome will then lead straight to Alert Level 1 or 2.

Differently from the ALF for drinking-water (Figure 5.1), the next step after prescreening in this ALF offers two points of entry, of which the simpler approach of visual inspection may well be used alone. The supplementary point of entry is analyses of cyanobacterial biomass – depending on the equipment and expertise available either by microscopy as biovolume or by chemical analysis as the concentration of chlorophyll-*a* (combined with qualitative microscopy to assess whether or not the biomass primarily consists of cyanobacteria or other phytoplankton). This adds a more objective trigger for action which may be important particularly where warnings or temporary site closure is likely to lead to concern or opposition, for example, because of substantial restrictions of site use and economic consequences for operators. Biomass should, however, be used in addition to – and not instead of – the visual assessment described on the left-hand side of Figure 5.5.

As discussed at the beginning of this chapter (and more specifically for drinking-water in section 5.1.2.2), for MCs, STXs and ATXs the toxin/biomass ratios proposed for triggering Alert Levels are quite conservative and thus highly protective of cyanotoxin occurrence, while for CYNs, with its low GV, they are somewhat more uncertain. Also note for CYNs that dissolved CYNs can persist well after CYN-producing taxa (i.e., particularly species of *Aphanizomenon*; in Australia and the Americas also *Raphidiopsis raciborskii*) are no longer conspicuously present in a sample, and where these have been observed, sampling should follow the guidance given in

Box 5.1, and/or CYN concentrations should be determined. In contrast, the WHO recreational GV for STXs and the health-based reference value for anatoxin-a are substantially higher, and it is unlikely that the Alert Levels for biovolume and chlorophyll-*a* will not be sufficiently protective to ensure that meeting these also ensures not exceeding the 30 μg/L for STX or 60 μg/L for ATX.

The Alert Levels Framework (ALF) given in Figure 5.5 does not address the concerns of individuals with an allergic predisposition, for example, atopy, who may experience acute cutaneous or respiratory allergies at quite low concentrations of cyanobacteria, whereas those without such predisposition will likely be unaffected. Also, the aggravation of cutaneous reactions due to accumulation of cyanobacterial material and enhanced disruption of cells under bathing suits and wet suits discussed above may occur even at densities below the Alert Level values used in the ALF.

The Vigilance Level addresses a situation with dominance of cyanobacteria in the phytoplankton, but at biomass levels too low to contain hazardous toxin levels and thus with fairly clear water that might show slight turbidity with greenish discoloration; transparency determined with a Secchi disc will usually be in the range of 1–2 m. However, because of the potential for rapid increase or even scum formation, it is appropriate to intensify surveillance and inform site users about their potential to increase to higher levels. In this range, that is, at a maximum of 4 mm^3/L cyanobacterial biovolume or 12 μg/L of chlorophyll-*a*, microcystins can reach concentrations in the range of 12 μg/L provided that the cyanobacteria present have a high content of this toxin. However, generally concentrations of toxin will be lower than this (see section 4.6).

Because of the potential for rapid increase of cyanobacterial biomass and thus toxin levels between monitoring occasions, dominance of cyanobacteria even at low levels should not be a cause for complacency, particularly if recreational site monitoring occurs only at intervals longer than once per week. Concentrations in this range are a cause for alertness and locally appropriate responses with a focus on improving the understanding of the specific situation.

Vigilance is particularly relevant for waterbodies with total phosphorus concentrations well above 20 μg/L (provided N is not reliably limiting) because cyanobacteria, once dominant, may reach a higher biomass within a few days. It is also particularly relevant for very large waterbodies because they have a potential for scum formation even at these rather low biomass levels, as scums can accumulate from very large water volumes. However, lakes and reservoirs with low phytoplankton density rarely show prolonged dominance of cyanobacteria, and such scums tend to be short-lived minor events.

Alert Level 1 addresses a situation in which cyanobacteria are clearly visible when inspecting the site, particularly as greenish turbidity or

discoloration and possibly also as minor green streaks or specs floating on parts of the water surface, but not as scum covering major parts of the surface area, with Secchi depth in the range of 0.5 – 1 m or even less (Figure 5.6). In such a situation, cyanotoxin concentrations can reach potentially hazardous levels even without scums, but typically they do not, and recreational use may be continued without exposure to cyanotoxins exceeding the recreational guideline values (GVs). This is particularly the case for scum-forming microcystin producers such as *Microcystis* or *Dolichospermum* which may be visible as slight streaks or small specs between which water is fairly clear.

Determining biomass and possibly toxin concentrations provides more precise information and is important in waterbodies with a history of supporting the growth of non-scum-forming species of cyanobacteria: for example, *Planktothrix agardhii* can reach very high densities, particularly in shallow waterbodies, up to 70 mm^3/L biovolume or 200 µg/L of chlorophyll-*a*. Secchi depth in such situations will be less than 0.5 m. *P. agardhii* may contain particularly high cell contents of microcystins (up to or exceeding 1 µg toxin per µg chlorophyll-*a*; see section 2.1).

Figure 5.6 Streaks, specs and Secchi disc reading depicting Alert Level 1 conditions.

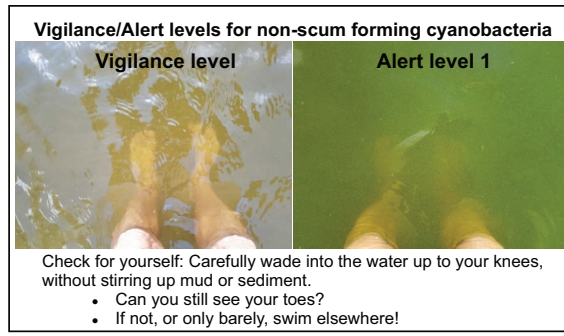


Figure 5.7 Simple and efficient guidance for recreational site users for checking whether non-scum-forming cyanobacteria are present at potentially unhealthy concentrations.

Though such extreme situations are rarely observed, in such situations, it is possible that microcystin concentrations range up to several hundred µg/L or more, without scum formation.

Informing site users to avoid exposure to high densities of such evenly dispersed cyanobacteria is less straightforward than informing them to avoid scums because the situation is harder to describe. Figure 5.7 shows one option for visualising a criterion for self-assessment of the situation.

Where data from visual inspection and quantifying cyanobacterial biomass can be supported by cyanotoxin analyses, this can serve to avoid undue restrictions of recreational site use in situations where cyanobacterial biomass is high but toxin content is low, rendering toxin concentrations in the water below Alert Level 1.

At Alert Level 1, the cyanobacteria present may well increase to a heavy bloom within a few days if conducive conditions prevail in the waterbody. Watching out for scums is therefore recommended, and increasing surveillance may therefore be appropriate, particularly for heavily used recreational sites, in order to rapidly detect if the situation escalates to Alert Level 2.

Alert Level 2 describes a situation with scums or very high cell density leading to substantial turbidity (Figure 5.8). While scums can be thick in parts of the waterbody, other parts may still show a Secchi depth ranging up to about 1 m. Whilst in such a situation the recreational GVs for cyanotoxins are not necessarily exceeded, this is quite likely. Cyanotoxin analysis can be used to confirm or downgrade the Alert Level status. As discussed above, if scum material is both very thick and highly toxic, the ingestion of 100–200 mL by a toddler can contain an acutely hazardous dose. The presence of cyanobacterial scums is a readily observable indicator of a high risk of adverse health effects.

Alert Level 2 situations call for immediate action to avoid scum contact and, in particular, oral uptake. Temporary banning of use may be

Figure 5.8 Examples of *Microcystis* scums depicting Alert Level 2 conditions.

appropriate, and intensified monitoring may be important to either confirm or downgrade the Alert Level status in order to not unnecessarily restrict use. Providing information to the site users is important to achieve an understanding of the hazard and thus compliance. Measures to reduce exposure that can be implemented quickly may include the installation of floating physical barriers to prevent the scum from being driven into the swimming area, provided that surface scums are the key issue (rather than dispersed suspended cells or colonies). If scums typically accumulate at certain sites while others largely remain unaffected, directing recreational use to another site may be an option. Removing drying scum accumulated on beaches may be necessary to avoid the development of dust (using personal protecting equipment if scum is already dry).

Misconceptions of what constitutes a scum are common for large, deep and usually clear lakes with low nutrient concentrations: in such lakes, cyanobacteria may become transiently dominant in the phytoplankton but only at low concentrations. Cells from the large water volume may rise to the surface and be swept into a downwind bay where they may form a surface film, typically thin and with cyanotoxin concentrations well below

hazardous levels. Site users not accustomed to any visible phytoplankton on the surface may interpret even a very thin and locally limited film as "scum" and be unduly concerned, and advisories may need to explain what amounts to a sufficiently pronounced scum to cause concern. Local information may be appropriate to dispel such concerns.

Rescinding warnings after a bloom, when recreational use is safe again, is important in order to avoid undue discouragement of healthy outdoor recreational activity as well as "warning fatigue": if warning signs remain posted even though the water is clear, it is likely that site users will tend to ignore them in the future. This is discussed in more detail in Chapter 15.

5.2.4 Assessing risks from recreational exposure to cyanobacteria on benthic and other surfaces

Death of dogs and livestock has been observed even where the water was clear and toxins from cyanobacteria growing attached to submerged aquatic plants ("periphyton"; including species of *Microcoleus* (*Phormidium*), *Tychonema* and possibly of other genera) or the sediment ("benthic cyanobacteria") have been identified as the cause (e.g., Puschner et al., 2010; Wood et al., 2017; Fastner et al., 2018; see also section 4.2.2). Some countries, for example New Zealand, report widespread occurrence of benthic mats of *Phormidium* found under a wide range of water qualities and proliferating during stable stream flow (Wood & Williamson, 2012). *Microcoleus* (*Phormidium*) is known to produce anatoxins. Benthic mats have become a concern because of the frequency of dog deaths, attributed to anatoxin-a, homoanatoxin-a and saxitoxins and in one case also to microcystins (Wood & Williamson, 2012). Likewise, ATX-producing *Tychonema* species may colonise submerged macrophytes such as *Fontinalis* (Fastner et al., 2018).

