Global Water Research Coalition

Management Strategies for Algal Toxins

An international review

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IWA affiliate

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Global Water Research Coalition: Global cooperation for the generation of water knowledge

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Management Strategies for Algal Toxins

An International Review

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Executive Summary

The *Management Strategies for Toxic Blue-green Algae: Literature Review* is a comprehensive literature survey, which consolidates current knowledge on the management of toxic blue-green algae (cyanobacteria). The survey covers all aspects of published research on the management of source water and all stages of the treatment process.

Chapter 1 provides an introduction to cyanobacteria including an outline of the health effects associated with toxic cyanobacteria. The guidelines and standards that relate to toxic cyanobacteria have been described as well as their application to management through a structured framework.

Chapter 2 covers source water management. This includes a description of the life cycle of cyanobacteria and factors affecting growth to help the water supplier understand the effect of management strategies for the control of cyanobacteria. Sampling and monitoring programs, including current benchmark monitoring protocols are covered. An overview of the best procedure for assessing the risk of toxic algal outbreaks in a water supply and a description of the alert levels framework are covered. An evaluation of aerators, mixing strategies and algicides cover topics for source water management practices.

Chapter 3 covers treatment strategies. This includes advice on conventional treatment, oxidation by chlorine and ozone and adsorption by granular activated carbon (GAC) and powdered activated carbon (PAC), biological filtration, UV and membranes and multiple barrier options.

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Abbreviations

ADWG	Australian Drinking Water Guidelines
ARMCANZ	Agriculture and Resource Management Council of Australia and New Zealand
ALF	Alert Levels Framework
CCL	Contaminant candidate list
DOC	Dissolved organic carbon
ELISA	Enzyme-linked immunosorbent assay
EPA	Environment Protection Authority
GV	Guideline value
HPLC	High performance liquid chromatography
LC-MS	Liquid chromatography-mass spectometry
LOAEL	Lowest observed adverse effect level
LPS	Lipopolysaccharides
MAC	Maximum acceptable concentrations
MAV	Maximum acceptable values
MCL	Maximum contaminant levels
NHMRC	National Health and Medical Research Council
NOAEL	No observed adverse effect level
NSWBGATF	New South Wales Blue-green algae task force
NZ	New Zealand
OECD	Organisation for Economic Co-operation and Development
PAR	Photosynthetically active radiation
PMAV	Provisional maximum acceptable values
PPIA	Protein phosphatase inhibition assay
PVC	polyvinylchloride
SRP	Soluble reactive phosphorus
STX-eq	Saxitoxin equivalents
TDI	Tolerable daily intake
TP	Total phosphorus
WHO	World Health Organization

1 INTRODUCTION

Toxic cyanobacteria have been reported in twenty-seven countries and are found on all continents, including Antarctica. Cyanobacteria are recognised as a significant water quality problem and a hazard in regard to their potential to contaminate water intended for a range of beneficial uses, including drinking, recreational activity and agricultural purposes. Drinking water authorities world-wide are faced with the challenge of treating contaminated water, or the possibility of a toxic bloom occurring sometime in the future. The increase in knowledge of cyanobacterial toxins has led to toxic cyanobacteria being identified as an issue of concerns in water supply reservoirs (CRC for Water Quality and Treatment Occasional Paper 2, 1998).

Cyanobacteria produce a range of toxins and odour compounds which have a deleterious effect upon drinking water quality. The water supply problems associated with cyanobacteria include offensive tastes and odours and the production of toxins and their possible effects on public health. To effectively deal with the problems posed by cyanobacteria drinking water authorities require an integrated approach involving risk assessment, monitoring and water treatment (CRC for Water Quality and Treatment, Occasional paper 2, 1998).

Catchment management is an important tool for the control and reduction of nutrients into source waters. However, it is not covered in this literature review. A comprehensive summary of this topic can be found in Chorus and Mur (1999).

Since the apparent increase in both the incidence and awareness of algal blooms in Australia and overseas, and the recognition of the issue of toxicity, significant research has been conducted into their management. As a result there exists a wealth of published materials, both scientific papers and reports. This review endeavours to consolidate the available current knowledge on the management of cyanobacteria.

1.1 Health Effects

Historically exist well-documented and anecdotal reports exist of animal poisonings and deaths and human poisonings from drinking water contaminated with cyanobacterial blooms. Epidemiological evidence has shown symptoms of poisoning or injury that has been attributed to the presence of cyanobacterial toxins in drinking water (Kuiper-Goodman et al., 1999). Toxicological studies carried out on animals have provided information on the role of the toxins in poisonings and on their comparative toxicity (Kuiper-Goodman et al., 1999). Little is known of the scale and nature of either long-term or short-term effects of these toxins (NHMRC ref vol 2). Therefore the health significance of algal toxins in water supplies is a key issue.

There are three main groups of cyanotoxins:

- cyclic peptides,
- alkaloids, and
- lipopolysaccharides.

Table 1 lists the major target organs of these toxins, and the cyanobacteria that produce them. Although the toxins listed are assumed to be the substances most significant for human health, it is unlikely that all cyanotoxins have been discovered. Researchers are also identifying many compounds from cyanobacteria and marine phytoplankton that could be used medicinally (Carmichael, 1997; Skulberg, 2000).

Table 1 General features of the cyanotoxins (Sivonen and Jones, 1999)

Toxin Group	Primary target organ in mammals	Cyanobacterial genera
Cyclic peptides		
Microcystins	Liver	*Microcystis, Anabaena, Planktothrix (Oscillatoria), Nostoc, Hapalosiphon, Anabaenopsis*
Nodularin	Liver	*Nodularia, Anabaena, Planktothrix (Oscillatoria), Aphanizomenon*
Alkaloids		
Anatoxin-a	Nerve synapse	*Anabaena, Planktothrix (Oscillatoria), Aphanizomenon*
Anatoxin-a(S)	Nerve synapse	*Anabaena*
Aplysiatoxins	Skin	*Lyngbya, Schizothrix, Planktothrix (Oscillatoria)*
Cylindrospermopsin	Liver	*Cylindrospermopsis, Aphanizomenon, Umezakia, Raphidiopsis, Anabaena*
Lyngbyatoxin-a	Skin, gastrointestinal tract	
Saxitoxins	Nerve axons	*Anabaena, Aphanizomenon, Lyngbya, Cylindrospermopsis*
Lipopolysaccharides (LPS)	Potential irritant; affects any exposed tissue	All

1.1.1 Cyclic peptides: microcystins and nodularin

Characteristics and occurrence
The cyclic heptapeptides contain a specific amino acid (ADDA) side chain, which to date has been found only in microcystins and nodularin (a cyclic pentapeptide toxin of cyanobacteria from brackish waters). Microcystins are the most frequently occurring and widespread cyanotoxins, with about 70 structural analogues identified so far (Rinehart et al., 1994; Sivonen and Jones, 1999). They vary with respect to their structure and this results in pronounced differences in toxicity.

Microcystins are found in most populations of *Microcystis* spp., which frequently form surface scums, and in strains of some species of *Anabaena*, which may also form scums. Furthermore high microcystin content has been observed in *Planktothrix* (syn. *Oscillatoria*) *agardhii* and *P. rubescens* (Fastner et al., 1999). However, *P. agardhii* never forms scums, and *P. rubescens* usually do not form scums. In Australia, microcystins are the most significant public health issue associated with cyanobacterial blooms in south-eastern Australia (NHMRC/ARMCANZ, 2001).

Toxicity varies between different populations of *M. aeruginosa*. However one extensive survey of toxicity across the Murray-Darling Basin indicated that 56% of field samples tested were hepatotoxic (Baker and Humpage, 1994). A natural population may consist of a mixture of toxic and non-toxic strains and this is believed to be the reason why population toxicity may vary over time and between samples (Chorus and Bartram, 1999).

For microcystins, it has been shown that toxicity of a strain depends on whether or not it contains the gene for microcystin production (Rouhiainen et al., 1995; Dittmann et al., 1996), and that field populations are a mixture of both genotypes – those with and without this gene (Kurmayer et al., 2002). Experience with cyanobacterial cultures also shows that microcystin production is a fairly constant trait of a given strain or genotype, only somewhat modified by environmental conditions (Chorus, 2001).

Nodularin is produced by the cyanobacterium *Nodularia spumigena*, which is regarded as primarily a brackish water species and is known to form blooms in estuarine lakes in Australia, New Zealand and Europe. It can also occur in brackish inland lakes in Australia (Wood, 1975). In addition to these saline environments, there are frequent blooms of toxic *Nodularia spumigena* in freshwater lakes of the lower River Murray, South Australia (Baker and Humpage, 1994). This is a relatively

rare circumstance where *N. spumigena* blooms in freshwater and is of particular importance as the water is used for potable supplies, irrigation and stock watering. Lake Alexandrina in South Australia was the site of the first scientifically documented animal poisoning by *N. spumigena*, and indeed by any cyanobacterium (Francis, 1878). It is likely that these poisonings and the toxic effects described by Francis were due to nodularin. Low numbers of *N. spumigena* have also been recorded in the other (freshwater) river systems of the Murray-Darling Basin. The limited geographic scope for blooms of this organism in freshwater in Australia makes the occurrence of nodularin a relatively minor public health threat in drinking water.

Toxic effects
The microcystins and nodularin are known to cause liver damage (hepatotoxins). They block protein phosphatases 1 and 2a, which are "molecular switches" in all eukaryotic cells, with an irreversible covalent bond (MacKintosh et al., 1990). For vertebrates, a lethal dose of microcystin causes death by liver damage within hours to a few days.

Fitzgeorge et al. (1994) demonstrated that microcystin toxicity is cumulative: a single oral dose showed no increase in liver weight (which is a measure of liver damage), whereas the same dose applied daily over 7 days caused an increase in liver weight of 84 percent and thus had the same effect as a single oral dose 16 times as large. This may be explained by the irreversible covalent bond between microcystin and the protein phosphatases, which leads to subsequent damage to cell structure (Falconer, 1993). Healing of the liver probably requires growth of new liver cells. Subacute liver injury is likely to go unnoticed for two reasons:

Liver injury shows externally noticeable symptoms only when it is severe. Acute dose-response curves for microcystins are steep. Therefore, little acute liver damage may be observed up to levels close to severe acute toxicity.

There are two potential mechanisms for long-term microcystin damage to the liver, progressive active liver injury as described above (Falconer et al., 1988), and the potential for promotion of tumour growth. Tumour-promoting activity of microcystins is well documented in animals, although microcystins alone have not been demonstrated to be cancer causing.

1.1.2 Alkaloids

The alkaloid toxins produced by cyanobacteria include a range of compounds that damage nerve cells (neurotoxins), including anatoxins, saxitoxins, and cylindrospermopsin, which is a recognised hepatotoxin, but also causes general cell damage (cytotoxin).

While the neurotoxins have a different modes of action, all have the potential to be lethal at high doses by causing asphyxia (an decrease in oxygen and increase in carbon dioxide causing unconsciousness and death) - anatoxin-a and anatoxin-a (S) through cramps, and saxitoxins through paralysis. However, no human deaths from exposure to neurotoxins are known.

A number of cyanobacterial genera can produce neurotoxins, including *Anabaena, Oscillatoria, Cylindrospermopsis*, *Cylindrospermum*, *Lyngbya* and *Aphanizomenon,* but to-date in Australia, neurotoxin production has only been detected from *Anabaena circinalis*, and the Australian isolates appear to produce only saxitoxins (Velzeboer et al., 1998). Blooms of *Anabaena circinalis* have been recorded in many Australian rivers, lakes, reservoirs and dams, and it is the most common organism in riverine blooms in the Murray-Darling Basin (Baker and Humpage, 1994). In temperate parts of Australia, blooms typically occur from late spring to early autumn. The first reported neurotoxic bloom of *A. circinalis* in Australia occurred in 1972 (May and McBarron, 1973). The most publicised bloom occurred in late 1991 and extended over 1,000 km of the Darling-Barwon

River system in New South Wales (New South Wales Blue Green Algae Task Force, 1992). A State of Emergency was declared with a focus on providing safe drinking water to towns, communities and landholders. Stock deaths were associated with the occurrence of the bloom but there was little evidence of human health impacts. Relatively low numbers of *A. circinalis* (below 2,000 cells/mL) can produce offensive tastes and odours in drinking water due to the production of odourous compounds such as geosmin.

Australian studies to date have indicated the presence of saxitoxins and cylindrospermopsin in cyanobacteria, while not finding any species containing the neurotoxins anatoxin-a or anatoxin-a (s) (NHMRC/ARMCANZ, 2001).

Cylindrospermopsin raciborskii is considered to be a tropical and subtropical species and it can be regarded as the major toxic cyanobacterium of concern in Queensland as it has been found in many water supply reservoirs in northern, central and southern Queensland. While *C. raciborskii* and *A. ovalisporum* are both predominantly tropical/sub-tropical in terms of habitat, both species occur in the Murray-Darling River system (Baker and Humpage, 1994) and have been shown to develop blooms in lakes in Sydney, Adelaide and also in south west Western Australia. Based upon international bio-geographic studies it has been proposed that *C. raciborskii* appears to be invading temperate regions (Padisák, 1997).

Cylindrospermopsin was first isolated from *Cylindrospermopsis raciborskii* (NHMRC/ARMCANZ, 2001; Ohtani et al., 1992). It is a general cytotoxin with a rapid onset of symptoms resulting in kidney and liver failure. Symptoms may become obvious only several days after exposure, so it will often be difficult to determine a cause–effect relationship. Results by Falconer and Humpage (2001) suggest that cylindrospermopsin may also act directly as a tumour initiator, which has implications for long-term exposure.