Such mats of *Microcoleus* or lumps of macrophytes with attached *Tychonema* may cause a lethal dose for animals ingesting substantial amounts. While dogs sometimes appear to be attracted to decaying material and to ingest substantial amounts, this behaviour is unlikely for humans, including small children. However, swimmers may also be in direct contact with such material after a storm breaks off clumps of material or it naturally detaches from the sediment and is accumulated in shallow water or on the shore.

Implications for managers deciding on whether to restrict recreational use of such waterbodies are challenging: while dead animals are a cause for concern, negligibly low cyanotoxin concentrations in the water would not be. Where water is very clear, that is, with Secchi depths of 2 m or more, concentrations of concern are unlikely, as dissolved toxin leaching from detached mats or macrophytes will dilute and/or degrade quickly. Such situations require a rapid assessment of the risk and its communication to site users – for example, assurance that concentrations in the water are indeed low. Confirmation through cyanotoxin analysis is the most convincing way forward for hazard analysis in such situations. Box 5.6 gives a case example.

BOX 5.6: DOG DEATHS ATTRIBUTED TO *TYCHONEMA* GROWING ON *FONTINALIS* IN A CLEAR LAKE

Lake Tegel is an important suburban resource for the city of Berlin, Germany, both for abstracting drinking-water via bank filtration and for intensive recreational use. Lake Tegel went through a history of eutrophication in the 1980s followed by restoration efforts resulting in the re-establishment of mesotrophic conditions. Since the begin of the millenium, the lake has become clear, with Secchi depths rarely less than several metres, and heavy cyanobacterial blooms no longer occur. Stands of submerged macrophytes now cover large areas of the lake bottom, and as these bind nutrients that thus are no longer available for phytoplankton, they contribute to keeping the water clear.

Against the background of this success story, in May 2017, the acute neurotoxicosis of 12 dogs, several of which died, after playing and swimming in Lake Tegel caused considerable concern. Intensive investigation detected high biomass of anatoxin-a-producing *Tychonema* spp. in detached and floating water moss (*Fontinalis*), which led to concentrations of anatoxin-a of up to 8700 μg/L detected in dog stomach content (Fastner et al., 2018). Interestingly, while the aqueous fraction of some samples of floating *Fontinalis* with *Tychonema* contained up to 1870 μg/L of anatoxin-a, concentrations in other *Fontinalis* samples were low, that is, in the range of 10–20 μg/L. Moreover, water samples – even those taken only a few metres away from the floating *Fontinalis*/*Tychonema* clumps – contained 1 μg/L or less of anatoxin-a. The available data indicated the occurrence of *Tychonema* on *Fontinalis* to be highly variable and the extremely high concentrations detected in May 2017 appear to be an isolated event.

This situation demonstrates a typical challenge for risk assessment: should recreational use of a lake in such a situation be discouraged or is it safe to continue activities? In this specific case, the public authority responsible for site surveillance initiated a monitoring campaign focusing on *Tychonema* and anatoxin-a for Lake Tegel and later also for other Berlin lakes, the removal of macrophyte clumps on the shoreline and information of the public advising against contact with the clumps. Based on the low toxin concentrations found outside the areas with dislodged macrophyte clumps, the lake's importance for recreation and its otherwise high water quality (as compared to the other, more eutrophic and bloom-ridden waterbodies available in and around the city), the authority did not discourage or ban recreational use.

New Zealand has introduced a three-tiered Alert Level Framework for benthic cyanobacteria which is similar to the guidelines for planktonic cyanobacteria, but based on the percent coverage of the waterbody's sediment as well as on detached material from benthic mats accumulating along the shore (Wood & Williamson, 2012).

5.2.5 Assessing risks from recreational exposure to marine dermatotoxic cyanobacterial

As discussed above, some marine beaches have been reported to cause widespread health problems due to the benthic marine cyanobacterium, *Moorea* sp., growing on rocks and in shallow embayments in tropical and subtropical seas. *Moorea producens* and possibly other species of *Moorea* can cause severe blistering when people swimming in affected coastal waters come into contact with strands of these filamentous cyanobacteria, particularly if trapped and macerated under bathing suits (section 2.6). This response may be due to acute toxicity, as *Moorea* can produce irritant toxins. The dermatotoxic alkaloids produced by *Moorea* are not considered in Table 5.4 because exposure patterns to them are different – that is, not unintentional ingestion of planktonic cells or colonies, but cutaneous contact with clusters of filaments (each 10–30 cm in length). Measures to protect site users include providing information about avoiding skin contact, removing bathing suits and showering after immersion to ensure removal of any *Moorea* from the skin (Osborne et al., 2007).

For example, the Moreton Bay Regional Council, Queensland, Australia, has established a three-level approach. Where *Moorea* deposits on beach and adjacent waters are small to moderate and away from built-up areas, they monitor and install warning signs for the public (level 1). Where large quantities of *Moorea* are washing ashore or beginning to form rafts adjacent to built-up areas, they advocate removal from beaches with tractors and excavators (level 2) and notify relevant stakeholders including other government authorities and media. Where very large quantities are washing ashore, in addition to the level 2 procedures, the beach will be closed to the public to safeguard against associated risk of wading or swimming (level 3) (Moreton Bay Regional Council, 2018).

5.2.6 Research to improve our understanding of recreational exposure

As discussed above, symptoms clearly caused by microcystins, cylindrospermopsins, anatoxins or saxitoxins following recreational exposure are not very likely; however, as compared to exposure through drinking-water uptake, recreational activities are more likely to lead to exposure to higher concentrations, possibly causing detectable symptoms. A larger body of thoroughly investigated cases is therefore valuable to improve our understanding

of the hazards that cyanotoxin exposure imply for human health. A key issue for this aim is the quantification of exposure. While rapid (preferably within hours) site inspection and bloom sampling mentioned above would be the best approach, this is often hampered by limited institutional capacity and communication between the institutions responsible for public health versus environmental monitoring. Continuous online monitoring of cyanobacterial biomass development with *in situ* fluorescence probes can greatly improve the understanding of the wax and wane of blooms, as can remote sensing if data can be obtained with sufficient frequency.

Biomarkers are a further helpful tool to assess exposure. Notable advances have occurred in the analytical detection and quantification of cyanotoxins in physiological fluids such as serum, blood, vomitus and urine from exposed groups using chemical and antibody-based methods, although for human blood these findings so far have only been reported for microcystins (Hilborn et al., 2007; Chen et al., 2009; Li et al., 2011). However, for other cyanotoxins such as anatoxin-a, similar advances have been reported from veterinary researchers investigating dog poisonings. While such investigations are usually conducted on necropsied tissues, particularly liver in the case of microcystins or nodularin, analytical chemists have confirmed the presence of anatoxin-a in dog urine (Puschner et al., 2010) and stomach contents (Hoff et al., 2007; Fastner et al., 2018). Such methods are useful in order to support or exclude diagnoses of cyanotoxin exposure and possible intoxication. Many laboratories can also identify cyanobacterial cells and trichomes in vomitus and stool samples or at least have the capacity to capture photomicrographs of stool or vomitus, which can be referred to expert phycologists for confirmation or exclusion of cyanobacterial cells.

The "ideal" case investigation would be triggered by one or several individuals exposed to significant levels of cyanobacteria in recreational waters presenting soon after symptom onset for medical assessment, providing samples of blood, stool, urine and potentially vomitus, a good estimate of the amount of water ingested and the time and location of exposure, from where water samples would be immediately collected for cyanobacterial and cyanotoxin analysis. The putative case would then be rapidly assessed by either an expert hepatologist for a comprehensive assessment of liver function, or, in the case of exposure to a cyanobacterial neurotoxin, for nerve conduction studies and detailed assessment of neuromuscular function. Likewise, the ideal assessment for those presenting with anaphylaxis or other allergic reaction, possibly due to cyanobacterial exposure, would be rapid referral (after recovery) to a clinical immunologist, asthma specialist or dermatologist for confirmatory challenge testing. Furthermore, ideal patients would be willing to consent to publication of their case history, and the attending clinicians will be keen to publish. Substantial public health benefits would arise from a better scientific understanding gleaned from a series of studies employing various subsets of the aforementioned "ideal" case criteria.

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5.3 FOOD

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Four chief sources of exposure to cyanotoxins through food for which data have been published include: (i) animals grown in aquaculture or harvested as food in brackish or freshwater containing cyanobacteria (for examples, see Table 5.5), (ii) so-called blue-green algal food supplements (BGAS, see section 5.5), (iii) food prepared using water contaminated with cyanotoxins (ineffectively treated or untreated) and (iv) crops irrigated with water from waterbodies with toxic blooms. Key mechanisms include toxin adsorption to the surface of plants or translocated to leaves and fruits after root uptake and trophic transfer to animals along food chains. Further sources for cyanotoxins and conceivable pathways into food for which, however, published data are largely lacking, include soil amended with sediment dredged from waterbodies with blooms and the use of algae, including cyanobacteria, as a cheap source of food for poultry or other farm animals.

5.3.1 General considerations on risk assessment and risk management

For assessing and managing health risks from food, the Codex Alimentarius provides the HACCP concept – Hazard Analysis Critical Control Points – which is very similar to the Water Safety Plan (WSP; see Chapter 6) approach. The WSP approach draws on many of the principles and concepts from other risk management approaches, including HACCP. Both approaches emphasise that monitoring the end product alone will not ensure safety. Rather, they focus on controlling the processes that are crucial for the safety of food or drinking-water. Both call on the managers and technical operators of a given facility to conduct a comprehensive analysis of the hazards that could occur in their system, to assess the human health risks they cause, to identify the key measures that are critical for safety ("*Control Measures*" in WSP terminology or "*Critical Control Points*", that is, CCPs in HACCP terminology), and to develop management plans to ensure that these measures are in place and properly functioning at all times.

Where the production of drinking-water and food draw on the same waterbody, the WSP for drinking-water and the HACCP plan for food may interface with respect to assessing and managing the waterbody and its catchment. Naturally, a close collaboration between the teams developing respective plans is desirable. This is important for risk assessment since exposure to cyanotoxins in water and food would add up. HACPP is being implemented in marine fisheries and shellfish harvesting, and it is interesting to note that even here, in the marine environment, one argument

Table 5.5 Examples of cyanotoxin concentrations in foods

A	*B*	*C*	*D*	*E*	*F*	*G*	*H*
Organism	*Organs or tissue*	*Cyanotoxin*	*Content [µg/kg ww]*	*Max. [µg] ingested with serving of 0.1 kg*	*% of TI for DW short term (MC; CYN) or acute (STX) exposure*	*Study type*	*Reference*
Crops							
Lactuca sativa	Leaf	MC-LR	2.4–147	14.7	61	Lab	Cordeiro-Araújo et al. (2017)
Lactuca sativa	Unknown	MC-YR, MC-RR	ND–108	10.8	45	Field	Li et al. (2014)
Ipomoea aquatica	Unknown	MC-RR, MC-LR	ND–68	6.8	29	Field	Li et al. (2014)
Brassica oleracea	Unknown	MC-RR	ND–20	2.0	9	Field	Li et al. (2014)
Brassica rapa var. *Parachinensis*	Unknown	MC-RR	ND–40	4.0	17	Field	Li et al. (2014)
Brassica oleracea var. Sabellica	Leaf	CYN	3.1	0.3	5	Lab	Kittler et al. (2012)
Brassica juncea	Leaf	CYN	4.0	0.4	7	Lab	Kittler et al. (2012)
Lactuca sativa	Leaf	CYN	3.1–8.2	0.8	13	Lab	Cordeiro-Araújo et al. (2017)
Eruca sativa	Leaf	CYN	5.5–11.5	1.1	18	Lab	Cordeiro-Araújo et al. (2017)
Spinacea oleracea	Leaf	CYN	9.5–120	12.0	200	Lab	Llana-Ruiz-Cabelo et al. (2019)[a]
Lactuca sativa	Leaf	CYN	2.4–42	4.2	70	Lab	Llana-Ruiz-Cabello et al. (2019)[a]

(*Continued*)

Table 5.5 (*Continued*) Examples of cyanotoxin concentrations in foods

A	*B*	*C*	*D*	*E*	*F*	*G*	*H*
Organism	*Organs or tissue*	*Cyanotoxin*	*Content [μg/kg ww]*	*Max. [μg] ingested with serving of 0.1 kg*	*% of TI for DW short term (MC; CYN) or acute (STX) exposure*	*Study type*	*Reference*
Molluscs							
Mytilus galloprovincialis	Whole	dmMC-RR[b]	ND–39	3.9	16	Field	Rita et al. (2014)
Patinopecten yessoensis	Whole	MC-LR	ND–4.3	0.43	2	Field	Cui et al. (2018)
Crassostrea virginica	Whole	MC-RR, MC-LR	ND–9.8	1.0	4	Field	Cui et al. (2018)
Mytilus galloprovincialis	Whole	CYN	28.1–41.6	4.2	70	Lab	Freitas et al. (2016)
Anodonta cygnea	Whole	CYN	247	24.7	412	Lab	Saker et al. (2004)
Anodonta cygnea	Whole	$STX_{t.eq}$	160–220	22	73	Lab	Pereira et al. (2004)
Alathyria condola	Muscle	$STX_{t.eq}$	144–179	18	60	Lab	Negri & Jones (1995)
Crustaceans							
Astacus astacus	Head/ thorax	[Asp^3,Dhb^7]MC-LR, [Asp^3]MC-RR, [Asp^3,Dhb^7]MC-RR	10	1.0	4	Field	Miles et al. (2013)
Astacus astacus	Tail	[Asp^3,Dhb^7]MC-LR, [Asp^3]MC-RR, [Asp^3,Dhb^7]MC-RR	<1	<0.1	<1	Field	Miles et al. (2013)
Procambarus clarkii	Abdomen	MC-RR, MC-LR	1.4–17.1	1.7	7	Field	Ríos et al. (2013)
Cherax quadricarinatus	Muscle	CYN	205	20.5	342	Field	Saker & Eaglesham (1999)

(*Continued*)

Table 5.5 (*Continued*) Examples of cyanotoxin concentrations in foods

A	*B*	*C*	*D*	*E*	*F*	*G*	*H*
Organism	*Organs or tissue*	*Cyanotoxin*	*Content [µg/kg ww]*	*Max. [µg] ingested with serving of 0.1 kg*	*% of TI for DW short term (MC; CYN) or acute (STX) exposure*	*Study type*	*Reference*
Fish							
Aristichthys nobilis	Muscle	MC-RR, MC-YR, MC-LR	177[2]	17.7	74	Field	Chen et al. (2007)
Hypophthalmichthys molitrix	Muscle	MC-RR	0.4[2]	0.0	0.2	Field	Chen et al. (2009b)
Hypophthalmichthys molitrix	Muscle	MC-RR, MC-LR	ND–249[c] (mean: 39.4)	24.9	104	Field	Chen et al. (2006)
Pomoxis nigromaculatus	Muscle	MC-LR	ND–70	7.0	29	Field	Schmidt et al. (2013)
Cyprinus carpio	Muscle	MC-LR	ND–4	0.4	2	FIeld	Schmidt et al. (2013)
Cyprinus carpio	Muscle	MC-RR	0.6[b]	0.1	0.3	Field	Chen et al. (2009b)
Oreochromis niloticus	Muscle	MC-LR[b]	4.2–5.2	0.5	2	Field	Greer et al. (2017)
Geophagus brasiliensis	Muscle	$STX_{t'eq}$	12–20	2.0	7	Field	Clemente et al. (2010)

For contextualising the health risk due to these concentrations, column "F" relates to the amount of toxin ingested with a serving of 0.1 kg to the percentage of the *tolerable intake for short-term (MCs, CYN) or acute (STX) exposure* (TI calculated from the NOAEL, UF and bodyweight of 60 kg as given in Chapter 2). Note that these short-term GVs were derived assuming drinking-water to be the major source of exposure to these cyanotoxins, not leaving a proportion to other sources, and that this comparison is intended merely to give a rough estimate of the health relevance of concentrations found in food, not for defining safe levels for food. This table does not take into account the relative toxicities of microcystin congeners.

ND = not detected above the detection limit. $STX_{t,eq}$ = saxitoxin toxicity equivalents.

[a] When applied with equal amounts of MC; without MC accumulation was fourfold lower.
[b] Secondary technique (i.e., MMPB, ELISA) indicated higher levels may have been present.
[c] Converted to wet weight using a wet-to-dry weight ratio of 5.

for implementing HACPP is the risk of microcystins present in mussels (Tzouros & Arvanitoyannis, 2000). HACPP in the seafood industry, as elsewhere, is based upon seven principles: (i) hazard analysis, (ii) identification of the critical points in the process, (iii) establishment of critical limits, (iv) requirements for CCP monitoring, (v) corrective actions, (vi) record keeping procedures and (vii) verification. Implementation of HCAPP based upon these principles in freshwater fisheries and harvesting greatly enhances the protection of the consumers.

5.3.2 Sources of exposure

Both plants and animals have shown highly variable accumulation of cyanotoxins. Table 5.5 shows results of concentrations measured in organisms collected in the field, or exposed experimentally in the laboratory, focusing on experiments using concentrations in a realistic range. To enable an estimate of the health implications of these concentrations, in face of the lack of guideline values (GVs) for concentrations in foods, Table 5.5 relates the exposure from a serving of 0.1 kg of these foods to the tolerable intake (calculated from the no observed adverse effect level (NOAEL), UF and bodyweight of 60 kg as given in Chapter 2) for short-term (MCs, CYNs) or acute (STXs) exposure. The data in Table 5.5 show that while in many cases concentrations in foods are low, some field observations and laboratory experiments found concentrations that would lead to a dose in the range of – or above – that which would be acceptable for up to 2 weeks for an adult consuming 2 L of drinking-water per day, using the short-term WHO GVs for drinking-water. Trends that can be discerned are that consuming molluscs and crustaceans collected from environments with blooms might cause higher risks, particularly because they are eaten with the viscera which can contain large amounts of toxic cyanobacteria. In contrast, the edible portions of higher trophic-level organisms (e.g., muscle tissue of fish), excluding viscera, have less chance of containing a large amount of free toxin. Livestock reports are, however, based on very few animals, rendering results uncertain.