1.1.3 Lipopolysaccharides

Lipopolysaccharides (LPS) are an integral component of the cell wall of all cyanobacteria (as well as other types of bacteria), and help to determine and maintain the size and shape of the cell (Bertocchi et al., 1990). As LPS are always present in cyanobacteria it would appear to make LPS a potential issue of concern for exposure in recreational situations, relative to the other known toxins. These compounds have been shown to produce irritant and allergenic responses in human and animal tissues (Sivonen and Jones, 1999). They are pyrogenic (fever-causing agents) and toxic (Weckesser and Drews, 1979). An outbreak of gastro-enteritis is suspected to have been caused by cyanobacterial LPS (Lippy and Erb, 1976). Interestingly, however, cyanobacterial LPS are considerably less potent than LPS from some other types of bacteria such as *Salmonella* (Keleti and Sykora, 1982; Raziuddin et al., 1983).

1.1.4 Exposure

Exposure to cyanobacteria and their toxins can arise through three routes:

- Direct contact of exposed parts of the body (ie skin), including sensitive areas such as the ears, eyes, mouth and throat, and the areas covered by a bathing suit (which may collect cell material)
- Accidental swallowing of water containing cells
- Aspiration (inhalation) of water containing cells.

Rapid onset, short-term health effects are the most obvious health risk associated with cyanobacterial toxins and have been well documented with animal toxicity studies and bioassays to characterise toxicity. In a population there are individuals who are at greater risk of suffering a

health effect than others; for example, children who drink a higher volume of water in proportion to body weight than adult, or individuals who are at risk of injury to organs such as dialysis patients, or patients with liver disease. Experiments have also shown that long-term exposure to microcystins can result in liver injury and the possibility of tumour promotion and growth. Therefore it is the long-term health effects, with low levels or short term exposure to high levels of toxins that may also represent a significant health risk (Kuiper-Goodman et al., 1999). It is important to know if drinking water contains cyanobacteria and associated toxins, as the health implications are potentially considerable.

1.2 Guidelines and Standards

In the Australian context drinking water guidelines are not mandatory standards but are designed to provide "guidance on what constitutes good quality drinking water (as distinct from water which is acceptable)" (NHMRC/ARMCANZ, 1996). The NHMRC/ARMCANZ (1996) document also states that "guidelines provide a reference for use within the Australian administrative and legislative framework to ensure the accountability both of water authorities, as managers, and of State health authorities, as auditors of the safety of water supplies. The guidelines should not however, be construed as legally enforceable standards." The definition and intention for use of guidelines issued by the World Health Organisation (WHO) is essentially similar. Chorus and Bartram (1999) indicate that the primary aim of guidelines is the protection of public health. World Health Organisation Guidelines for Drinking Water Quality (WHO, 1993, 1996) represent a scientific consensus on the health risks presented by microbes and chemicals in drinking water and are used to derive guideline values. The aim of these guidelines is to protect public health, to ensure the safety of drinking water supplies by monitoring the constituents of water that are known to be hazardous to health. The guideline values are intended to be used in the development of risk management strategies. These values are associated with guidance on monitoring and management practices. The guideline values may be amended locally or nationally according to specific circumstances (Falconer et al., 1999).

In order to establish guideline levels it is necessary to determine the Tolerable Daily Intake (TDI). This is the dose level in humans that is considered to be without adverse effects when taken daily over a lifetime. This value is derived either from human studies, experimental databases or often from animal studies by using a lowest observed adverse effect level (LOAEL) or no observed adverse effect level (NOAEL) (from human or animal studies), divided by appropriate safety or uncertainty factors (Kuiper-Goodman et al., 1999). The guideline value for lifetime consumption of a chemical contaminant of drinking water can be calculated by applying the derived TDI to a typical daily water intake in litres by an individual of given body weight. The proportion of total daily intake of the contaminant which is ingested is considered, because some intake may come from other source ie. food (Falconer et al., 1999).

The WHO has set a provisional guideline value of 1.0 μg L^{-1} for microcystin-LR. The WHO determined that there was insufficient data to date to calculate a TDI and therefore derive a guideline value for cyanotoxins other than microcystin-LR. The basis for a provisional guideline value for microcystin-LR was that there was evidence of a potential hazard, but the information on health effects was limited (WHO, 1998).

The WHO guideline values are used as a basis for the Australian Drinking Water Guidelines (ADWG) as part of the National Water Quality Management Strategy coordinated by the National Health and Medical Research Council (NHMRC) and the Natural Resource Management Ministerial Council (NRMMC). They provide a framework for standards of acceptable drinking water quality. The guidelines aim to provide the Australian community and water supply industry with guidance on what constitutes good quality drinking water. They include the safety of water

from a health point of view (including microbiological, physical, chemical and radiological quality) and its aesthetic quality (including turbidity, colour, taste and odour) (ADWG, 1996).

In relation to cyanobacteria a working group completed a review of information on cyanobacteria and their toxins in relation to drinking water and public health in April 2000. This has resulted in the production of four "Fact Sheets" for individual classes of toxins: microcystins, nodularin, saxitoxins, and cylindrospermopsin (Fact Sheets 17a-17d) (NHMRC/ARMCANZ, 2001). At this time a guideline value recommended only for total microcystins (Fact Sheet 17a). The guideline recommends that the concentration of total microcystins in drinking water should not exceed 1.3 µg L^{-1}. No guideline values could be set for concentrations of nodularin, saxitoxins or cylindrospermopsin due to the lack of adequate data. In relation to lipopolysaccharides (LPS) produced by cyanobacteria, the working group concluded at an early stage of their work that there was insufficient information to carry out a critical assessment on occurrence and significance of LPS and did not produce a fact sheet.

The ADWG fact sheet for saxitoxins, which is produced predominantly by *Anabaena circinalis* in Australia, notes that taste and odours are also important water quality parameters for this cyanobacterium. *Anabaena circinalis* is well known to produce the offensive odorous compound, geosmin. This compound is detectable even at low numbers (below 2,000 cells mL^{-1}), but poses no known health risks.

Until 2002 there was insufficient data to derive guideline values for cyanotoxins other than microcystins (ie. nodularin, saxitoxins and cylindrospermopsin). However, a recent study was designed to determine the No observed adverse effect level (NOAEL) for cylindrospermopsin in male mice (Humpage and Falconer, 2002). The study followed the Organisation for Economic Co-operation and Development (OECD) protocols for NOAEL determination. From the NOAEL a TDI and from that a Guideline Value (GV) for cylindrospermopsin in drinking water was determined. The study has proposed that the guideline value for cylindrospermopsin in drinking water be set at 1.0 µg L^{-1} (Humpage and Falconer, 2002).

The guideline value is important for water supply authorities, as this value sets the amount of toxin tolerable in drinking water ie. at the tap. For some countries the level is a guideline level that is in the form of a recommendation from the health authorities. For other countries the level is a standard that must be complied with and compliance is monitored. Table 2 shows the current status of guidelines worldwide in relation to cyanobacteria and their toxins for drinking water.

As was previously described the health implications of cyanobacterial toxins require that the toxins in drinking water are detected when present. To do this water managers need to monitor by taking water samples of the water supply to test for the presence of cyanobacterial cells and toxins (refer Section 2.2 Sampling and Monitoring Programs).

Table 2 Guideline values or standards for cyanobacterial toxins in drinking water from various countries. (Information derived from websites unless otherwise stated)

Country	Guideline Value/Standard	Comments/Explanations
Africa	None found	Clean drinking water supply to all people main current focus
Asia Pacific Region	None found	Clean drinking water supply to all people main current focus
Australia	1.3 µg L^{-1} total microcystins GV	From the Australian Drinking Water Guidelines see section above for further explanations on the process of guideline derivation
Canada	1.5 µg L^{-1} cyanobacterial toxins as microcystin-LR MAC	Canada uses guidelines as the standard of water quality. The guidelines are expressed with the unit of Maximum acceptable concentrations (MAC). These are derived from tolerable daily intake (TDI) which in turn are derived from a calculated no-observed adverse effect level (NOAEL) from data from human or animal studies. To derive a MAC from a TDI adjustments are made for average body weight and drinking water consumption, as well as other considerations. In terms of health the guidelines ensure that the MACs are far below exposure levels at which adverse effects have been observed. For the case of cyanobacterial toxins the guideline is considered protective of human health against exposure to other microcystins (total microcystins) that may also be present
European Union	Assumed to be the same as WHO Drinking Water Guidelines. No specific values for cyanobacterial toxins found	Under microbiological parameters stated that water "should not contain algas" The standards are generally based on the WHO Guidelines for Drinking-water Quality. Parameters in terms of Maximum Admissible Concentration (MACs). No information found on the derivation of a MAC.
New Zealand	For cyanobacteria: <1 potentially toxic cyanobacterium present in 10mL of sample. PMAV for cyanobacterial toxins: Anatoxin (as STX-eq): 3.0 µg L^{-1} Anatoxin-a (S): 1.0 µg L^{-1} Cylindrospermopsin: 3.0µg L^{-1} LPS endotoxins: 3.0 µg L^{-1} Microcystins: 1.0 µg L^{-1} Nodularin: 1.0 µg L^{-1} Saxitoxins:1.0 µg L^{-1}	Maximum acceptable values (MAVs) for micro-organisms or organic determinands of health significance. Provisional MAV (PMAV) for cyanobacterial toxins. MAVs are based on the WHO 'Guidelines for Drinking Water Quality'. They are the concentration of a determinand, which is not considered to cause any significant risk to the consumer over a lifetime of consumption of water. The method of derivation varies according to NZ conditions and the way in that the determinand presents a risk. However they are derived with the use of a TDI. The MAVs are standards in NZ. The Standards provide compliance criteria and compliance is routinely monitored
South America (Brazil)	1.0 µg L^{-1} for microcystins 3.0 µg L^{-1} for saxitoxins 15 µg L^{-1} for cylindrospermopsin	Guideline values for microcystins, saxitoxins and cylindrospermopsin, along with biomass monitoring programs. Guideline value for microcystins adopted as mandatory. Guideline values for equivalents of saxitoxins and for cylindrospermopsin included as recommendations. Use of algicides prohibited. (Azevedo, 2001).
United Kingdom	See European Union	
United States of America	None currently known.	Maximum Contaminant Levels (MCLs) are the highest level of a contaminant that is allowed in drinking water. They are enforceable standards. Cyanobacteria and their toxins are listed as microbiological contaminants on the contaminant candidate list (CCL). This means that they are currently recognised as unregulated contaminants, but are known to occur in public water systems and may require regulation under the Safe Drinking Water Act. Contaminants on the CCL are a priority for the US Environmental Protection Agency with the aim to set MCLs
World Health Organisation	1.0 µg L^{-1} for microcystin-LR GV	Refer to World Health Organisation Guidelines for Drinking-Water Quality, 1996

2. SOURCE WATER MANAGEMENT

2.1 Growth of Cyanobacteria

Cyanobacteria (blue-green algae) are microscopic procaryotic photosynthetic organisms that form a common and naturally occurring component of most aquatic ecosystems (Van den Hoek et al., 1995). They can occur singly or grouped in colonies (Whitton and Potts, 2000) and occur in many and varied environments. In low numbers they are an important contributor to the aquatic biology of waterways but can increase to such large numbers that they can dominate when conditions become favourable, causing unsightly colouring of the water, thick scums, and at times unpleasant smells (Blue-green algae task force, 1992). A complex interaction of environmental factors has been shown to contribute to cyanobacterial growth. A detailed discussion on these processes is provided by Mur et al., (1999).

2.1.1 Nutrients (*adapted from Utkilen et al., 1999; Mur et al., 1999)*

Mass developments of cyanobacteria are generally associated with high nutrient concentrations. High phytoplankton density leads to high turbidity and low light availability, and cyanobacteria are the group of phytoplankton organisms that can grow best under these conditions.

Phosphorus is usually the key nutrient controlling proliferation of cyanobacteria in freshwater. Total phosphorus (unfiltered samples) determines the capacity of a water body to form cyanobacterial blooms. If dissolved phosphate (soluble reactive phosphate determined from filtered samples) is detected at concentrations of only a few micrograms per litre, cyanobacterial growth and biomass are not limited by phosphate and phosphorus may continue to be released from sediments for many years after external inputs have been minimised. Water exchange rates, sediment chemistry, temperatures, mixing conditions, and sediment disturbance govern the amount of phosphorus contained in the sediments. Thermal stratification can also result in substantial release of phosphorus from sediments. During stratified conditions, iron-bound phosphorus in the sediments can become a major source of phosphorus for cyanobacteria. Under oxygenated conditions (ie. well mixed water body) phosphorus-rich sediments are sealed by an oxidised surface layer involving an iron phosphorus complex. However, under stratified conditions (ie. unmixed water body) the sediment surface becomes anoxic due to bacterial decay of organic matter falling from upper layers. Under these conditions the complex breaks down, resulting in phosphorus release from the sediments.

As a simple guide, the influence of nutrient levels on cyanobacterial growth can be measured in terms of total phosphorus levels in the water body. In general, a total phosphorus level of 10–25 μgL^{-1} presents a moderate risk in terms of the growth of cyanobacteria. For levels of less than 10 $\mu g\ L^{-1}$ there is a low risk of cyanobacteria growth, and a level greater than 25 provides high growth potential.

Potential sources of nutrients include both human related activities and natural inputs such as:

- sewage outfall
- on-site or other private sewage disposal systems
- intensive agricultural activities resulting in possible run-off from untreated animal effluent
- rivers and streams
- urban stormwater.

2.1.2 Light *(adapted from Mur et al., 1999)*

Cyanobacteria contain the photosynthetic pigment chlorophyll *a*, but unlike other phytoplankton they also contain phycobiliproteins. These are light harvesting pigments that are able to harvest light in the green, yellow and orange part of the spectrum (500-650 nm). This enables cyanobacteria to harvest light energy efficiently. Cyanobacterial growth rates in high turbidity water are higher than that of green algae, which allows them to out compete green algae in highly turbid waters.

Both turbidity and stratification can influence the amount of light received by cyanobacteria in a water body. The light conditions in a given water body determine the extent to which the physiological properties of cyanobacteria will be of advantage in their competition against other phytoplankton. Generally, the zone in which photosynthesis can occur is termed the euphotic zone. By definition, the euphotic zone extends from the surface to the depth at which 1 percent of the surface light intensity can be detected. The euphotic zone can be estimated by measuring the transmittance of the water with a Secchi disk and multiplying the Secchi depth reading by a factor of approximately 2-3. Those cyanobacteria that can regulate their buoyancy via gas vesicles are able to overcome these problems by moving to water depths with optimal light conditions.

2.1.3 Temperature *(adapted from Utkilen et al., 1999)*

Cyanobacterial and algal growth rate are temperature dependent. Growth can occur at low temperatures although there is significant potential for growth above about 15^{o}C and maximum growth rates are attained by most cyanobacteria at temperatures above 25^{o}C. Temperature also has a large role in stratification of a water body.

2.1.4 Stratification of water bodies

Thermal stratification of a water body influences the depth at which cyanobacteria are likely to be found, the light levels they receive, and the concentrations of nutrients in the water body.

The shape and structure of lakes and reservoirs, the latitude, weather conditions and the physical nature of the water influence thermal stratification. Stratification can be determined by measuring vertical profiles of temperature within the water body. Where thermal stratification occurs, the water body functions as two separate non-mixing layers (the epilimnion and the hypolimnion), with a transitional layer (thermocline) in between (Figure 1). The two layers have different physicochemical characteristics. The upper, warmer, epilimnion can become wind-mixed and because of its exposure, can freely exchange dissolved gases (such as O_2 and CO_2) with the atmosphere. The hypolimnion, the bottom layer of colder water, is isolated from the epilimnion by the thermocline. The density change at the thermocline, caused by the temperature difference, acts as a physical barrier that prevents mixing of the upper and lower layers.

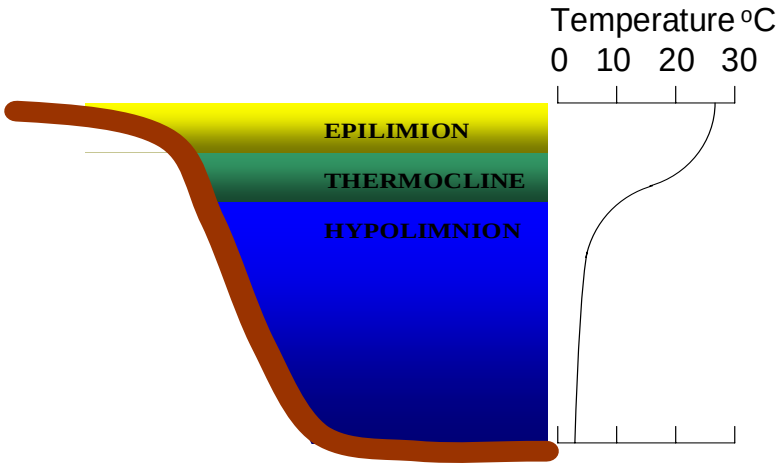

Figure 1 Cross section of a thermally stratified lake showing location of the epilimnion and hypolimnion and associated temperature changes

In temperate climates, thermal stratification generally occurs seasonally (summer-autumn) in water bodies of appropriate depth, whereas in tropical climates it often follows diurnal time patterns.

Stratification can also result in substantial release of phosphorous from sediments, causing an increase in the internal loading of the water body. This, in turn, can result in an increase in cyanobacterial biomass. During stratified conditions, sediment-bound phosphorous can become a major nutrient source for cyanobacteria. Under oxygenated conditions (ie. well mixed water body) phosphorous rich sediments are sealed by an oxidised surface layer of an iron phosphorous complex. However, under stratified conditions (ie. unmixed water body) the sediment surface becomes anoxic due to microbial activity in the sediments. Under these conditions the complex breaks down resulting in phosphorus release from the sediments (NHMRC, 2001).

Usually, shallow (eg. 2-3m), wind-exposed lakes are unstratified. Lakes of intermediate depth (eg. 5-7m) may develop transient thermal stratification for a few calm and sunny days, which is then disrupted by the next rain or wind event. In temperate climates deeper lakes can exhibit a stable stratification from spring to autumn. The formation of stratified conditions can influence light intensities experienced by cyanobacteria, bloom formation and nutrient levels in the water.

2.1.5 Cyanobacterial life cycle

The cyanobacterial life cycle involves the planktonic population and benthic resting stages or akinetes (Baker, 1999). It is important to note that only one type of cyanobacteria produces akinetes, these are the filamentous, heterocystous cyanobacteria (Order *Nostocales*). Akinetes are thick-walled reproductive structures (Fogg et al., 1973). They are found in sediments and are thought to provide a resting stage that may enable the survival of a species. They germinate when environmental conditions are appropriate, thereby providing a source of inoculum for subsequent populations, particularly from one season to the next (Baker, 1999).

The life cycle of akinete producing cyanobacteria can be summarised in a number of steps. First, the filaments of cyanobacteria growing by cell division. Akinete production and release follows, usually for the population to survive over winter. Finally, growth from the akinetes occurs, which is triggered by environmental factors, including light, with new cyanobacteria maturing and growing by cell division for the new season's population (Fogg et al., 1973; Baker, 1999).

Other filamentous or single cell/colonial cyanobacteria are not known to form akinetes or other resting stage structures. It has been suggested that some vegetative cells may rest over winter in a state of senescence in the sediment. New populations grow by cell division (P.Baker pers. comm.).

2.1.6 Potential for cyanobacterial growth and bloom formation

There are several factors that enable cyanobacteria to have a competitive advantage over other phytoplankton and become dominant. These are:

- Gas vacuoles for buoyancy regulation, which enable them to overcome spatial separation (due to stratification) between light and nutrients in the water column
- Nitrogen fixing capabilities in low nitrogen waters
- Some produce akinetes which reseed the waterbody.

Basic conditions that provide a measure of the likelihood of a cyanobacterial bloom occurring in a water body are summarised in Table 3. The values in this table are a guide only, other conditions may occur that support the formation of a cyanobacterial bloom.

Table 3 Potential for cyanobacterial growth

	Environmental factor			
Potential for Cyanobacterial Growth	History of Cyanobacteria	Water Temperature (^oC)	Nutrients: Total Phosphorus ($\mu g\ L^{-1}$)	Thermal Stratification
Very Low	No	<15	<10	No
Low	Yes	<15-20	<10	Infrequent
Moderate	Yes	20-25	10-25	Occasional
High	Yes	>25	25-100	Frequent and persistent
Very High	Yes	>25	>100	Frequent and persistent/strong

Previous identification of cyanobacteria in a water body is an important indicator of potential for future occurrence. For example:

- Have blooms of cyanobacteria been identified in the past?
- Have specific incidents, such as animal deaths or human illness, been suspected of being associated with exposure to cyanobacteria and their toxins?

The environmental conditions that favour the development of a bloom are:

- High nutrient levels, particularly phosphorus (P)
- Low N:P ratios (less than 29:1)
- High water temperature (above 20^oC)
- Abundant zooplankton (which may graze on other phytoplankton)
- Low flows (long retention times and calm weather)
- Reduction in turbidity to moderate levels and increased light intensity.

When all the above environmental conditions coincide it is possible for a bloom to occur. Under calm weather conditions many excessively buoyant cells or colonies may accumulate at the surface. Light winds drive them to leeward shores and bays, where they form scums. In extreme cases, such agglomerations may become very dense and even acquire a gelatinous consistency. More frequently, they are seen as streaks or slimy scums that may even look like blue-green paint or jelly.

These have been termed "blooms." Blooms distributed evenly throughout the upper water layer may be dense enough to cause visible discolouration. Scums, however, have frequently been reported to accumulate cells by a factor of 1000 or more; one-million-fold accumulations to pea-soup consistency are observed (NHMRC, in draft).

Bloom development consists of the seeding and initial development phase, followed by a rapid growth phase. The plateau phase and die-off phase follows this. The plateau phase can last for some time if the appropriate environmental conditions persist.

2.2 Sampling and Monitoring Programs

A complete review and evaluation of monitoring, sampling and laboratory analysis of cyanobacteria (identification and enumeration) and their toxins (detection and quantification) can be found in 'National Protocol for the Monitoring of Cyanobacteria and their Toxins in Surface Fresh Waters' (Burch et al., in review). National benchmark protocols for sampling and monitoring of cyanobacteria are described in detail. This section is taken directly from that document.

Monitoring covers the two major components of sampling of the water body and analysis of the samples collected, in the laboratory. This provides an early warning system for the development of cyanobacterial blooms (Lawton et al., 1999). An overview of the two components follows.

There are many different reasons and objectives for monitoring cyanobacteria. Each objective may require a different level of effort and resources to achieve the desired monitoring outcome. For example, the complexity of sampling and the level of counting precision required by a community monitoring group may be quite different from that required by a health authority officer or drinking water treatment plant operator. As a monitoring method is refined to provide a higher level of certainty and confidence in the result, there will be a necessary increase in human resource effort and cost. At times the increase in cost may be exponentially related to the desired level of confidence in the outcome. Therefore it is important to structure and conduct a monitoring program that is tailored to meet the objectives of the program.

Regardless of the monitoring program carried out, the quality aspects associated with each process must be considered carefully. Each level of sampling and sample analysis will require an appropriate level of quality input to ensure the delivery of accurate and reliable results. The safety aspects of a monitoring program must also be considered when designing a program, so that all risks associated with safety are identified and minimised before a monitoring program is implemented.

2.2.1 Sampling

The appropriate sampling strategy will depend most importantly on the objective of the monitoring program. For most purposes, the clear aim should be to obtain samples that are representative of the water body as a whole or the part of a water body that is in use (eg. water off take site). The sampling strategy will also depend on the water body type and in some circumstances (ie. public health surveillance) on the current health alert status that applies to a particular water body. Water body types may be defined as storage reservoirs and lakes, service reservoirs, rivers and river weir pools.

The three levels of sampling effort (high, moderate or low) are based on the following components:

- The access point for sample collection
- The method used to collect a sample

- The number of samples collected at any one time
- The frequency of sampling.

Access point for sample collection
Cyanobacteria tend to be extremely patchy in distribution, both vertically and horizontally. Vertical patchiness results from the development of a stratified water column in warm calm weather, allowing those cyanobacteria that are buoyant to maintain their position at the surface for extended periods. Horizontal patchiness is common for most phytoplankton, but can be particularly pronounced in cyanobacteria due to the effect of prevailing winds, which cause accumulation downwind along shorelines of reservoirs or bends in river reaches (See Figure 2). Benthic cyanobacteria are also known to cause problems associated with water use and therefore may sampling may be required.

Depth integrated sampling in open water provides, in general, a better representation of the 'true' or average cyanobacterial population in a water body and is therefore the preferred option. Open water, mid-stream or benthic sampling is normally achieved by working from a boat, but can also be achieved in some circumstances from a bridge over a river, or from an open water structure such as a reservoir off-take. For drinking water supplies, sampling the appropriate depth next to, or from, the water off-take tower may be highly desirable. Due to the resources required for open water sampling (ie. boat and two people), it is often reserved for high priority public health surveillance (ie. drinking water supplies) or for high quality ecological studies.

If open water sampling is not possible due to the unavailability of a boat or off take structure, the second option for monitoring drinking water supplies is to sample from reservoir/lake shorelines. Such samples may not be representative of the 'true' cyanobacterial population due to the bias in spatial distribution discussed above and the limited choice of suitable locations. In choosing a location for sampling the likely effects of the prevailing winds and water currents should be taken into account.