A key problem in using published literature is the uncertainty of many results: The extensive literature survey on cyanobacterial toxins in food by Testai et al. (2016) concluded that the majority of publications had significant flaws in toxin extraction, sample cleanup and/or the analytical methods which undermine the confidence in the data on toxin levels in food. These impair the quality of the analytical data, due to inefficient extraction, poor quality controls and downstream matrix effects resulting in a loss of sensitivity and inaccurate quantification, as well as missing reporting of how quantification was achieved (Testai et al., 2016). For accumulation in plants, information on how toxins were applied, that is, via the soil (enabling only root uptake) or irrigated also on leaves (thus possibly adhering to leaf surfaces), is important but often not clearly described. A further

challenge is accounting for metabolised or protein-bound toxins, as it is yet unclear whether they represent a potential reservoir of toxin that may be released in the gut. Strategies to account for metabolised or protein bound toxins, such as Lemieux oxidation or protein deconjugation techniques, require careful calibration and intimate knowledge of analyte chemistry to avoid producing data that may lead to overestimating the hazard. Dionisio Pires et al. (2004) used Lemieux oxidation to extract microcystins from mussels and found that the bound fraction was always smaller than free microcystin. They contrast this with earlier mussel studies which reported a 10 000-fold in the bound fraction compared to the free fraction. Also, beyond experimental data, more data on levels of cyanotoxins found in food items in markets are needed in order to assess actual rather than perceived risk, with testing geared towards capturing the "total" cyanotoxin pool in order to remain conservative.

5.3.2.1 Microcystins

Information on microcystins detected in crops is limited, with a few studies conducted in the laboratory showing accumulation via uptake through roots and/or leaves (Table 5.5). None of these data indicate a dose substantially above that which can be tolerated from drinking-water for up to 2 weeks unless a serving size significantly above 100 g is assumed. Interestingly, one study did not result in detectable microcystin in spinach and lettuce even though it confirmed cylindrospermopsin uptake (Llana-Ruiz-Cabello et al., 2019) and another resulted in detections in lettuce leaves but not in arugula (Cordeiro-Araújo et al., 2017). These results indicate that other factors (e.g., plant variety, physiology, morphology) influence accumulation and require consideration when monitoring. Only one field study was identified confirming microcystins in crop irrigated with water (Dianchi Lake, China; Li et al. (2014). None of these studies addressed bound microcystin (e.g., conjugated) content in crops.

Among the studies with mussels or crustaceans assessed by Testai et al. (2016) as having been performed with reliable methods, sufficiently comprehensively reported, maximum concentrations in wet weight ranged up to 3400 (±1000) µg/kg for *Mytilus edulis* and up to 329 ± 95 µg/kg for crayfish, while other authors found only low concentrations of MCs (Table 5.5). For fish muscle, the review by Testai et al. (2016) includes a study that found up to 2860 µg/kg in silver carp from China and one with up to 340 µg/kg for *Odontesthes bonariens*is from Argentina. However, most analyses targeting specific microcystin congeners via LC-MS/MS have rarely found MCs in fish muscle, even though they reported microcystins in the source water and other organs (e.g., liver; Kohoutek et al., 2010; Hardy et al., 2015), and if MCs were found, in most cases, concentrations were low. This is likely due to rapid microcystin elimination as well as the remaining fractions in

muscle tissue being unextractable (bound to proteins) or, to a lesser degree, extractable but inactivated by metabolism (e.g., conjugated with thiols; Williams et al., 1997a; Williams et al., 1997b). While extractable conjugated microcystins are detectable with ELISA and MMPB ((2S, 3R)-2-methyl-3-methoxy-4-phenylbutanoic acid), which is also able to detect protein bound fractions), many other methods are too specific to detect these fractions. This partially explains why methods targeting free microcystins (i.e., not bound or degraded) such as LC-MS/MS tend to report lower values than ELISA and MMPB (Li et al., 2014; Foss et al., 2017; Greer et al., 2017).

Studies reporting high microcystin levels in fish muscle (>12 µg/kg wet weight) frequently employed ELISA (Freitas de Magalhães et al., 2001; Berry et al., 2011; Poste et al., 2011). Not only do some of these assays react with microcystin conjugates, but they are also prone to nonspecific binding resulting in overestimation (Hardy et al., 2015; Foss et al., 2017). This could be considered a welcome conservative approach for the case that protein-bound microcystins become released (Smith et al., 2010) and/or metabolised MCs become deconjugated (Miles et al., 2016), regaining some toxicity. However, little is known about these processes, and undue restrictions of food use may also impair health (see section 5.3.4). Research addressing these potential reservoirs, geared to resolving the disparity between fractions of total MC burden (bound, free and conjugated), is therefore important, particularly for molluscs and crustaceans which are a relevant protein source in some regions.

Testai et al. (2016) assess the few results available for livestock as insufficient because they rely on a very limited number of animals and insufficient analytical method that does not include bound MCs; thus, the possible transfer of MCs to the milk or meat cannot be assessed. Chen et al. (2009b) found low levels of MC-RR, MC-YR, MC-LR in muscle tissue of the common duck (*Anus platyrhynchos*) and Chinese softshell turtle (*Pelodiscus sinensis*), that is, 3 and 0.6 µg/kg ww, respectively.

In spite of the analytical limitations of many studies, a general trend in literature indicates higher microcystin content in liver and viscera than in muscle. Further, the available evidence supports microcystins to be biodiluted rather than biomagnified in the aquatic foodweb (Ibelings et al., 2005; Ibelings & Havens, 2008), indicating that properly cleaning meat in higher trophic level animals reduces the risk of exposure to microcystins.

5.3.2.2 Cylindrospermopsin

As for microcystins, plant studies have addressed the uptake of cylindrospermopsin into leafy vegetables such as lettuce, arugula and mustard, sometimes at higher levels than microcystins (Table 5.5), with one study finding CYN in spinach at levels higher than other leafy greens, possibly resulting in exposure at levels relevant to health. Llana-Ruiz-Cabello et al. (2019) found high concentrations in spinach and lettuce, but only when

applying CYN in concentrations of 25 µg/L together with 25 µg/L of MCs; applying 25 µg/L CYN alone resulted in fourfold lower concentrations in the plant material. However, such levels have not been reported from field studies or market acquired vegetables. In animals, CYN has been studied less than MCs, with accumulation reported from bivalves and crustaceans, in one case at levels potentially relevant to health (Table 5.5). For fish, studies with sufficiently selective methods (e.g., LC-MS/MS) are largely lacking; the review by Testai et al. (2016) includes three studies that found no or only very low concentrations in fish.

5.3.2.3 Saxitoxins

Saxitoxins (STXs) in food products are well documented in the marine environment, including numerous cases of human illness and death. To date, there have not been any reports of paralytic shellfish poisoning caused by freshwater cyanobacteria even though STX accumulation in freshwater mussels has been demonstrated (Negri & Jones, 1995). Freshwater fish, *Oreochromis niloticus* and *Geophagus brasiliensis*, were found to accumulate STXs from the environment, but not in concentrations that would lead to exposure in a health-relevant range (Table 5.5; Galvão et al., 2009). Testai et al. (2016) include one study finding up to 30.6±14.5 µg/kg of PSP toxins in Cichlidae. Interestingly, intraperitoneal dosing of the tropical freshwater fish *Hoplias malabaricus* four times with STX at 800 µg/kg did not result in accumulation in muscle tissue (da Silva et al., 2011).

5.3.2.4 Anatoxins

Very little is known regarding the accumulation of anatoxin-a and/or homoanatoxin-a, with studies lacking on crops or invertebrates. One study has shown anatoxin-a to bioaccumulate in fish (Osswald et al., 2011), but others have shown it to rapidly eliminate from fish and mussels (Osswald et al., 2008; Colas et al., 2020).

5.3.2.5 Conclusions on exposure via food

In summary, as preliminary assessment considerering all types of cyanobacterial toxins, the data available by 2019 do not point to a high level of short-term exposure to cyanotoxins in crops or muscle tissue of fish and crayfish, whereas exposure may be more significant if viscera are eaten, as is the case for small fish, crustaceans and mussels. If for instance crops are sprayed or irrigated with lake water containing scums or high levels of cyanotoxins and in particular if foods are not sufficiently washed or prepared, risks may be higher. However, data obtained with reliable methods are insufficient for drawing clear conclusions.

Where crop irrigation with scum material is widespread (as described, e.g., in Li et al., 2014) or fish, mussels and crayfish from bloom-ridden waterbodies constitute staple foods, screening cyanotoxin concentrations in such foods is recommended, with attention to the methodological requirements described in section 5.3.4.

Hazard analysis for any of the above settings may indicate that when cyanotoxins in foods cannot be excluded because of – substantial – cyanobacterial blooms in the waterbody used for the production of the food, a more detailed analysis becomes important. Checklist 5.2 provides guidance for conducting such an analysis.

CHECKLIST 5.2 FOR ASSESSING THE RISK OF CYANOTOXIN EXPOSURE THROUGH FOOD

1. Are blooms of potentially toxic cyanobacteria present in the waterbodies used for collecting, producing or preparing food (see Chapters 4 and 8)?
   - 1.1. Inspect these waterbodies to collect information on the presence of surface blooms or scums, strong greenish discoloration and turbidity.
   - 1.2. Collect samples for species identification and quantification, particularly if these observations indicate cyanobacteria could be present.
   - 1.3. Particularly if potentially toxic cyanobacteria are found and if feasible, have toxin content of the cells and bloom analysed (see point 2.3).
   - 1.4. If cyanotoxins are present currently or were present during the previous month, further risk analysis in food becomes relevant. Clarify the time pattern of toxin occurrence – is it sporadic for a few days, or continuous for many weeks or months?
2. Are organisms (e.g., fish, shellfish, snails, bivalves) harvested for food from the impacted waterbodies? If so,
   - 2.1. Find out whether these species are likely to filter-feed particles, including cyanobacteria, and whether they have been reported to contain cyanotoxins.
   - 2.2. Find out whether viscera and gonads are removed prior to consumption or whether the organisms are consumed whole.
   - 2.3. Check whether analyses of their cyanotoxin content are feasible, and if so, together with experts derive a plan for sampling and analyses.
3. Are crops irrigated with water containing high amounts of cyanobacteria?
   - 3.1. If so, check whether the use of alternative water sources, free of blooms, is feasible or run a programme of sampling and analyses to assess whether the practices used lead to cyanotoxins in the crop.
   - 3.2. Investigate whether substantial amounts of cells cling to the surface of fruits or vegetables which are potentially consumed without sufficient treatment to remove them.