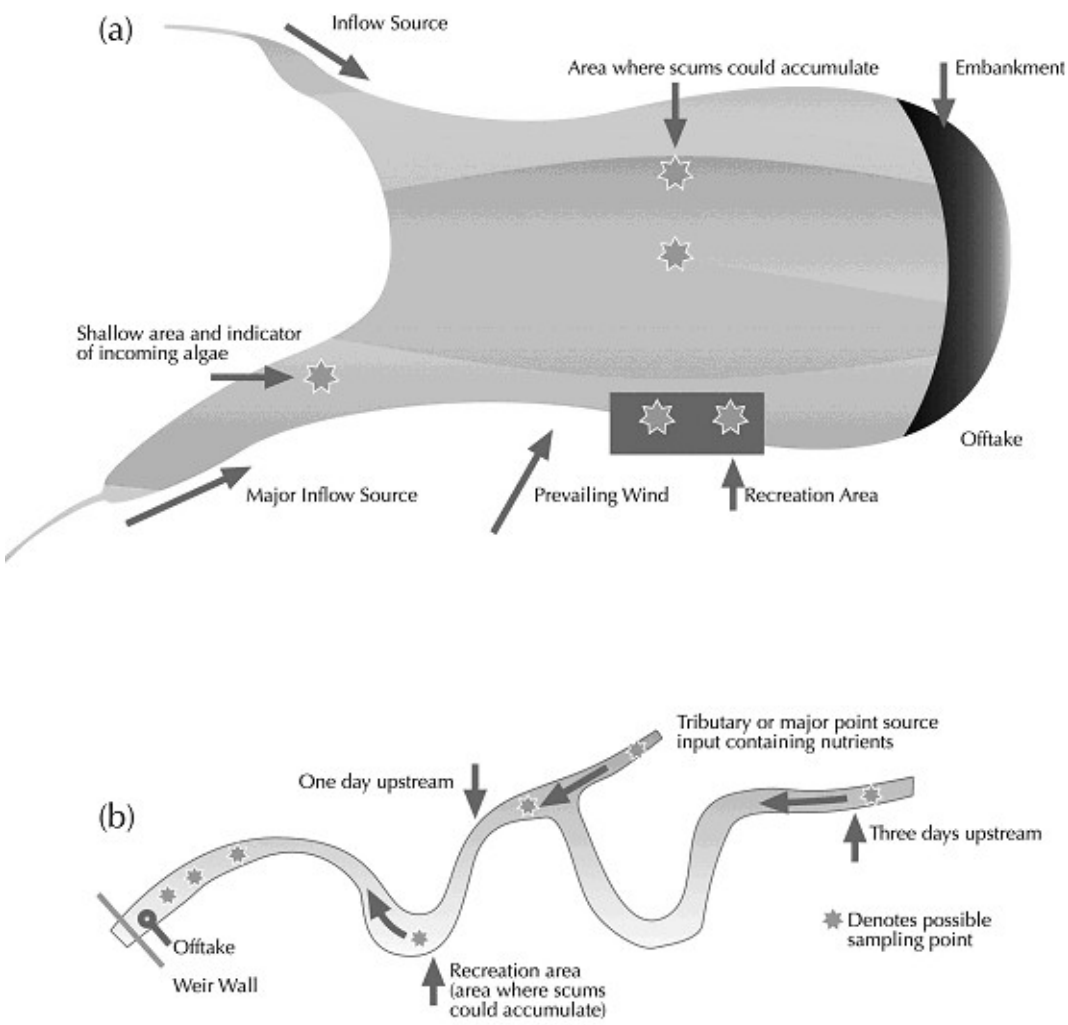

Figure 2 Typical sampling sites in a) lakes/reservoirs b) rivers

Table 4 Scale of sampling effort and procedures for monitoring cyanobacteria (for operators)

Water Body Type	Sampling Effort	Sampling Access	Sample Type (method)	Number of Samples	Frequency of Sampling
Reservoirs & lakes	High	Open water by boat	Integrated depth	Multiple sites	Weekly or bi-weekly
	High	Supply offtake	Offtake depth or integrated depth	Single site	Weekly or bi-weekly
	Moderate-Low	Shoreline	Surface	Single or multiple sites	Weekly or fortnightly
Rivers and weir pools	High	Midstream by boat or bridge or weir	Integrated depth	Multiple sites	Weekly or bi-weekly
	High	Supply offtake	Offtake depth or integrated depth	Single site	Weekly or bi-weekly
	Moderate-Low	River bank	Surface sample	Single site or multiple sites	Weekly or fortnightly
All water bodies	Low	Near to offtakes, bank or shorelines	Visual inspection for water discolouration or surface scums	Single or multiple sites	Fortnightly to daily depending on season and frequency of use

Method for sample collection

Reservoir/River sampling by boat. Reservoir sampling is ideally conducted using a boat. The sampling stations in a reservoir should preferably be chosen in a stratified random pattern; that is, randomly within several defined sectors, representing the entire water body. For boat sampling the use of permanent moorings with marker buoys placed in each of the sectors is the most practical approach and makes open water sampling easier, especially in windy weather. Having permanent sampling sites also gives consistency, which enables the comparison of results at each site over a given time frame. If permanent marker buoys are unable to be placed in a water body, then a GPS should be used to ensure the consistency of sampling points over time. One way to introduce randomness when boat sampling is to move sampling station moorings within sectors on a yearly basis.

Integrated samples. Depth integrated samples are recommended for open water sampling, where a representative sample of the water column is desirable. The samples should be collected using a flexible hosepipe or rigid plastic pipe. A rigid pipe can be fitted with a one-way valve, which tends to simplify the operation of withdrawing the pipe and sample from the water. The depth that the sample pipe is dipped should reflect the approximate depth to which cyanobacterial cells are likely to be mixed. In Australian water bodies, this can vary from approximately 2-10 metres, depending on the degree of stratification and exposure of the reservoir to winds. When the stratification status is uncertain, a temperature probe, if available, may be used to determine the depth of any thermocline present. If this equipment is not available, a 5 metre long flexible pipe is recommended, but a 2 metre long pipe may be more appropriate in shallower water bodies (those that are less than 3 metres deep). The inner diameter of the pipe should be at least 2.5 cm and flexible pipes are probably more practical than rigid pipes for pipe lengths greater than two metres.

Discrete samples. Open water sampling for public health surveillance is often required at the raw water abstraction point for reticulation to a drinking water treatment plant. For this purpose, samples are often collected with a sampling bottle apparatus (eg. Van Dorn or Niskin samplers), that can be filled at a specific depth below the surface corresponding to the off take depth. The rationale for this is to determine the total load of cyanobacteria (and their toxins) to the water treatment plant. In addition, the degree of cell lysis and toxin release through the reticulation system can be measured from an accurate assessment of intact cells at the off take point. This is important information for determining the appropriate strategy for cell and toxin removal in the treatment plant. When choosing a sampling site near the water abstraction point in a reservoir the size of the off take and the abstraction pumping rate should be considered. If pumping rates are high, vortices may occur around the off take or abstraction valves, which indicate that surface water is being drawn down into the off take. If this situation is present in the reservoir, a number of samples at depths ranging from the surface to the off take depth should be taken to determine the total load of cyanobacteria cells and toxins entering the water treatment plant.

Extension pole. Sampling from a bank or shoreline is comparatively simple, but introduces a risk of excessive biasing of samples by shoreline accumulations. A ‘pole-type’ sampler can be used, where the bottle is placed in a cradle at the end of an extendable pole of at least 2 metres length. Alternatively, a spear sampler as described in Hötzel and Croome (1999) is a useful sampling device for collecting an integrated depth water sample when standing on the bank or shoreline. It is also important to note that in using either the pole or spear sampler, scum accumulations near to the shoreline will not be sampled. A separate dip sample of any accumulations may be needed for further testing such as toxin analysis.

Samples for benthic cyanobacterial surveys. In some instances it may be necessary to collect benthic samples for identification of cyanobacteria. In most cases benthic samples are not collected routinely and are generally for qualitative analysis only. This may be the case when high levels of

taste and odour compounds are detected in water bodies or supplies when little or no cyanobacteria are detected in water samples. Samples can be collected using a benthic sampler such as an Eckman grab or a rigid plastic corer (eg. PVC or polycarbonate pipe).

An alternative cause of taste and/or odour problems may be due to cyanobacteria growing attached on dam walls or off take structures. Cyanobacteria attached to these structures can be scraped off, most easily when water levels drop.

If a more quantitative assessment of benthic cyanobacteria growth is required, the use of removable artificial substrates is recommended. Artificial substrates of known surface area should be situated in appropriate positions within the water body and allowed to rest to allow benthic cyanobacterial growth. Once removed, the benthic cyanobacteria can be scraped off and measured quantitatively. Substrates can then be returned to the water body until the next sampling occasion.

Sampling from a pipeline. Water samples are generally collected from a tap situated along, or at the end of a pipeline. In this case, a tap should be opened and allowed to run for a sufficient period of time to allow a fresh water sample to be collected. It is recommended that water samples collected from a pipeline are used for qualitative (ie. identification) purposes only as the water in the pipeline may be treated with chemicals such as chlorine or copper sulphate.

Number of samples

For monitoring trends in cyanobacterial abundance, an indication is required of the 'true' cyanobacterial population, representing the entire water body. This can be achieved by collecting a suite of discrete samples from different sampling sites, which are counted separately and then may be averaged. As an alternative to undertaking separate counts on samples collected at several sites, samples may be pooled or composited. These samples are collected at three or more individual sites but pooled into one container. The sub-sample for counting is then taken from the container after its contents have been thoroughly mixed. If composite samples are made, then the individual samples being pooled must be of equal volume to prevent bias. An alternative to pooling samples in the field, is to send discrete samples to a laboratory, where they can be sub-sampled, pooled and analysed. Using this process, a portion of the original discrete sample can be retained for further analyses if required.

The trade off from compositing is a decrease in statistical power for subsequent data analysis against a three-fold or greater reduction in counting costs.

The number of sampling sites in a water body will be, in most cases, dictated by time and financial considerations. However, a minimum of three sites should be used when cyanobacterial counts exceed national Alert Level 1 conditions (> 2000 cells mL^{-1}) for both open water sampling and shoreline sampling (see section on Alert levels). For lakes and reservoirs, the sampling stations should be at least 100 m apart (where possible), while for rivers, replicate samples should represent different 'parcels' of water. When sampling from a boat, replicate samples should preferably be taken at the downstream end first to avoid re-sampling the same 'parcel' of water. At lower cyanobacterial abundances, a single site may be sufficient, although the loss of statistical power for data analysis must be taken into consideration.

Frequency and timing of sampling

The appropriate frequency of sampling will be dictated by the category of use, the current alert level status, the cost of monitoring, the season and the growth rate of the organism(s) under consideration. Apart from cost, the underlying consideration in operations monitoring is the possible health consequences of missing an early diagnosis of a problem. Growth rates are generally related to seasonal conditions and previous studies have shown that cyanobacteria in the field can

exhibit growth rates from 0.1-0.4 d^{-1} (equivalent to population doubling times of nearly a week (6.93 days) to less than two days (1.73 days), respectively.

It is recommended that sampling for high risk/high security supplies (ie. drinking supplies) should occur on at least a weekly basis and probably bi-weekly when national Alert Level 1 status (cyanobacterial counts > 2000 cells mL^{-1}) is attained. For supplies where the public health risk is deemed to be low (ie. low cell counts in non-supply reservoirs), fortnightly sampling may be adequate, but caution is advised given the rate at which the cyanobacterial population may increase.

The timing of sampling for buoyant cyanobacteria can be important during calm, stratified periods if depth integrated samples are not collected. Buoyant cyanobacteria tend to accumulate near or at the water surface overnight, which can result in an over-estimation of cell concentration in surface samples collected early in the morning or an under-estimate in those collected at depth at the same time. Temporary surface scums may be observed early in the morning, but they tend to disperse as winds increase and may even be mixed back into the water column during the day. Thus, a sample that is less biased by scum formation is, on average, more likely to be obtained later in the day. If the option exists, it is preferable to delay sampling to later in the day, but whatever time is chosen it is best to adhere to the same sampling times for each location on each sampling occasion if possible.

Visual inspection
Visual inspection for water discolouration or surface scums of cyanobacteria is an important part of any monitoring program. Using this form of monitoring, a limited number of samples are initially collected which are analysed basically, for example a simple qualitative analysis and then generally no continued sampling or analysis is carried out. Be warned however, that the visual inspection method does not apply to all cyanobacteria, for example *Cylindrospermopsis*, which does not form a scum.

In situations where non-bloom forming cyanobacteria are present it is essential that samples are collected for analysis to determine the abundance of cyanobacteria in the water body. In cases where bloom forming cyanobacteria are present, a qualitative assessment of cyanobacterial presence can be a useful indicator of water quality and the relative hazard of a water body.

Another tell-tale sign of cyanobacterial blooms is their odour. Some cyanobacteria produce a distinctive earthy/musty odour that can often be smelt at some distance before the bloom/scum can be seen. Therefore it is useful to conduct ‘odour surveillance’ in conjunction with any visual inspection program.

2.2.4 Laboratory analysis of samples

There are two components to the laboratory analysis of cyanobacterial samples: estimating phytoplankton abundance by microscopic examination and detection and quantification of cyanotoxins.

Estimating cyanobacterial abundance
Species determination and quantification by microscopic examination provide relatively rapid, useful information on the composition of the sample and estimates of cell numbers. This is often sufficient for an early assessment of any potential hazard and for initial management decisions (Lawton et al., 1999).

Enumeration protocols require a satisfactory level of precision in estimates of cell abundance for colonial and filamentous cyanobacteria and the detection of trends in cell abundance with

reasonable confidence and the minimum counting effort. The levels of analytical effort for estimating abundance of cyanobacteria are based on the following components:

- Qualitative or quantitative assessment
- The level of taxonomic identification of specimens (genus or species)
- Incorporation of a pre-concentration step
- Standard of equipment used (ie. microscope and counting chambers)
- Specified level of counting precision, including reproducibility and repeatability.

For public health surveillance, it is important that potentially toxic cyanobacteria be identified to species level. This information is necessary to determine the analytical technique appropriate for determining toxin levels (Lawton et.al., 1999).

Many monitoring programs are established to detect cyanobacteria that are known to taint water supplies with disagreeable tastes and odours. Detection of these odours are often possible at quite low cell concentrations (>500 cells mL^{-1}) and identification of nuisance taxa at an early stage can alert operators to the need for remedial measures in either raw water or treated water.

Detection and quantification of cyanotoxins.
There are several analytical methods available for the detection and quantification of cyanotoxins, which vary in their manner of detection, the information they provide and sophistication. (Harada et al., 1999). For a complete overview and review of methods please refer to the "National Protocol for the Monitoring of Cyanobacteria and their Toxins in Surface Fresh Waters," 2004 (Burch et al., in review) and "Evaluation of Analytical Method for the Detection and Quantification of Cyanotoxins in Relation to Australian Drinking Water Guidelines" (Nicholson and Burch, 2001).

Microcystins, for example, range from immunological or biochemical screening techniques based on enzyme-linked immunosorbent assays (ELISA) and enzyme activity (protein phosphatase inhibition, PPIA) assays respectively, to quantitative chromatographic techniques based on high performance liquid chromatography (HPLC) and more sophisticated (and expensive) liquid chromatography-mass spectrometry (LC-MS).

Analytical techniques based on either HPLC or LC-MS can also be used for determining saxitoxins or cylindrospermopsin in water. Animal bioassays (mouse tests), and in some cases assays based on isolated cell lines, are also available for screening the entire range of toxins. It is important that the method selected gives the information that is required of the sample being tested so that appropriate management decisions can be made.

2.3 Risk Assessment for Cyanobacterial Growth in Reservoirs

2.3.1 Risk assessment model

A simple risk assessment procedure has been developed to predict scenarios for cyanobacterial growth based on a series of modelled empirical relationships. These in turn can be used to derive the likely levels of toxins or odours for a prescribed set of circumstances. The intention is to use this to provide a measure of the challenge to water quality and therefore the treatment process from cyanobacterial contamination for a certain level of nutrients (in this case phosphorus) in the source water. More sophisticated deterministic water quality models are also available to predict cyanobacterial growth, however the relationships presented here provide a simple indication of the likely threat from toxins and odours.

The relationships are derived from published information and relate nutrients to cyanobacterial growth and to rates of odour/toxin production. The resulting growth predictions can then be compared with actual cyanobacterial growth data from historical records to verify the calculations used in the model. This simple model can be used to estimate "worst case challenges," ie. to answer the question "how bad could it get in my reservoir, and what scale of treatment might I need?" In reality, the actual magnitude of the risk over a season is determined by the period of time that favourable growth conditions persist, the carrying capacity of the reservoir (the total algal biomass that the physico-chemical conditions that the reservoir will support) and the types and effectiveness of management operations implemented.

More complex models, particularly deterministic models, incorporate many factors that limit the growth of phytoplankton, and therefore cyanobacteria, and include temperature, degree of stratification, and availability of light, phosphorus and nitrogen. To determine levels of growth it is necessary to determine which factor is limiting. Once the limiting factor has been identified then individual case scenarios can be calculated to determine the carrying capacity of the reservoir. For example, if a reservoir experiences low turbidity (ie. light not limiting) and the cyanobacteria involved are nitrogen fixers (ie. nitrogen not limiting) but there is limited phosphorus available, then phosphorus becomes the limiting factor for growth. In many cases the yield-limiting variable that ultimately determines the size of the cyanobacterial population is phosphorus, and the concentration of phosphorus underpins the calculations in this simple model (refer Table 5 for an example to calculate the cyanobacterial hazard in a reservoir and Appendix 1 for a more detailed example).

Within the calculations for the model three different algal growth (and therefore toxins/odours) scenarios have been developed around the degree of availability of phosphorus as the yield-limiting variable. These are:

- *Most favourable case:* assumes that only small concentrations of the limiting factor are available for the purpose of cyanobacterial growth, and so problem cyanobacteria are not dominant and toxin/odour production occurs at low levels
- *Most likely case:* based on variations to the current reservoir conditions, with no significant changes to the limiting factor over an extended period
- *Worst case:* assumes that 80% of the limiting factor is available (ie significant increase) and solely available for the purpose of cyanobacterial growth. The problem cyanobacteria become dominant and toxin/odour is produced at maximum reported rates.

Initial conditions for the scenarios should use historical and current water quality data where it is available. This information is combined with projections from the scenarios for the identified limiting factor, which can then attempt to predict future risk scenarios.

The outputs from this simple model are cyanobacterial numbers, and taste, odour and toxin concentrations. These can then be compared with drinking water guidelines and alert levels to assess drinking water quality. Safety and management practices can be adjusted accordingly.

Table 5 Example of a simple modelled correlation between phosphorus and cyanobacterial contamination by odours and toxins.

		Concentration in Reservoir				
	Total Phosphorus µg L^{-1}	**From Table Below**	**Dissolved Geosmin ng L^{-1}**	**Microcystin µg L^{-1} (intracellular)**	***Anabaena* cells mL^{-1}**	**Saxitoxin µg L^{-1} (total)**
Lowest Level	40	Most Favourable Case	1.80	0.03	1000	0.07
		Most Likely Case	96.0	1.15	13,300	0.88
		Worst Case	720.00	12.8	44,400	2.93
Current Level	80	Most Favourable Case	3.60	0.06	2000	0.13
		Most Likely Case	192.0	2.30	26,700	1.76
		Worst Case	1440.00	25.60	88,900	5.87
Highest Level	160	Most Favourable Case	7.20	0.12	4000	0.26
		Most Likely Case	384.0	4.61	53,300	3.52
		Worst Case	2880.00	51.20	177,800	11.73

Table 6 Categories used to calculate geosmin, microcystin and saxitoxin levels in Table 5. TP is total phosphorous, chl *a* is chlorophyll *a*

	Most Favourable Case	**Most Likely Case**	**Worst Case**
Proportion of bioavailable TP	0.36	0.6	0.8
Proportion bioavailable P converted to chl *a*	0.5	0.8	1.0
The proportion of chl *a* that is either *Anabaena* or *Microcystis*	0.1	0.5	1.0
The chl *a* content of *Anabaena circinalis* (pg $cell^{-1}$) (Reynolds, 1984)	0.72	0.72	0.72
The ratio of geosmin to *Anabaena* chl *a*	50	100	150
The proportion of extracellular geosmin	0.05	0.1	0.15
The possible production of saxitoxins from *Anabaena*	0.33	0.33	0.33
The ratio of microcystin to *Microcystis* chl *a*	0.04	0.12	0.4

The determination of the final algal population biomass in a reservoir by theoretical calculations involves the use of selected published empirical variables and assumptions as follows:

- The proportion of bio-available phosphorus will be converted into chlorophyll *a*
- If conditions are appropriate for cyanobacterial growth then a proportion of chlorophyll *a* will be for cyanobacterial growth

By using published values for chlorophyll *a* per cell the maximum cell concentration for a species can be determined. The values for *Anabaena circinalis* and *Microcystis aeruginosa* from Reynolds (1984) are as follows:

- *Anabaena circinalis*: 0.72 pg $cell^{-1}$
- *Microcystis aeruginosa*: 0.36 pg $cell^{-1}$

Bowmer et al. (1992) published a geosmin:chlorophyll *a* ratio of 59-360 ng μg^{-1} at 70-17 µmol m^2 s^{-1} photosynthetically active radiation (PAR). Chorus and Bartram (2000) published a microcystin:chlorophyll *a* ratio of 0.12. A cell density of 20,000 cells mL^{-1} of *Anabaena circinalis* would produce approximately 1.32 µg L^{-1} saxitoxin (Humpage and Falconer, 2003 unpublished).

These relationships are used to estimate the maximum geosmin or microcystin concentration given the chlorophyll *a* yield in the reservoir.

Two main assumptions are made using this model:

- that the climatic conditions are favourable for cyanobacterial growth. The population is determined by the carrying capacity of the reservoir
- that given stable conditions the phosphorus concentration is likely to determine cell biomass.

The levels of total phosphorus (TP) in the example were derived by using the average current levels in a known reservoir. To obtain the lowest level for TP the current level was halved and to obtain the highest level the current level was doubled. If historical data is available then it is possible to determine an average current level.

2.3.2 Information used for model calculations

1) When the level of total phosphorus (TP) has been determined the TP:SRP (soluble reactive phosphorus) ratio is selected. The ratio is 1:0.36 for most favourable case, 1:0.6 for most likely case and 1:0.8 for the worst case. This is based on the assumption that some of the total P is unavailable due to binding to particles etc.
2) From the SRP the proportion of bioavailable P converted to chlorophyll *a* can be determined. The assumptions are that some bioavailable P will be taken up by other organisms, but most bioavailable P is taken up by phytoplankton.
3) The proportion of chlorophyll *a* that is either *Anabaena* or *Microcystis* depends upon the degree of dominance the cyanobacteria has achieved.
4) The chlorophyll *a* content of *Anabaena circinalis* is based on the published value from Reynolds (1984). This is used to determine the number of cells mL^{-1}.
5) The possible production of saxitoxins can then be determined from the number of cells mL^{-1} using the estimated saxitoxin load of 0.33 µg L^{-1} for every 5000 *Anabaena* cells (Humpage & Falconer, 2003, unpublished).
6) The proportion of extracellular geosmin is derived from the published range (Bowmer et al., 1992) and depends upon the strain and environmental conditions.
7) The ratio of microcystin to *Microcystis* chlorophyll *a* was derived from the published range (Chorus and Bartram, 1999) and also depends upon the strain and environmental conditions.
8) To calculate the geosmin, saxitoxin and microcystin levels it is necessary to first calculate the proportion of chlorophyll *a* that is either *Anabaena* or *Microcystis*. The further calculations are based on this value.

2.3.3 Risk modification

The output from the simple model should be considered in the light of a number of factors that will modify and reduce the risk from odours and toxins. For example:

- Are the cyanobacteria present toxin or odour producers?
- Is the monitoring program sufficient to detect cyanobacterial growth?
- What management strategies are available in the reservoirs when required (eg. variable off take height, algicide use, destratification)
- What are the weather patterns and how will they affect cyanobacterial growth?

Some factors such as weather patterns and the type of cyanobacteria can obviously not be controlled, however some management strategies can be implemented to reduce the risks of cyanobacterial growth.

The authors would like to acknowledge Leon Linden, Fiona Harvey and Greg Ingleton, SA Water, AWQC for the information on which this risk assessment has been based.

2.4 Alert Levels for Action

2.4.1 Alert levels framework

An ‘Alert Levels Framework’ (ALF) is a monitoring and management action-sequence that operators and regulators can use for graduated response to the onset and progress of a potentially toxic cyanobacterial bloom in drinking waters. Although the ALF is intended to assist in the management of potentially toxic cyanobacterial blooms, the approach of systematic and precautionary monitoring and assessment is applicable to the occurrence and growth of all cyanobacteria in drinking water supplies. This is because all cyanobacteria should be treated with caution, until either the absence of toxicity is confirmed by testing, or advice based upon past local knowledge indicates the absence of hazard.

Please note that the ALF described here is a generic model for drinking water only. The ALF is intended as a situation assessment tool primarily based around data from cyanobacterial cell counts and equivalent cell biovolume, which are to be used in conjunction with the drinking water guidelines for toxins. The rationale for the use of cell counts to prompt management actions is that for most practical purposes, cell counting is still used by most water authorities to detect algal-related water quality problems. This is because the testing is widely available and provides relatively rapid and cost-effective information. By contrast, toxin testing is still not generally widely available and has a slow turn-around time for results. The cell counts (and biovolumes) must however be regarded as an indicator or "surrogate" for a potential toxin hazard. The counts can be used to prompt actions, such as toxin monitoring, which can then be assessed in relation to the Drinking Water Guidelines, to assess the hazard and risk in consultation with health regulators. The cell numbers that define the Alert Levels are recorded from samples that are taken from the source water, adjacent to, or as near as possible to, the water supply offtake (ie. intake point).

The following section has been taken directly from the ‘National Protocol for the Monitoring of Cyanobacteria and their Toxins in Surface Fresh Waters’ (Burch et al, in review).

2.4.2 How to use the alert levels framework

The framework assesses the development of a potentially toxic cyanobacterial bloom through a monitoring program with associated actions in four stages that are linked to ‘Alert Levels’. The actions accompanying each level cover categories such as additional sampling and testing, operational options, consultation with health authorities and other agencies, and media releases. The sequence of alert levels is based upon initial detection of cyanobacteria at the Detection Level, progressing to moderate cyanobacterial numbers at Level 1, where consultation, additional sampling and assessment of toxicity may occur. The next stage at higher cell numbers is defined by the potential for detection of toxins above guideline concentrations at Level 2. Alert Level 2 represents the point where the operators and health authorities may decide to issue a health warning or notice in relation to suitability of the water for consumption. This would follow a full health assessment and depend upon circumstances such as availability and efficacy of water treatment, consumption patterns, etc. It is also possible that an operator may decide to issue advice or a notice at cell numbers lower than that equivalent to the guideline (Table 7). The sequence can then escalate to Alert Level 3 at very high cyanobacterial biomass in raw water. This level represents the situation where the potential risk of adverse health effects is significantly increased if treatment is unavailable or ineffective. Alert Level 1 and 2 ideally require an assessment of toxicity and toxins

in raw water and assessment of both the drinking water and the efficacy of the treatment system for toxin removal.

The intention of the Alert Levels Framework is to provide a best-practice operating guide based around a monitoring program with cell counts. The cell counts are employed as supplements and surrogates in addition to toxin measurement, which may or may not be required at frequent intervals. It is important to note that cell counts do not replace toxin analyses, which are required for health risk assessment, but rather are used as relatively conservative triggers in the management plan.

2.4.3 Levels of the framework

Detection level
This level encompasses the early stages of bloom development, when cyanobacteria are first detected at low levels in raw water samples. The indicative cell numbers for this detection level are somewhat arbitrary, and are >500 cells mL^{-1}. Taste and odours may become detectable in the supply, although their absence does not indicate absence of toxic cyanobacteria. Presence of cyanobacteria in low numbers (and sometimes detection of characteristic tastes and odours) constitutes an early warning, and if a routine monitoring program is not in place, it is appropriate at this time to sample and dispatch the samples to a laboratory for confirmation of the presence of cyanobacteria. The recommendation for a monitoring program is to commence weekly sampling and cell counts at a representative location(s) in the water body. The presence of low population densities of cyanobacteria could still indicate the potential for the formation of localised surface scums, and operators should regularly inspect raw water offtakes for scums or discoloured water.