4. Are soils augmented with sediment dredged from systems containing high amounts of cyanobacteria? If so, check dredged material for cyanotoxins and – depending on the results – also the crop.
5. Find out whether other exposure pathways to these cyanotoxins are likely (drinking-water or recreation)? If so, estimate the dose from these and determine the proportion from food which is most appropriate for your setting.
6. Estimate the contribution of the affected foods to the local diet and the time spans of their contamination with cyanotoxins.
   6.1. Is it consumed seasonally or year-round? On a daily basis, or occasionally? Are exposure patterns likely to be short term and occasional (justifying assessing exposure in relation to a short-term tolerable daily intake, TDI) or more likely to be continuous for many weeks on end and several times a week (necessitating application of a TDI for chronic lifetime exposure)?
   6.2. Estimate the amounts consumed and the impact of local traditions for collecting and preparing these foods on exposure pathways.
7. Clarify the tolerable cyanotoxin dose from food in the local setting together with toxicologists, taking points 5 and 6 into account. Note that in deriving the WHO guideline values for chronic exposure via drinking-water, WHO apportioned 20% of intake to other sources, including food, while the short-term values are based on exposure only to drinking-water. As discussed above (see point 5 of this checklist), this apportionment may need to be adjusted locally, depending on other exposure routes and the contribution of foods containing cyanotoxins to the local diet.
   7.1. From the results of local analyses and/or published data on the potential toxin content of these foods (see Table 5.5 and section 5.3.2) and the dose found tolerable for food in your setting, estimate how likely the cyanotoxin contents in the edible parts of these organisms are to exceed that tolerable dose and by how much.
   7.2. If restricting access to fish, mussels and shellfish is considered, what are the consequences for overall local diet? Are suitable alternatives available, accessible and accepted?
   7.3. If restricting access to fish, mussels and shellfish is considered and access to alternative protein food sources is poor or in question, how high is the uncertainty of the information base on cyanotoxin content in these foods? Does the information show a sufficiently substantial risk to justify the loss of this food source?
8. Are measures in place to control cyanotoxin contamination of food or exposure to potentially contaminated food (see Table 5.5)? Are they sufficient, or are further measures needed?

5.3.3 Assessing and managing exposure via food

Cyanobacterial metabolites may also cause a musty or earthy taste of fish ("tainting"; see section 2.9). While this is a mere quality issue with no direct health relevance, it does indicate that cyanobacteria – and thus cyanotoxins – may be present. This may serve as a warning signal, but it is not a reliable one: cyanotoxins may well occur without the presence of taste-and-odour compounds, and other organisms such as Actinomycetes may also cause tainting. Therefore, the absence of a musty taste is not a reliable indicator of the safety from cyanotoxins.

The use of waterbodies for aquaculture or fisheries usually is not the primary cause of excessive nutrient concentrations leading to cyanobacterial blooms and cyanotoxin occurrence. However, these activities may augment nutrient loading to the waterbody they use, particularly where aquaculture or fisheries are intensive (Rickert et al., 2016). Flow-through aquaculture systems may drain into the waterbody which they also tap to feed their basins or ponds, thus contributing to the waterbody's nutrient load. Fisheries may involve fertilising ponds and lakes (including with manure, organic wastes or agricultural byproducts) in order to augment fish production. Feeding may significantly contribute to the nutrient load to the waterbody, thus enhancing cyanobacterial blooms. Cage culture ("net-pen") systems rear animals in cages or nets floating within the waterbody, thus adding feed directly into the waterbody.

For commercial food production, control measures can be taken in planning, design and during operation (Table 5.6). In planning, they may involve land-use and waterbody management to avoid cyanobacterial proliferation (see Chapters 7 and 8), or, where this is not sufficiently successful, (re)locating aquaculture to sites where cyanotoxin levels are low. Where fisheries or aquaculture are a major cause of eutrophication, permits limiting size of stock and amounts of feeding may be appropriate in order to control eutrophication of the waterbody. Fish rearing systems may be designed to recirculate the used water back to the fish rearing unit through a treatment system which removes nutrients (and other harmful substances such as antibiotics). Control measures for the operation of fish rearing systems include regular removal of sludge from basins, ponds and water treatment units in order to remove nutrients which otherwise would supply cyanobacterial growth (see also Rickert et al., 2016).

Where food production is continued even though cyanobacteria occur, further control measures may be required in order to keep toxin concentrations in food below hazardous levels. Typically, they involve public information and creating awareness, particularly for subsistence fisheries. Keeping the live animals in clear water for a depuration period of a few days may be a Critical Control Point in a producer's HACCP plan. Depuration of microcystins in various marine and freshwater mussels has been shown

to occur within days to a few weeks – although small amounts may remain for periods longer than this (Dionisio Pires et al., 2004). Thus, possibly a couple of weeks after blooms have disappeared, eating shellfish may be safe again, but the fragmented knowledge about depuration does not allow this to be generally assumed, and it is therefore important to verify that concentrations are safe with appropriate analytical techniques.

Another control measure may be to remove the body parts of the animals which contain high cyanotoxin concentrations, that is, viscera and liver of fish or the guts and hepatopancreas of crayfish and mussels, before they are sold on the market, or to inform consumers of the need to do so. This control measure, however, cannot always be applied, so that some animals will be eaten whole (e.g., bivalves, snails, small fish such as smelt). In such cases, where cyanobacteria occur seasonally, harvesting animals can be restricted to seasons with low cyanobacterial occurrence, and operational monitoring will check that they are not marketed during these seasons. Where seasonal patterns are less reliable, harvesting may be restricted when simple indicators show that levels of cyanobacteria – or cyanotoxins – have exceeded a predefined limit. Monitoring of cyanotoxin concentrations in the animals harvested may also be an option to control exposure, as is the case in Victoria, Australia, where authorities advise to refrain from consumption when concentrations exceed Alert Levels (Van Buynder et al., 2001; Saker et al., 2004).

Not many countries have regulated cyanotoxins in food from freshwaters and between those that do, values vary considerably: For MCs, five authorities give values ranging from 5.6 µg/kg for fish (France) to 51 µg/kg for molluscs; for CYN, three authorities give values from 18 µg/kg (two states in Australia) to 70 µg/kg (California, USA); for STX in fish, prawns and shellfish, the value of 800 µg/kg is the same in these two Australian states and in Canada and for ATX, only California, USA, gives a value set at 5000 µg/kg for fish (see Table 3.1.3.3 in Testai et al., 2016). Choices of control measures can be optimised depending on the conditions in the specific aquatic setting that lead to blooms, the local patterns of consumption potentially leading to exposure and the available institutional capacity for operational monitoring.

Table 5.6 shows some examples of measures to control cyanotoxin levels in food collected or farmed in waterbodies. It is important to emphasise that cooking (e.g., boiling, frying, microwave) offers no reliable protection against cyanobacterial toxins in food. Contrasting results published for MC vary from a decrease to no effect or even an increase after cooking (Testai et al., 2016). Table 5.6 also gives options for monitoring to ensure that the intended control measures are being implemented and that they are functioning during day-to-day operations, as required both for HACCP and Water Safety Plan (WSP). Further information can be found in Rees et al. (2010).

Table 5.6 Examples of control measures for the commercial production of fish, crayfish and mussels and of options for monitoring their implementation and functioning

Process step	***Examples** of control measures for food*	*Options for their operational monitoring and/or verification 3.4*
Planning	For measures to control cyanobacteria through catchment management, land-use planning and waterbody management, see Chapters 7–9	
	Designate sites with low levels of cyanobacteria for harvesting and/or farming aquatic organisms as well as for abstracting water for irrigation	Conduct periodic site inspections during the cyanobacterial growing season
	Require permits for location, design and operation of aquatic farming operations (e.g., net-pens) and fish stocking	Review (application for) permit with respect to adequacy of choice of site, planning and operation
	Plan intensive land-based aquaculture systems with treatment of the outflow (e.g., in a wetland) to avoid eutrophication	Inspect outflow and check for illicit direct flow to the waterbody
	Plan irrigation schemes to avoid direct contact between water containing cyanobacteria and the crop to be consumed	Review plans
Design, Construction and Maintenance	Design aquaculture as closed recirculation system with treatment, aeration, sustainable stocking rates and controlled feeding rates	Conduct visual site inspection; review management plan for stocking and feeding rates (require development, if nonexistent)
	Avoid discharge of untreated effluent – treat it or use it as liquid fertiliser on crops	Monitor effluent flow; review information about its designation
	Construct and maintain particle traps in tanks (with separate sludge outlet) and collect waste from cages	Inspect structures; require records of waste collection and review them regularly
	Design irrigation systems as drip or ditch systems without direct crop contact; abstract water for irrigation outside of scum areas and depths as discussed in Chapter 9	Inspect abstraction points for irrigation water

(*Continued*)

Table 5.6 (*Continued*) Examples of control measures for the commercial production of fish, crayfish and mussels and of options for monitoring their implementation and functioning

Process step	***Examples** of control measures for food*	*Options for their operational monitoring and/or verification 3.4*
Operation	For cultures of aquatic organisms (e.g., net-pens), limit stock density and feeding to levels not likely to enhance eutrophication and thus cyanobacterial development	Inspect sites and enterprises for compliance with permits, for example, farm records for fish stock and food application
	Use low-polluting feed, high levels of lipid, lowered protein content, typically with high digestibility value, low in phosphorus	Inspect feed used; discuss criteria for its choice with operators
	If manure, fertilisers or wastewater are applied, base amounts on nutrient budget and optimise application times in relation to animal demand	Inspect materials applied; discuss practices with operator; if available, inspect records of application
	Remove viscera, liver or guts from organisms before marketing	Inspect products marketed
	Allow for depuration times of animals after exposure to toxin-containing cyanobacteria	Inspect enterprises for availability and functioning of facilities for depuration in clear water and for records of their use
	Restrict food collection during specific seasons for which cyanotoxin contamination is known and/or when Alert Levels (for cyanobacteria or for cyanotoxins) are exceeded	Monitor compliance with seasonal marketing restrictions or an indicator of cyanobacterial biomass or cyanotoxins in the water

Source: Modified from Rickert et al. (2016).