Alert Level 1
Alert level 1 represents the level at which the cyanobacteria population is established, and localised high numbers may occur. The threshold for this level is a cell number ≥2,000 cells mL^{-1} of total cyanobacteria for a sample taken at the source water intake for the drinking water supply. The actual number for a cell count estimate of 2,000 cells mL^{-1} is likely to be in the range of 1,000-3,000 cells mL^{-1}. This is based on a likely minimum precision of ± 50 per cent for counting colonial cyanobacteria such as *Microcystis aeruginosa* at such low cell densities. For counting filamentous cyanobacteria such as *Anabaena circinalis* the precision is likely to be much better at these cell densities (approximately ± 20 %), giving an actual cell density in the range of 1,600-2,400 cells mL^{-1}. The definition for Level 1 is relatively conservative and has been chosen to indicate a point that represents a cell density providing a buffer, or time margin, of 4-6 days before the guideline for toxin in raw water could be exceeded (ie. Level 2 conditions) if the population is toxic and is actively growing (based upon a doubling rate of 4 days which is equivalent to a growth rate, μ of 0.17 d^{-1}).

Alert Level 1 may require notification and consultation with health authorities and other agencies for ongoing assessment of the status of the bloom. Contact with health authorities may be made initially when this level is reached, but may not need to be made weekly if local conditions deem this unnecessary. For instance, if the dominant cyanobacterium present is not known to be problematic based on prior testing and experience, this alert level can be adjusted to suit the local situation.

The requirement for information on toxicity assessment at this level will depend upon advice and discussion with health authorities. It will also depend upon circumstances such as whether the cyanobacteria are known toxigenic species, past history of toxicity, nature of the supply and associated water treatment, local sensitivity in relation to this supply, etc. This consultation should

be initiated as early as possible and continue after the results of toxicity testing and/or toxin analysis become available.

The bloom population should be sampled to establish the extent of its spread and variability. Special samples (concentrated scums and/or grab samples representative for the raw water intake) should be collected and dispatched for toxicity testing or toxin analysis.

This level may warrant operational intervention in drinking water supply, such as the deployment of booms adjacent to offtakes, or changing the depth of drinking water abstraction. Mixing or destratification may be successful. Treatment with algicides may be an emergency measure in some situations and should be restricted to reservoirs only; its applicability also depends upon local environmental regulations.

Alert Level 2
Alert Level 2 is the next stage at moderate and slightly higher cell numbers of potentially toxic cyanobacteria, defined by the potential for the occurrence of toxins above guideline concentrations if treatment is ineffective.

The threshold for Level 2 (in the absence of toxin information) are cell numbers and/or biovolumes that could indicate the potential for a toxin hazard around the guideline level if:

- the population was highly toxic, and
- all toxins were released into the raw water and passed through treatment.

This level is characterised in general terms by an established bloom with moderately high numbers showing an upward trend over several successive samples at sampling frequencies of, for instance, twice per week. The cyanobacterial population is likely to have developed to the extent that localised surface scums may form where scum-forming species are prevalent.

Two thresholds definitions are given for Level 2 (Table 7). These are:

1. Cell numbers $\geq$5,000 cells mL^{-1} *Microcystis aeruginosa* or *Anabaena circinalis* (Range: 5,000-10,000 cells mL^{-1})
2. Total biovolume of other cyanobacteria of 1 mm^3 L^{-1} (Note that this is given at 1 significant figure).

The cell numbers were selected as those that are equivalent to the preliminary "hazard surrogates" given in the Australian Drinking Water Guidelines for toxic *Microcystis aeruginosa* (Fact Sheet 17a). The concentration of $\geq$5,000 cells mL^{-1} (5,000-10,000 cells mL^{-1}) for Alert Level 2 has been set to span the surrogate guideline cell concentration of 6,500 cells mL^{-1} for *Microcystis aeruginosa*, which is equivalent to the toxin guideline of 1.3 μg L^{-1} microcystin-LR, if the toxin was fully released from the cells (NHMRC/ARMCANZ, 2001). The same threshold is recommended for *Anabaena circinalis*. The rationale for this recommendation for *Anabaena circinalis* is provided below.

The rationale for the choice of this approximate biovolume of 1 mm^3 L^{-1} for other cyanobacteria (toxigenic or of unknown status) that is equivalent to 5,000-10,000 cells mL^{-1} of *Microcystis aeruginosa*, is that this biovolume of toxic cells could potentially be equivalent to the ADWG guideline for microcystins if the cyanobacteria was found to be toxic and produced microcystins. Furthermore, it is assumed that for blooms and populations of unknown cyanobacteria, the hazard from toxicity is unlikely to exceed the worst case for an equivalent biovolume of highly toxic

Microcystis aeruginosa containing microcystin. This should allow protection from significant risk while assessments are made.

This biovolume may represent relatively low cell densities for some species of cyanobacteria, and is cautious and conservative to account for unknown toxic properties in particular cyanobacteria.

Alert Level 2 represents the point where the operators and health authorities may decide to issue a health warning or notice in relation to suitability of the water for consumption. This would follow a full health assessment and depend upon circumstances such as availability and efficacy of water treatment, consumption patterns, etc. It is also possible that an operator may decide to issue advice or a notice at cell numbers lower than those equivalent to the recommended thresholds (Table 7).

It may certainly be acceptable to continue to supply drinking water from a water body providing a positive toxicity result, dependent upon a risk assessment by the health authorities who may recommend specific action to protect more susceptible population groups.

The operational interventions at this level are the same as those for Alert Level 1.

Alert Level 3

The threshold definition for Alert Level 3 is total cyanobacterial cell numbers of $\geq$50,000 cells mL^{-1} of the toxic species Microcystis aeruginosa or Anabaena circinalis (ie. toxins confirmed by analytical or bioassay techniques) in the raw water (ie. adjacent to the offtake). Alert Level 3 is alternately defined by the total biovolume of other toxic cyanobacteria $\geq$10 mm^3 L^{-1} (see Table 7). This describes an established toxic bloom with high cell numbers and possibly localised scums. The sampling program will have indicated that the bloom is widespread with no indication of a cyanobacterial population decline in the short term.

Conditions in Level 3 are indicative of a significant increase in the risk of adverse human health effects from the supply of water that is untreated, or treated by an ineffective system, even for short-term exposure. Detection of cyanobacteria at this level warrants immediate notification of health authorities, and appropriate health risk assessment. In making decisions on activation of an alternative supply for human consumption, it should be noted that generally drinking water guidelines are based on lifetime exposure to a toxicant. For many substances, higher concentrations may be safely tolerated for short periods on an infrequent basis.

The cell count in Level 3 is really intended as a trigger for the immediate notification and assessment of a situation where there is limited prior information from an ongoing monitoring program, and treatment is limited or its efficacy for toxin removal is untested. A typical scenario is the initial discovery of a major bloom in a source water. This warrants immediate contact with health agencies, followed by a health risk assessment and preparation for an alternative supply. The circumstances for Level 3 are where, dependent upon the nature of the treatment, the population sensitivity, and their consumption patterns, there is some potential for adverse public health outcomes if these high numbers are present in the source water or supply. Cell numbers of cyanobacteria >50,000 cells mL^{-1} indicate the potential for much higher localised concentrations, ie. surface scums. This can mean that much higher peak cell densities could be entering the supply for short periods, which are not captured by the monitoring program.

If activated carbon treatment (powdered or granular) or an advanced oxidation process such as ozone treatment is available, they should be used in the treatment process. The treated water should be monitored for the specific cyanotoxins occurring in the source water to confirm their removal. If water quality has changed under these circumstances, it may be warranted to issue a media release and to reinforce warnings about recreational use of the source water. It is important to bear in mind

that water treatment systems may have originally installed granular activated carbon filtration for the removal of other substances (such as pesticides or dissolved organic carbon). The high load of organic substances usually associated with a cyanobacterial bloom may lead to rapid saturation of the adsorption sites of the carbon, and monitoring for breakthrough of toxins is therefore critical.

The application of algicides in this phase can enhance the problem by causing high concentrations of dissolved toxins as a consequence of cell lysis. Whereas filtration systems may have removed cell-bound toxins, dissolved toxin is more likely to break through treatment systems.

If activated carbon or other advanced treatment is not available, and toxins are present in supply at concentrations significantly above the guideline then Level 3 may result in the activation of a contingency water supply plan that is appropriate for the operator and the user/community. This may involve switching to an alternative supply for human consumption, or in some circumstances the delivery of safe drinking water to consumers by tanker or in bottles. More extensive media releases and even direct contact with consumers via letterbox drops of leaflets with appropriate advice to householders may be necessary. Where advice is provided to the public because of a cyanobacterial hazard to human health it will generally be advisable to emphasise that the water would be suitable for purposes such as washing, laundry, toilet flushing, etc. Closure of a public drinking water supply because of a cyanobacterial hazard in a source water is not justifiable since the adverse health effects of disruption of supply are likely to substantially outweigh the cyanobacterial hazard.

Monitoring of the bloom should continue to determine when it is in decline so that normal supply can be resumed. Monitoring is usually only warranted at 3-7 day intervals. Experience suggests that the toxicity of a cyanobacterial population can change, but it is unlikely to become completely non-toxic or to decline in a period of a few days.

The sequence at Level 3 follows through to deactivation of an emergency with media releases to confirm this. It is possible that the collapse of a bloom, or management action such as flushing and control of scum, could lead to a rapid decline from Level 3 back to Level 1 or beyond. Likewise the sequence might escalate rapidly, by passing Level 1 & 2, if adequate monitoring and early warning information is not available.

Table 7 Threshold definitions for the national Alert Levels Framework

~ Note: for Drinking Water only ~

Level	Threshold Definition [1.]	Recommended Actions
Detection Level	>500 cells mL^{-1} cyanobacteria *Cyanobacteria detected at low levels*	*Have another look* Regular monitoring Weekly sampling and cell counts Regular visual inspection of water surface for scums adjacent to offtakes
Alert Level 1	≥2,000 cells mL^{-1} total cyanobacteria, (Individual species or combined total). (Range: 1,000–3,000 cells mL^{-1})[2] *Trigger value for this level can be adjusted for local conditions (see text)* *Cyanobacteria detected at levels that indicate population is established, and localised high numbers could occur.*	*Talk to the health regulators* Notify agencies as appropriate Increase sampling frequency to 2x weekly at offtake and at representative locations in reservoir to establish population growth and spatial variability in source water Establish the representativeness (ie variability) of the offtake sample over time Decide on requirement for toxicity assessment or toxin monitoring
Alert Level 2	≥5,000 cells mL^{-1} *Microcystis aeruginosa or Anabaena circinalis* (Range: 5,000-10,000 cells mL^{-1}) [3] *-or-* the total biovolume of other cyanobacteria ≥ 1 mm^3 L^4 *Established bloom of cyanobacteria (may be potentially toxic); potential for toxin concentration to exceed guideline if treatment is ineffective.*	*Decide on the significance of the hazard re the guidelines* Advice from health authorities on risk to public health, ie. health risk assessment considering toxin monitoring data, sample type and variability, effectiveness of treatment Consider requirement for advice to consumers if supply is unfiltered Continue monitoring as per Level 1 Toxin monitoring of water supply (finished water) may be required, dependent upon advice from the relevant health authority
Alert Level 3	≥50,000 cells mL^{-1} *Microcystis aeruginosa, Anabaena circinalis* *-or-* the total biovolume of other cyanobacteria ≥ 10 mm^3 L^{-15} *In circumstances without water treatment, or ineffective treatment, there may be an elevated risk of adverse human health outcomes if alternative water supplies or contingency advanced water treatment are not implemented*	*Assess potential risk immediately if you have not already done so* Immediate notification of health authorities for advice on health risk for this supply May require advice to consumers if the supply is unfiltered Toxicity assessment or toxin measurement in source water and drinking water supply if not already carried out Continue monitoring of cyanobacterial population in source water as per Level 1 In absence of treatment and subject to health risk assessment may require alternative contingency water supply

1. The cell numbers that define the Alert Levels are recorded from samples that are taken from the source water, adjacent to, or as near as possible to, the water supply offtake (ie. intake point).
2. The actual numbers for a cell count estimate of 2,000 cells mL^{-1} is likely to be in this range. This is based on a likely minimum precision of +/-50% for counting colonial cyanobacteria such as *Microcystis aeruginosa* at such low cell densities. For counting filamentous cyanobacteria such as *Anabaena circinalis* the precision is likely to be much better at these cell densities (~+/-20%), giving an actual cell density in the range of 1,600-2,400 this count.
3. The concentration of 5,000-10,000 cells mL^{-1} for Alert Level 2 has been set to span the surrogate guideline cell concentration of 6,500 cells mL^{-1}, which is equivalent to the toxin guideline of 1.3 μg L^{-1} microcystin-LR if the toxin was fully released from the cells.
4. This biovolume (≥1 mm^3 L^{-1}) is approximately equivalent to those numbers of *M. aeruginosa* for Level 2.
5. This biovolume (≥10 mm^3 L^{-1}) is approximately equivalent to those numbers of *M. aeruginosa* for Level 3.

2.5 Control Techniques

A major problem in reservoirs experiencing periods of warm stable conditions is that the water becomes stratified (see sections 2.1.3 and 2.1.4). These conditions provide cyanobacteria with optimum conditions for growth and the potential for cell numbers to increase (see section 2.1 Growth of cyanobacteria). Management options for reducing the risk of cyanobacterial growth include aeration and mixing. However, once cell numbers have begun increasing, immediate control of cyanobacteria may be achieved by the use of algicides.

Naturally flowing rivers have mixing and aeration that is associated with their flow. Water that has been impounded in storages, especially in summer months, does not experience natural mixing. Aeration from the atmosphere occurs only in the top few meters of the water column, leaving the lower levels to become depleted of dissolved oxygen (Kirke, 2000). This results in the well-known and documented water quality problems and cyanobacterial growth.

Circulation of water provides two functions. The first is destratification. This results in uniform temperature and density through the water column. The second is aeration, which maintains adequate levels of dissolved oxygen through the water column. Artificial mixing also aims to mix the cyanobacteria below the level of light penetration as they are susceptible to light limitation, which may potentially reduce growth. When natural methods are not available, artificial means can be used to mimic the natural convection when it is absent. Artificial circulation may be achieved in two ways using either aerators or mechanical mixers as described below (Burch et al., 2000; Kirke, 2000).

2.5.1 Aerators

Aeration is a method of destratification that uses an air bubble plume or conventional air curtain (Figure 3). Compressed air is released as air bubbles near the bottom of the storage, and as the air bubbles rise they entrain water from the lower levels. The rapid upward flow of bottom water mixes with the surrounding water. Once the water reaches the surface it plunges back down entraining some warm, oxygen rich surface water downward where it spreads out at a neutral density level (Burch et al., 2000; Kirke, 2000).

This method of destratification has been widely used to oxygenate the hypolimnion and control the remobilisation of nutrients and metals from the reservoir sediment (Burch et al., 2000). Bubble plumes are not thought to assist directly with aeration. The bubble plume sets up large-scale circulation that weakens the temperature gradient increasing mixing by wind and surface cooling.

While a bubble plume may be efficient for reducing the mobilisation of metals and nutrients from sediments, during hot calm weather a bubble plume may not provide enough destratification and mixing to prevent the growth of cyanobacteria. Even small temperature gradients ($<1.0^{o}C$), are thought to provide cyanobacteria with the stability required to become dominant (Burch et al., 2000).

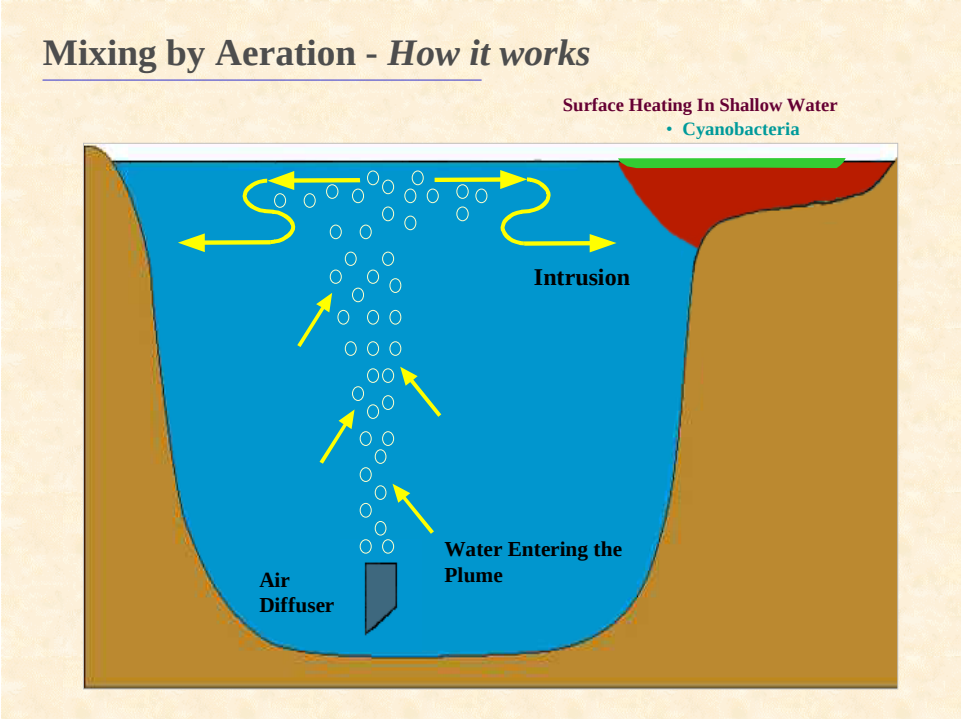

Figure 3 Schematic of bubble plume aeration

2.5.2 Mixing strategies

Mechanical mixers are a method of destratification, which work by creating a downward flow from the surface by an impeller (with or without a draft tube), displacing bottom water very slowly upward (Figure 4). The mixers tend to be physically large, with diameters of the impellers of five meters being used (Burch et al., 2000; Kirke, 2000). The draft tube forces vertical exchange to the required depth.

Water is drawn slowly radially inward at the surface up to many meters away from the impeller. This effectively “skims off” the warm surface layer where the cyanobacteria tend to bloom. This water is pumped down to levels below the photic zone. Oxygen is also transported down to the oxygen depleted lower levels. An adequate flow for the size of the storage and penetration to the full depth of the storage of the downward flow is important to achieve destratification (Kirke, 2000). The entrained surface layer mixes into the main circulation pattern of the reservoir (Burch et al., 2000).

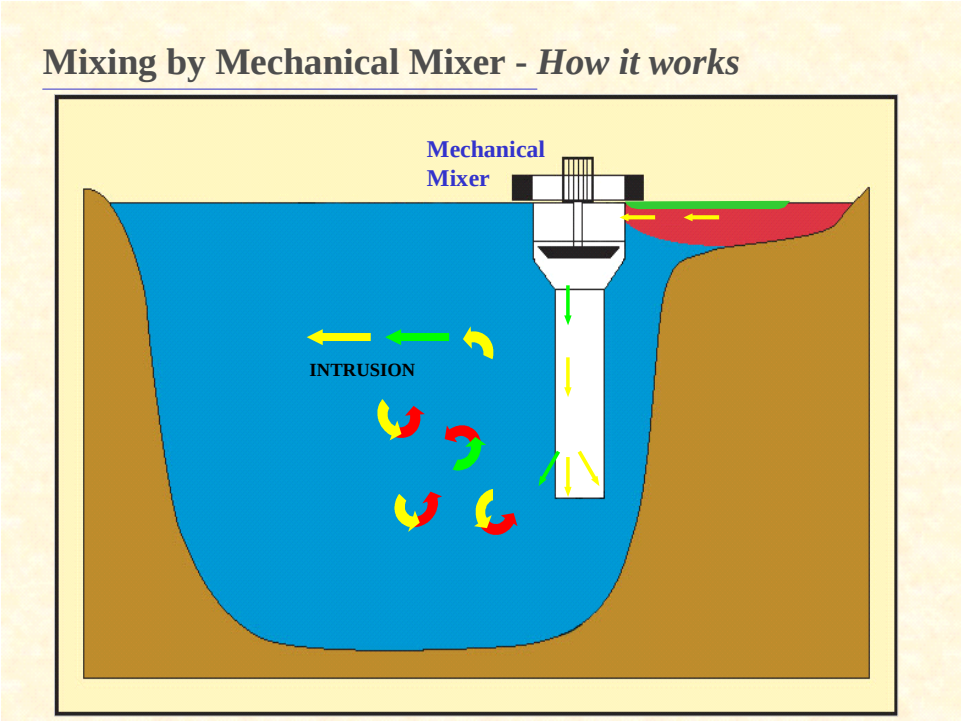

Figure 4 Schematic of mixing by mechanical mixer

2.5.3 Algicides

Algicides have been widely used as a management tool to control cyanobacterial blooms. They can provide immediate and cost effective control of algae. Algicide treatment of water bodies most commonly involves the use of copper based compounds, mainly copper sulphate ($CuSO_4.5H_2O$). However other compounds that have been used as algicides include (Burch et al., 1998):

- Cooper-ethylenediamine complex
- Copper-triethanolamine complex
- Copper citrate
- Potassium permanganate
- Chlorine
- Lime
- Barley straw.

Copper sulphate is the algicide most widely used to control cyanobacterial blooms as it is regarded as effective, economical and safe for operators to use, although copper can have adverse environmental impacts on the aquatic ecosystem (Burch et al., 2001).

The mechanism of copper toxicity is, at high concentrations, thought to be by cell lysis (McBrien and Hassall, 1965). At lower concentrations copper is transported into the algal cells where it exerts physiological and toxic effects (Florence, 1986).

The effectiveness of copper as an algicide is determined by a combination of factors. Chemical indicators such as pH, alkalinity and dissolved organic carbon (DOC) of the receiving water control copper speciation and complexation, which reduces copper toxicity. Thermal stratification affects the distribution of copper after application, which may then affect contact with the algae (Burch et al., 2001).

It is important, when treating cyanobacteria with algicides that they are applied in the early stages of bloom development when cell numbers are low. This reduces the potential release of high concentrations of toxins or odour compounds. The lysis of cells by algicides can compromise the effectiveness of toxin removal by conventional water treatment strategies. When algicides are used it is good practice to isolate the reservoir for a period to allow the toxins and odours to degrade (Burch et al., 2001).

Copper sulphate is a broad-spectrum aquatic biocide. Cyanobacteria are generally regarded as being sensitive to copper sulphate. Table 8 summarises the relative toxicity of copper sulphate to a range of different cyanobacteria (Palmer, 1962).

Table 8 Relative toxicity of copper sulphate to cyanobacteria. Modified after Palmer (1962)

Group	Very Susceptible	Susceptible	Resistant	Very Resistant
Cyanobacteria	*Anabaena, Microcystis, (Anacystis), Aphanizomenon, Gomphosphaeria, Rivularia*	*Cylindrospermum, Planktothrix, (Oscillatoria), Plectonema*	*Nostoc, Phormidium*	*Calothrix, Symploca*

The effective dose rate for copper sulphate for most commonly occurring planktonic algae and cyanobacteria is in the range of 0.5-2.0 mg L^{-1} which would be equivalent to 0.125-0.5 mg Cu L^{-1} (Holden, 1970, Burch et al., 2001). The effective dose can be affected by factors of pH, alkalinity, DOC, and temperature of the water body and the abundance and physiological state of the algae (Palmer, 1962; Holden, 1970).

It is important to note the environmental impacts that copper dosing may cause. Copper is known to be toxic to non-target organisms such as zooplankton, other invertebrates and fish (Burch et al., 2001). It is also known to accumulate in lake sediments (Sanchez and Lee, 1978, Hanson and Stefan, 1984). In many countries there are national or local regulations to control the use of algicides due to their adverse environmental impacts (Burch et al., 2001).

Recommendations for copper sulphate dosing

There is a range of methods used for copper sulphate dosing. The most commonly used methods involve variations on delivery of dry copper sulphate alongside or behind powerboats. The method of application of copper sulphate may have important effects on copper dispersal and ultimately the toxicity and success of treatment. The most common and traditional copper sulphate application method used in North America has been the dragging of burlap bags filled with $CuSO_4$ crystals alongside or behind motorboats (Goudey, 1936; Monie, 1956; McKnight, 1981). In some cases, metal cages have been used to replace sacks (Goudey, 1936). The procedure used by SA Water in South Australia involves applying copper sulphate via a compressed air stream (ie. blower) to the water surface on either side of powerboats. Alternative application methods include pre-solution and spraying (Mackenthun, 1960; Toth and Riemer, 1968); dry feeding via a belt-conveyor to the propeller stream and wake of the boat (Monie, 1956); the use of modified agricultural spreaders on barges (McGuire et al., 1984); and dry scattering by a specially designed blower system (Goudney, 1936). The application of dry copper sulphate from crop duster aircraft has also been reported (Brouse, 1966).

3. TREATMENT STRATEGIES

Blue-green algal or cyanobacterial toxins can enter a water treatment plant (WTP) in two forms, as intracellular toxins (bound within the cells of cyanobacteria) or as extracellular or dissolved toxins. In the case of the former, removal of intact cells is desirable. Studies have shown that the coagulation process can achieve this without lysing the cells and thus minimising the release of dissolved toxins. However, in certain situations, preoxidation is practised to aid in the removal of algal cells by inactivating the motility of the algal cells prior to flocculation. This causes cell lysis, releasing additional amount of dissolved toxins into the system. Therefore, it is recommended that preoxidation is not practised if toxin removal is a priority. Only when the total toxin concentration (both intra- and extra-) is so low that lysing is irrelevant, should preoxidation be considered acceptable.

The following is a summary of the various treatment methods which have been employed to remove cyanobacterial toxins (cyanotoxins) in a WTP.

3.1 Coagulation, Flocculation and Clarification

Coagulation and flocculation are defined as the process whereby suspended particles are aggregated through the addition of a chemical coagulant.This process is ineffective for the removal of extracellular cyanotoxins (Keijola et al., 1988; Himberg et al., 1989; Lambert et al., 1996; Chow et al., 1998). However, it can be effective in removing intracellular cyanotoxins through the removal of the whole cyanobacterial cell. Studies by Chow et al. (1997, 1998, 1999) and Drikas et al. (2001) have shown that alum coagulation and flocculation does not compromise the membrane integrity of cyanobacterial cells, therefore the process does not release dissolved cyanotoxins into the water. In addition, the authors showed that the use of ferric chloride as a coagulant did not lyse any cells, rather, the addition of this coagulant was found to stimulate the growth of *Microcystis aeruginosa* and *Anabaena circinalis*. Jiang et al. (1993) evaluated alum, ferric sulphate, polyferric sulphate (PFS) and polyaluminium chloride (PACl) for the removal of *Anabaena flos-aquae* and *Asterionella formosa* and found that PFS was the best coagulant for the removal of algal cells, with alum displaying a similar, but slightly lower, removal. The authors attributed this to the presence of more highly charged cation species in the PFS. Coagulation/flocculation of smooth and spherical algal cells has been documented to occur via charge neutralisation while it is suggested that filamentous algae are removed by sweep coagulation (Bernhardt and Clasen, 1991). Therefore, identification of cyanobacteria may be desirable in optimising the coagulation/flocculation process.