Which of these control measures – or others – are to be implemented in a given setting needs to be determined locally, depending on the specific natural and socioeconomic conditions. Implementation is most effective if the stakeholders involved collaboratively develop their specific management plans (e.g., WSP or HACCP Plans or a combination of both) in which they define the control measures and how their performance is to be monitored, as well as responsibilities, lines of communication and documentation requirements. For situations in which operational monitoring shows that a control measure is not operating adequately (i.e., within its predefined limits), management plans should include a description of the

corrective action to take. Note that the options for monitoring suggested in Table 5.6 focus on the functioning of the control measures rather than on cyanotoxin levels in food.

5.3.4 Verification monitoring of cyanotoxin levels in food from aquatic systems versus operational monitoring

As mentioned above, monitoring cyanotoxin levels in food from aquatic systems is most useful for risk assessment, that is, to inform planning and to adapt management strategies in the medium to longer term. It is also valuable for verifying whether the whole set of control measures implemented in a given situation is meeting its target. As discussed above, some countries have regulated cyanotoxin concentrations in food (Testai et al., 2016) which trigger immediate action: for example, food exceeding them may be banned from further marketing. Such consequences of violating limits may be useful to enforce improved control measures. However, the basis for day-to-day management is operational monitoring that checks the functioning of the control measures by methods such as regular inspection, where HACCP is implemented, in the context of HACCP management plans. This allows quick responses if it shows a measure not to be functioning within its boundaries, and many operational monitoring approaches are possible at low costs.

As discussed above, monitoring food products for cyanotoxin levels is more challenging than water, with most analytical techniques compromised by matrix (see Testai et al. (2016) for an extensive literature survey describing the chemico-analytical and biological methods available for sample preparation and detection in detail). Although readily available and easy to use, the ELISA format is inadequate for food testing without, at minimum, proper cleanup, quality controls (e.g., spiking) and confirmatory testing, particularly for commercially available ELISAs specifically intended to be used for water testing. ELISA should be considered a screening tool, requiring confirmation of identity, and quantity, with strategies employed to address extraction efficiency and bound analytes. Spiking subsets of material prior to extraction allows for an assessment of extraction efficiency. Adding analyte to aliquots immediately prior to testing (after extraction) will help determine if the extract matrix causes inhibition or nonspecific binding. If sample cleanup using solid-phase extraction and/or liquid–liquid extraction does not prevent matrix effects, a dilution series can be employed to assess such effects (although dilution can compromise the detection limit). When using ELISAs, the following need to be addressed, at minimum:

- inhibition resulting in underestimation and false negatives;
- nonspecific binding of matrix components to antibodies or antigen resulting in overestimations and false positives;

- varying reactivity to nontarget (but related) analytes, such as degradation products and metabolites resulting in overestimation;
- varying reactivity to target analytes of similar structure (e.g., microcystin congeners) resulting in overestimation or underestimation.

Other methods for the analysis of cyanotoxins (discussed in more detail in Chapter 14) include liquid chromatography (HPLC, LC, UPLC) coupled with various detectors, such as photodiode array/ultraviolet (PDA; UV), mass spectrometer (MS) or fluorescence detector (FL). LC-UV has been employed for microcystin (223–238 nm), cylindrospermopsin (262 nm) and anatoxin-a (227 nm), but detection limits may be insufficient, and if identification is based solely on peak retention time (without another in-line detector such as MS), this increases the chance of misidentification in complex matrices such as food. Higher interferences from matrix also hinder quantification, making LC-UV techniques inadequate for monitoring most food items. Even the use of single-quadrupole mass spectrometry (LC-MS) is prone to over-reporting microcystin in matrices such as fish tissues (Kohoutek et al., 2010). Highly specific LC-MS/MS methods are useful for the analysis of complicated matrices, which, with proper calibrations, are recommended or anatoxin-a/homoanatoxin-a and cylindrospermopsin. It is more difficult to fully account for all saxitoxins (STXs) (>57 analogues) using a targeted LC-MS/MS approach, with the sum of toxins detected possibly underrepresenting totals, although improvements to this analysis have been made (Turner et al., 2019). Therefore, until methods have been adequately developed to address STXs in freshwater-related food contamination, it is recommended that accepted methods for monitoring PSP in shellfish be used (e.g., Lawrence et al., 2005; AOAC, 2011a; b). Similar to STX, as discussed in Chapter 14, targeting microcystin congeners with MS is limited by the availability of standard reference material, unless the water source has been thoroughly characterised and the microcystin congeners are known. In order to assess fractions bound to either proteins or thiols, thiol-deconjugation (Miles et al., 2016) or the MMPB technique (see above) can be used. However, careful calibration for the MMPB method requires preoxidation spiking with intact microcystin to properly account for oxidation efficiency and recovery, increasing the time needed for preparation and analysis.

In summary, while monitoring cyanotoxins with relatively few structures (e.g., cylindrospermopsin) can be easily achieved, monitoring the microcystins and saxitoxins is significantly more complicated. A useful approach is to screen for food items with, for example, ELISA (which are available for most cyanotoxins) and if this indicates levels of concern in food items and quality assurance controls indicate the test is performing properly, to confirm the data with a further test. These can be related to toxicity (e.g., receptor-binding assay or protein phosphatase inhibition

assay; see Chapter 14) or more targeted analyses of cyanotoxins (e.g., LC-MS/MS). Appropriate calibration standards (including internal standards) should be utilised, with certified reference materials used where available.

5.3.5 Balancing cyanotoxin risks against the risk of malnutrition

A critically important public health aspect when deciding which control measures to implement is their possible impact on the nutritional status of the population which may depend on fisheries and/or aquaculture as key protein source. This needs to be balanced against the health risks through cyanotoxins possibly contained in these foods. Restriction may well prove scarcely feasible where intensive aquafarming or angling is needed as basis for the population's protein supply. On the other hand, the published information on cyanotoxin concentrations in food (Table 5.5) indicates a fair likelihood that these may well be below concentrations of potential concern, although in exceptional cases like fishermen on Lake Chaohu exposure clearly is likely to be elevated (Chen et al., 2009a). In consequence, before taking measures with a potentially major impact on peoples' livelihoods and nutritional status, it may be worthwhile to assess the relevance of such foods as staple protein source for a population and to invest in a survey to sample and analyse cyanotoxin concentrations in the local produce from fisheries and aquaculture in order to avoid undue restrictions causing more harm than good. A critical issue to consider here is that cyanotoxin concentrations in food produce varies greatly between points in time. A further point to consider, albeit challenging, is the risk of exposure to multiple toxins as well as to multiple sources, that is, exposure via food augmented by toxins in drinking-water and/or recreational use of waterbodies with blooms.

5.3.6 Public awareness and information

For small-scale commercial and particularly for recreational, noncommercial angling and harvesting of invertebrates from aquatic systems, effective controls are difficult to implement, and creating public awareness of potential risks may be a more effective or the only feasible approach. In contrast to cyanotoxins in freshwater, for the marine environment, public awareness of "algal toxins" is well developed in many regions: for example, native Americans already warned early settlers in the USA not to eat shellfish in the summer months. Today, among tourists or other non-natives, marine bivalves cause disproportionately high numbers of cases of paralytic shellfish poisoning, and this is attributed to tourists' disregard for either official quarantines or traditions of safe consumption, both of which tend

to protect the local population (see Ibelings & Chorus, 2007). Many of the states in the USA and Australia and countries in Europe host hotlines with information for shellfish collectors. South Australia classifies collecting sites for shellfish in four categories: approved, conditionally approved, restricted and fully restricted. This approach is familiar from other contaminants: for example, banning fishing in certain waterbodies to avoid consumption of pathogen-contaminated or of mercury-contaminated fish. A further option is issuing quantitative advisories on the amount that may be safely consumed or the frequencies at which fish may be eaten (e.g., US EPA, 2017).

Public awareness approaches that have been successful for seafood from marine environments can be similarly applied to cyanotoxin risks from freshwater environments, from collecting shellfish and snails or catching fish where water is visibly greenish or covered by scums. Information campaigns successful elsewhere are best adapted locally or regionally, since the type of food varies greatly between different geographic regions. Information particularly needs to reach specifically sensitive subpopulations, for example, in the case of cyanobacterial hepatotoxins persons with chronic hepatitis or other liver disorders. Also, information campaigns about using food from waterbodies with cyanobacteria may be effectively combined with information on their recreational use. See Chapter 15 for more information on public communication and participation targeting toxic cyanobacteria.

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5.4 RENAL DIALYSIS

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Renal dialysis patients are a group of the population with a specific and increased risk of cyanotoxin poisoning. The exposure pathway through haemodialysis is intravenous and to a large water volume – approximately 120 L are used in each treatment, three times per week. Hence, this group can be affected even by cyanotoxin concentrations far below the lifetime guideline values (GVs) for drinking-water.

According to Couser et al. (2011), approximately two million people are receiving haemodialysis worldwide, of which 90% live in North America, Japan and Europe. Dialysis is not regularly available in low-income countries, mainly due to a limited access to medical assistance.

In a disastrous incident early in 1996 in Caruaru, Brazil, 131 dialysis patients were exposed to cyanotoxin-contaminated water. Of these, 116 people experienced symptoms, including visual disturbances, nausea and vomiting, 110 developed acute liver failure, and 60 deaths were attributed to acute intoxication by cyanotoxins (microcystins and cylindrospermopsin) from water used for haemodialysis treatment (Jochimsen et al., 1998; Carmichael et al., 2001; Azevedo et al., 2002).

In a second episode of human microcystin exposure by the intravenous route documented among patients undergoing dialysis (Soares et al., 2006), a complete water treatment system including reverse osmosis, operating according specific procedures for dialysis use, proved insufficiently safe to prevent microcystin exposure. Notably, in this case, the microcystin concentration in drinking-water distribution system of the city was below the provisional WHO drinking-water guideline value (GV) of 1 μg/L.

In face of the 100-fold higher water volume to which dialysis patients are exposed, tolerable concentrations in dialysis water would correspondingly need to be at least 100-fold lower. Additionally, however, with oral exposure, only a fraction of the cyanotoxins is efficiently absorbed by the gastrointestinal tract through an active transport involving organic anion transporting polypeptides (OATP; Shitara et al., 2013). This process is saturable and affected by the presence of other chemicals and dependent on the relative affinity of individual compounds (Fischer et al., 2005; Fischer et al., 2010). In contrast, if exposure is intravenous, the systemic bioavailability is close to 100%. Therefore, in face of the present sparse quantitative understanding of the kinetics of sublethal doses of cyanotoxins in humans, especially for renal disease patients, it is not possible to establish threshold values for the induced adverse effects, and thus, no GVs for cyanotoxins in water used for dialysis can be derived. Certainly, however, the GVs for cyanotoxins in drinking-water are not sufficiently protective.

Hazard analysis for cyanotoxins in water used for hemodialysis therefore needs to assess the source of the raw water. Surface water potentially containing even traces of cyanotoxins needs to be avoided whenever possible.

5.4.1 Assessing and controlling the risk of cyanotoxin exposure

The WHO guidelines for Drinking-Water Quality (WHO, 2017) 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 effective in removing cyanobacterial cells, but may not be sufficiently effective in removing or destroying dissolved cyanotoxin concentrations to below GVs, especially from water supplies with a high organic content and cyanobacterial dominance (see Chapter 10). Consequently, clinics and hospitals with special water needs, such as for dialysis treatment or for transfusions (intravenous administration), often apply additional water treatment, for example, for the removal of cyanotoxins. Such treatment ranges from granular activated carbon filtration, followed by reverse osmosis, to more elaborate treatment, including membrane filtration. 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 manufacturer's specifications. It is important that manufacturer specifications should be assessed under local conditions for their adequacy in maintaining performance. Activated carbon, for example, may be exhausted for its ability to remove cyanotoxins long before it reaches saturation for the removal of other organic compounds, and some manufacturers may be unaware of this.

As emphasised above, the present knowledge about toxicity of different cyanotoxins does not allow establishment of any safe concentration for intravenous exposure. Therefore, a monitoring programme for water quality used for dialysis procedure needs to be performed with methods of utmost sensitivity (see Chapter 14).

Contingency plans and actions for prevention or management of health hazards from cyanotoxins for this specifically susceptible subpopulation are usually developed and managed at local or regional level. Additionally, national authorities may have important roles in organising, supporting and facilitating plan formulation, particularly after an event of suspected or

proven intoxication. Some key actions for preventive management of these special water uses include the following:

- Establishment of a multiagency and multidisciplinary regional committee with participation of public health authorities, water supply managers, hospital and dialysis clinic technically responsible for elaborating an effective plan of communication about incidents of cyanobacteria blooms in water supplies and cyanotoxins levels in drinking-water system used to supply health units. This communication plan needs to guarantee information about cyanotoxins concentration in drinking-water distributed to hospital and dialysis clinics within less than 24 h.
- A compilation of information about reservoirs or rivers used as water supplies to each community and a comprehensive map of the water distribution system, including location of hospitals and dialysis clinics needs to be available to health authorities to support any contingency plan when it is needed. The data of basic limnological parameters monitored in water supplies, including phytoplankton density (with special emphasis on cyanobacterial biovolume or cell numbers per litre) should be up to date. Interagency cooperation, especially between the drinking-water supplier and the health authority, is crucial to prevent an incident.
- If cyanotoxins are detected in drinking-water used to directly prepare water for dialysis or infusions, even in concentration well below lifetime GVs, a contingency plan to supply alternative safe water to health units needs to be implemented immediately. It should be previously developed and established, including specific actions and responsibilities of different actors. It needs to include previous identification of potential alternative water supplies, preferably from uncontaminated groundwater; plans for transporting safe water from other areas or deploying portable water treatment systems.
- In case an alternative safe water supply is not available, the dialysis service should be interrupted and patients should be transferred to other health units with no risk of exposure from the dialysis water. In this situation, the dialysis unit potentially exposing patients to cyanotoxins needs to be thoroughly cleaned, including exchange of activated carbon and the cleanup of all filters and membrane systems used.
- A regular monitoring programme for cyanotoxin analysis in the in-house water treatment systems of a dialysis unit should be implemented in regions where cyanobacterial blooms occurrence in water supplies cannot be excluded because no source water without potential contamination is available, particularly if blooms were detected during the past 12 months. This analysis needs to include sampling of

water before and after the treatment steps in order to assess treatment performance. It requires a highly sensitive methodology which can detect cyanotoxins in the nanogram per litre range.
- Preparing a standardised press release (previously agreed between the authorities which need to be involved) and an agreement on the triggers for its publication can help inform patients early in an incident, if one occurs.

Guidelines for quality assurance of dialysis equipment and fluids generally are more focused on (heterotrophic) microbial and chemical contaminations (e.g., Kawanishi et al. (2009); Penne et al. (2009)). However, best practice standards for the production of pure or ultrapure water for renal dialysis do apply to all chemicals (Ledebo, 2007).With respect to cyanobacterial toxins, dialysis units need to inquire from the water supplier whether there is a risk of cyanotoxin contamination in drinking-water, either seasonally or for extended periods. In this case, periodic use of an alternative water source may be a way forward if the water source cannot permanently be altered.

More information on quality control for dialysis, including fluid quality, is available on the websites of the US National Kidney Foundation (https://www.kidney.org) or the European Renal Association – European Dialysis and Transplant Association (https://www.era-edta.org). Guidelines of the latter can be found in a supplement issue of "Nephrology Dialysis Transplantation" (ERA-EDTA, 2002). Further information and guidelines are given in ISO 11663 (ISO, 2009) and the standards cited therein.

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5.5 CYANOBACTERIA AS DIETARY SUPPLEMENTS

Daniel Dietrich

Cyanobacteria, specifically *Arthrospira* sp. (previously classified as *Spirulina* sp.; see Chapter 3), were used as a food staple by indigenous people in Central America and in the Rift Valley of Africa. Large-scale production of cyanobacteria and microalgae for marketing in western society started about 50 years ago. Much of the early research work dealt with the basic photosynthetic properties of microalgae, their possible therapeutic, antibiotic and toxic properties and their potential as an agricultural commodity for human consumption. The microalgae biomass industry now provides biomass for pigments and speciality chemicals used primarily in the food industry and, more recently, as food supplements, also termed health foods, nutraceuticals, esoteric foods or simply blue-green algal supplement (BGAS). These mostly originate from three filamentous genera of cyanobacteria: *Arthrospira* (*Spirulina*), including *A. platensis* and *A. maxima* (Belay & Ota, 1994), *Nostoc* (*N. commune* and *N. flagelliforme*) and *Aphanizomenon flosaquae*.

While *Arthrospira* is grown in cultures, often in outdoor ponds, mainly in the USA (southern California and Hawaii), Chad, France, Mexico, Myanmar, Thailand, Taiwan and Japan, *Nostoc* (*N. commune*) is either grown by indigenous people as food staples, also known as *llullucha* (Johnson et al., 2008) or as dietary food supplements in South-East Asia (Saker et al., 2007) and China (Gao, 1998) while *Aphanizomenon* is primarily harvested from a dammed natural lake (Klamath Lake, Oregon, USA; Carmichael et al., 2000). Production of food-grade "*Spirulina*" largely depends on the production region, for example, the UN estimates approximately 250 tons/year to be produced in Chad for sale on local markets, while Henrikson (2011) estimated the internationally oriented commercial enterprises to produce more than 500 tons/year. The production volumes of *Nostoc* are presently unknown and cannot be extrapolated from sales or consumption data, as these are missing as well.

Aphanizomenon production is also substantial; however, data on production volumes have not been possible to obtain. The only indication of the amounts of *Aphanizomenon flosaquae*-based dietary supplements is their annual sales, which range in the tens of millions US dollars (ODA, 2017).

5.5.1 Cyanotoxins potentially present in cyanobacterial food supplements

Cyanobacteria used as dietary supplements can be a source of cyanotoxins even when the main ingredient is considered nontoxic, such as *Arthrospira maxima*. Nonetheless, some studies suggest a potential for "*Spirulina*" products to contain cyanotoxins, possibly via contamination of cultures with

other, toxigenic cyanobacteria: the anatoxin-a analogs epoxyanatoxin-a and dihydrohomoanatoxin-a have been identified at concentrations ranging from nondetectable to 19 μg/g dry weight in "*Spirulina*"-based dietary supplements (Salazar et al., 1996; Salazar et al., 1998; Draisci et al., 2001). A market analysis demonstrated concentrations of anatoxin-a ranging between 2.50 and 33 μg/g, whereby these included products intended for human and animal consumption (Rellán et al., 2009). In alkaline crater lakes in Kenya, *Arthrospira fusiformis* was found to produce small amounts of both microcystins and anatoxin-a (Ballot et al., 2004; Ballot et al., 2005), and ELISA results were positive for microcystins in "*Spirulina*" food supplements, suggesting a contamination with a microcystin producer (Gilroy et al., 2000). There are no proven cases of human injury as a result of ingesting "*Spirulina*"-based food supplements, although these were proposed as the cause of liver injury of a 52-year-old Japanese (Iwasa et al., 2002). However, consumption of "*Spirulina*" as well as other cyanobacteria-based food supplements are frequently accompanied by massive diarrhoea, nausea, abdominal pain and skin rash (Rzymski & Jaśkiewicz, 2017).

Nostoc commune produced by indigenous people of Peru were found to contain β-methyl-amino-alanine (BMAA; Johnson et al., 2008). However, the analytical method used is now known to substantially overestimate BMAA concentrations, and the toxic potential of BMAA is debated highly controversially. The conclusion of section 2.7 of the present volume is that, at present, the weight of evidence suggests that BMAA is present in insufficiently high concentrations to cause neurogenerative diseases.

Aph. flosaquae can contain cylindrospermopsins, anatoxin-a and saxitoxins as well as toxicity not attributable to any of the known cyanotoxins (see Heussner et al., 2012, and Chapter 2). Although microcystin production has not been observed for *Aphanizomenon* sp., in natural blooms, *Aphanizomenon* sp. is often found associated with other cyanobacteria which are known to be toxigenic.

Common cyanobacteria associated with blooms of *Aphanizomenon* sp. are *Microcystis* sp. and *Dolichospermum* sp., that is, species that potentially produce microcystins (Ekman-Ekebom et al., 1992; Teubner et al., 1999; Wood et al., 2011; Shams et al., 2015; Chapter 4). Analysis of *Aph. flosaquae* samples taken from Lake Klamath for dietary supplement production demonstrated that approximately 80% of the samples taken between 1994 and 1998 contained >1 μg MC-LR equivalents per gram dry weight, which is the maximum acceptable content established by the state of Oregon in the USA (Gilroy et al., 2000). Further studies showed higher as well as lower microcystin contents (Table 5.7), which is partly attributed to shifts in taxonomic composition within the blooms in Lake Klamath dominated by *Aph. flosaquae*, in particular, the variable share of toxigenic *Microcystis* sp. in bulk phytoplankton biomass. The studies summarised in Table 5.7 show a trend to lower maximum microcystin contents over time.

Table 5.7 Microcystin concentration in *Aphanizomenon* sp. dietary supplements from the market

Number of Samples	*% samples exceeding 1.0 µg/g DW*	*Microcystin content µg/g DW*	*Detection method*	*Reference*
87	72	2.2–10.9	ELISA	Gilroy et al. (2000)
52	50	0–35.7 0–49.0 0–35.7	ELISA cPPA LC-MS/MS	Lawrence et al. (2001)
6	100	11–24.7	ELISA, cPPA HPLC	Schaeffer et al. (1999)
18	80	0.3–8.3 0.5–5.9	Adda-ELISA cPPA	Hoeger & Dietrich (2004)
12	33	0.1–4.7	ELISA	Saker et al. (2005) Saker et al. (2007)
26	35	<LoD–5.2	LC-MS/MS	Vichi et al. (2012)
10	60 50 40	<LoD–6.1 <LoD–11.0 <LoD–5.8	Adda-ELISA cPPA LC-MS/MS	Heussner et al. (2012)
60	6 7	0–3.0 <0.25–2.8	LC-MS/MS PPA	Marsan et al. (2018)

DW: dry weight; LoD: limit of detection; ELISA: enzyme-linked immunosorbent assay; cPPA: colorimetric protein phosphatase inhibition assay, HPLC: high-pressure liquid chromatography; LC-MS/MS: liquid chromatography–mass spectrometry; Adda-ELISA: enzyme-linked immunosorbent assay with a recognition antibody specifically directed against the Adda-moiety of microcystins.

5.5.2 Assessing and managing the risk of cyanotoxin exposure through food supplements

In the studies summarised in Table 5.7, maximum contents of microcystin per gram dry weight range between 3.0 and 49 µg/g, and therefore, a risk of exposure to cyanotoxins cannot be ignored. A detailed assessment, however, is difficult, firstly, because the manufacturer's recommendations for daily consumption vary widely from 0.5 to 15 g/day with some products indicating no maximum limit (Marsan et al., 2018) and, secondly, because individual consumption also varies and may largely exceed recommendations. However, based on reported possible toxin contents and a consumption of a few grams per day, exposure may well be at levels exceeding the provisional tolerable daily intake (TDI) of 0.04 µg/kg (see section 2.1) for adults and especially for children. Further, in deriving its drinking-water guideline values (GVs) for lifetime exposure, 20% of intake are allocated to sources other than drinking-water, which may not be appropriate for persons consuming cyanobacterial products on a regular basis (see sections 2.1 and 2.2). Dietrich and Hoeger (2005) discuss these aspects for

varying levels of microcystin contamination of food supplements and propose corresponding maximum amounts that can be safely consumed by infants, children and adults.

As with other health risks, animal poisoning indicate potential adverse health effects in humans (Hilborn & Beasley, 2015). The case of an 11-year-old female spayed pug dog, weighing 8.95 kg and presenting with abnormally high alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) activities and serious liver dysfunction, indicates uptake of a hepatotoxin. This dog was fed single to multiple daily rations of 1 gram of 100% certified organic *Aph. flosaquae* for approximately three and a half weeks. The analysis of the powder via LC-MS/MS revealed 0.166 µg/g of MC-LR and 0.962 µg/g of MC-LA, while no other MCs were reported (Bautista et al., 2015). Thus, the MC content would approximate the Oregon provisional guidance value of 1 µg/g dw (Gilroy et al., 2000). However, with an analytical method including more microcystin variants, as suggested in section 14.3, a higher actual total MC content may have been found. Further, neither the number of daily rations nor any further potential source of the dog's exposure – such as cyanobacterial blooms in a waterbody – are known, making it difficult to estimate retrospectively whether the undoubted exposure to microcystins through dietary supplements was enough to explain the observed symptoms in this single study on one animal.

A further issue in this context is the as of yet very incomplete understanding of the bioactivity of cyanobacterial metabolites beyond the known toxins. Underdal et al. (1999) found protracted toxic response in test animals exposed to extracts of *Aph. flosaquae* but could not identify any toxins. Similarly, Heussner et al. (2012) found cytotoxicity in *Aph. flosaquae* product extracts that were not associated with any of the known cyanobacterial toxins. Indeed, particularly *Aphanizomenon* species are known for inducing effects not yet explained by any identified cyanobacterial metabolite, for example, malformation of fish embryos (Oberemm et al., 1997; Berry et al., 2009). While such effects cannot be quantitatively used for a human health risk assessment, they do indicate potential presence of further hazards to clarify.

Further, field collections of cyanobacteria and, possibly to a lesser extent, cyanobacteria harvested from open tanks contain a high diversity of heterotrophic bacteria, including human pathogens (Berg et al., 2009) that may cause further health hazards.

5.5.3 Approaches to assessing and controlling the potential cyanotoxin hazards

The regulation of dietary supplements is generally less strict compared to regulations for food, pharmaceutical or drinking-water, and only few regulatory schemes are in place. For example, since 1994, dietary supplements

have been regulated in the USA under the Dietary Supplement Health and Education Act (DSHEA; FDA, 2017). Because cyanobacteria are capable of producing toxins and their presence has been confirmed in some dietary supplements, it is appropriate to regulate and monitor these toxins in dietary supplements, including the provision of adequate information to consumers. Considerations include the following:

Testing for cyanotoxin content: Biomass collected from natural blooms or open tank incubators should be tested, lot by lot as recommended by the regulatory authority, for possible contamination with potentially toxigenic cyanobacteria, for example, *Microcystis* sp. in blooms dominated by *Aph. flosaquae*. Production lots should be managed by unique identifying numbers and production dates. For potential subsequent reanalysis by regulatory authorities, producers should be mandated to retain representative samples of each charge produced and to make these available upon official request.

Testing for other contaminants: Dietary supplement products should be tested for other potential contaminants, including indicators for pathogenic bacteria and protozoa, where and when contamination is expected. This is best based on an assessment of contamination risks from the catchment or the culture conditions. Examples of contamination sources include excreta of migrating birds or surface runoff following rainfall.

Claims on possible effects: The proposed beneficial effects of the consumption of cyanobacterial food supplements have not been demonstrated in scientifically sound studies; only subjective and anecdotal evidence is proposed by the vendors. Therefore, product information should not suggest that consumption of larger amounts would produce more positive effects.

Consumer information: Producers should clearly inform the consumers which quality control procedures are in place and give access to the test results. Further they should give a clear maximum daily doses, specified for infants, children and adults. None of these measures, however, can serve to protect from negative effects of known and yet unknown bioactive substances in cyanobacteria, as discussed in section 2.10.

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