In contrast, Pietsch et al. (2002) reported that the coagulation/flocculation process (using alum at a dose of 4 mg L^{-1} as Al and ferric chloride sulphate at a dose of 6 mg L^{-1} Fe) caused extracellular cyanotoxins to be released from *Mircocystis aeruginosa* and *Planktothrix rubescence* cells. However, cyanotoxin release only occurred when using algal cells in the stationary growth phase. No release of cyanotoxin was evident when using cells in the exponential growth phase.

The clarification stage is also important for the management of cyanobacterial cells. Cyanobacterial cell lysis has been documented to occur in the clarifier sludge, releasing extracellular cyanotoxins (Drikas et al., 2001). This becomes a problem during the sedimentation processes, and in particular where recycling of the supernatant from the sludge to the head of the WTP is practised.

In some studies, dissolved air flotation (DAF) has been found to be superior to traditional sedimentation for the removal of cyanobacteria (Edzwald and Wingler, 1990; Edzwald, 1993; Nakamura et al., 1993). This is because some cyanobacteria can produce gas vacuoles which allow the cells to be buoyant, whereas during sedimentation, cyanobacterial cells have a higher propensity

to die and lyse. This is also dependent upon the water quality conditions since, in general, waters of high colour and low turbidity are conducive for DAF processes.

Rapala et al. (2002) looked at various water treatment processes for the removal of endotoxin. They determined that at one WTP, coagulation/clarification/sand filtration were effective in removing 86 % of the endotoxin (initial concentration of 434 EU mL^{-1}). In addition, slow sand filtration was able to remove an additional 36 % of endotoxin. At another WTP, they showed that coagulation and initial settling resulted in 83 % endotoxin removal (initial concentration of 345 EU mL^{-1}). However, secondary settling resulted in no endotoxin removal. Flotation resulted in an additional 26 % removal. Both oxidation and activated carbon processes were found to be ineffective at both WTPs for endotoxin removal implying that only processes used to remove particulate material in drinking water are effective.

3.2 Oxidation

3.2.1 Chlorine

Contrary to the results reported by Hoffman (1976) and Himberg et al. (1989), chlorine has been demonstrated as being an effective oxidant for the destruction of microcystins (Nicholson et al., 1994; Tsuji et al., 1997; Senogles-Derham et al., 2003, Newcombe et al., 2004). Nicholson et al. (1994) showed that provided a chlorine residual of at least 0.5 mg L^{-1} was present after 30 minutes contact time, chlorination was effective in the destruction of microcystin-LR (m-LR). Similarly, Tsuji et al. (1997) showed that a chlorine dose of 2.8 mg L^{-1} for a contact time of 30 minutes was sufficient for 99 % destruction of m-LR. Nicholson et al. (1994) also showed that the destruction of m-LR was found to be pH dependent with the most efficient m-LR destruction occurring between pH 5-8. Values of pH above 8 resulted in decreasing efficiency of m-LR oxidation.

Cylindrospermopsin (CYN) and saxitoxin have also been shown to be readily destroyed by chlorine provided a chlorine residual of 0.5 mg L^{-1} was present after 30 minutes (Senogles et al., 2000; Newcombe and Nicholson, 2002; Senogles-Derham et al., 2003). However, pH was again, an important parameter with a reduction in the efficiency in the chlorination of CYN observed at pH values less than 6. This implies that the hypochlorite ion was important for the destruction of CYN. Similarly, Senogles-Derham et al. (2003) showed that the pH for efficient saxitoxin destruction by chlorination must be greater than 8 with decreasing efficiency at lower pH values.

Chlorine was determined to be ineffective for the removal of anatoxin-a and endotoxin (Carlile, 1994; Rositano and Nicholson, 1994; Rapala et al., 2002). Rositano and Nicholson (1994) showed that at a concentration of 20 μg L^{-1} in raw water, a chlorine dose of 15 mg L^{-1} for 30 minutes only resulted in 15 % destruction of anatoxin-a. Although Rapala et al. (2002) showed no removal of endotoxin by chlorine, a recent study by Anderson et al. (2003) found that endotoxin, derived from bacteria, was efficiently degraded by chlorine. However, high chlorine doses (100 mg L^{-1}) and long contact times (122 hours) were required, conditions which are far removed from those used in practice.

Table 9 Conditions for chlorination recorded in the literature for the destruction of several classes of cyanotoxins

Cyanotoxin (concentration)	Cl_2 Dose (mg L^{-1})	Cl_2 Residual (mg L^{-1})	Contact Time (min)	NOM Present	pH	Destruction (%)	Reference
Cylindrospermopsin μg L^{-1}							
185	1.0	0.83	30	No	>6	>99	Senogles et al. (2000)
130	4.0	0.34	30	Yes (5.8 mg L^{-1})	>6	100	Senogles et al. (2000)
100	0.6	0.53	30	No	7.5	100	Senogles-Derham et al. (2003)
90	4.0	0.66	30	Yes (algal extract)	7.5	100	Senogles-Derham et al. (2003)
LPS-Endotoxin EU mL^{-1}							
35	-	-	-	Yes	-	0	Rapala et al. (2002)
200	100	97	121 hours	Yes (tap water)	7.45	92	Anderson et al. (2003)
300	2.0	1.5	169 hours	Yes (tap water)	7.45	72	Anderson et al. (2003)
Microcystin μg L^{-1} Unless otherwise stated							
192	1.0	0.5	30	Yes (algal extract)	<8	>97	Nicholson et al. (1994)
60	0.6	0.59	30	No	6.7	>99	Senogles-Derham et al. (2003)
50	2.3	0.65	30	Yes (algal extract)	6.3	96	Senogles-Derham et al. (2003)
10 mg L^{-1}	5.0	0.8	30	Yes (tap water + clay)	8.5	Mice died	Hoffman (1976)
10 mg L^{-1}	2.8	-	30	No	<7.2	99	Tsuji et al. (1997)
1 mg L^{-1}	2.0	-	10	No	7	>70	Rositano et al. (1998)
20	5.0	3.4	-	Yes	-	100	Jones et al. (1993)
20	2.0	0.75	-	Yes	-	90	Jones et al. (1993)
>150	High	-	4 days	Yes	-	100	Lam et al. (1995)
4.8	10	1.9	2 hours	Yes (5.7 mg L^{-1})	7.9	29	Hart and Stott (1993)
6.9	1.7	0.7	30	Yes (treated water)	5	>93	Carlile (1994)
6.9	1.7	0.7	30	Yes (treated water)	7	6	Carlile (1994)
6.9	1.7	0.7	30	Yes (treated water)	9	19	Carlile (1994)
6.9	1.7	-	22 hours	Yes (treated water)	5	>93	Carlile (1994)
6.9	1.7	-	22 hours	Yes (treated water)	7	88	Carlile (1994)
6.9		-	22 hours	Yes (treated water)	9	39	Carlile (1994)

Table 9 Conditions for chlorination recorded in the literature for the destruction of several classes of cyanotoxins (continued)

Cyanotoxin (concentration)	Cl_2 Dose (mg L^{-1})	Cl_2 Residual (mg L^{-1})	Contact Time (min)	NOM Present	pH	Destruction (%)	Reference
Nodularin μg L^{-1}							
128	1.0	0.5	30	Yes (algal extract)	<8	>95	Nicholson et al. (1994)
440	10	2.0	5	Yes (9.6 mg L^{-1})	<8	95	Nicholson et al. (1994)
440	10	0.5	30	Yes (9.6 mg L^{-1})	<8	100	Nicholson et al. (1994)
Anatoxin-a μg L^{-1}							
3.1	1.7	-	22 hours	Yes (treated water)	5	0	Carlile (1994)
3.1	1.7	-	22 hours	Yes (treated water)	7	0	Carlile (1994)
3.1	1.7	-	22 hours	Yes (treated water)	9	0	Carlile (1994)
20	15	-	30	Yes (raw water)	7	15	Rositano and Nicholson (1994)
Saxitoxin μg L^{-1}							
10-175	-	0.5	30	Yes	>8	>90	Newcombe and Nicholson. (2002)
91	6.0	0.89	30	Yes (algal extract)	8.7	94	Senogles-Derham et al. (2003)
73	4.0	0.76	30	Yes (algal extract)	8.0	>97	Senogles-Derham et al. (2003)

3.2.2 Ozone

Ozone has been found to be the most effective oxidant for the removal of dissolved microcystins and nodularin provided an ozone residual is present (Rositano, 1996; Rositano et al., 1998, 2001; Shawwa and Smith, 2001; Hoeger et al., 2002). However, the effectiveness of ozone for the destruction of cyanotoxins is expected to be highly dependent upon the characteristics of the water. In particular, natural organic material (NOM) has been found to compete with toxins for the reaction with ozone, resulting in incomplete toxin removal (Hart and Stott, 1993; Carlile, 1994; Rositano, 1996; Mouchet and Bonnélye, 1998; Rositano et al., 1998, 2001; Shawwa and Smith, 2001; Hoeger et al., 2002; Newcombe, 2002). Of these studies only Rositano and coworkers suggested that once the O_3 demand of the water was met, destruction of microcystin to below detection by high performance liquid chromatography (HPLC) could be achieved with a detectable O_3 residual. Even at a dissolved organic carbon (DOC) level of 27 mg L^{-1} an ozone dose of 1 mg L^{-1} with a reaction time of 5 minutes was sufficient for destruction of m-LR and -LA to below detection (Newcombe et al., 2003).

The ozone doses required for anatoxin-a and the saxitoxins have been demonstrated to be higher than those required for microcystin (Carlile, 1994; Rositano et al., 1998, 2001). Rositano et al. (2001) stated that a contact time of 5 minutes with an ozone residual of >0.2 mg L^{-1} was sufficient for the destruction of anatoxin-a to below detection limits, and therefore below the concentration considered of concern for water suppliers. In contrast, the authors showed that four classes of saxitions, GTX3, GTX2, C1 and C2, were quite difficult to destroy requiring considerably higher ozone doses than m-LR, m-LA and anatoxin-a. The authors attributed this to the structural differences of the toxins. Microcystins contain a number of readily oxidisable double bonds, while anatoxin-a and the saxitoxins contain no conjugation or aromaticity, resulting in fewer sites for oxidant attack. This recalcitrance of saxitoxins to ozonation was also found by Brooke (2002, unpublished data). In contrast, Kaeding et al. (1999) showed that an ozone dose of 6 mg L^{-1} oxidised STX, C1, C2 and GTX2 to below HPLC detection, from initial toxin concentrations less than 170 μg L^{-1}. Only at higher concentrations were these toxins difficult to remove requiring in excess of 20 mg L^{-1} of ozone. The cause of the discrepancy between this study and those of Rositano et al. (2001) and Brooke (2002, unpublished data) is not known. All studies showed that the saxitoxin variants reacted differently to ozone treatment with the ease of oxidation following the trend: STX toxins>C toxins>GTX toxins.

Only a few studies have focussed on the ozonation of emerging toxins such as cylindrospermopsin (CYN) and endotoxin. Craig et al. (1998) showed that cylindrospermopsin was efficiently degraded using an ozone dose of 1.5 mg L^{-1} and a contact time of 5 minutes in Grahamstown Dam water (4 mg L^{-1} DOC). However, ozonation was found to be ineffective for endotoxin removal with only 8 % removal (initial endotoxin concentration of 60 EU mL^{-1}) (Rapala et al., 2002).

Table 10 lists the ozonation conditions that have been documented in the literature for the destruction of several classes of cyanotoxins.

Table 10 Ozonation conditions employed in the literature for cyanotoxin destruction

Cyanotoxin (concentration)	O_3 Dose (mg L^{-1})	O_3 Residual (mg L^{-1})	Contact Time (min)	NOM Present	pH	Destruction (%)	Reference
Cylindrospermopsin mg L^{-1}							
24.6	1.5	<0.2	5	Yes (4.0 mg L^{-1})	6.9	100	Craig et al. (1998)
LPS-Endotoxin EU mL^{-1}							
60	-	-	-	Yes		8	Rapala et al. (2002)
Microcystin μg L^{-1} Unless otherwise stated							
166	0.2	-	<4	No	-	100	Rositano et al. (1998)
220	1.0		5	Yes (algal extract)	-	100	Rositano et al. (1998)
1 mg L^{-1}	0.22	-	15 sec	No	7	100	Rositano et al. (1998)
10	0.5	0.4	9	No	-	100	Hoeger et al. (2002)
~40	1.0	-	5	Yes (27 mg L^{-1})	7.5	100	Newcombe et al. (2003)
20	0.5	0.0	5	Yes (5.3 mg L^{-1})	7.8	100	Rositano et al. (2001)
20	0.6	0.0	5	Yes (4.6 mg L^{-1})	7.5	100	Rositano et al. (2001)
20	0.7	0.0	5	Yes (5.7 mg L^{-1})	7.8	100	Rositano et al. (2001)
20	1.1	0.0	5	Yes (15.5 mg L^{-1})	7.1	100	Rositano et al. (2001)
58	1.0	-	30	Yes	-	100	Keijola et al. (1988)
1.4	2.7	0.01	5	Yes (raw water)	-	>71	Carlile (1994)
1.0	1.5	0.02	5	Yes (treated water)	-	50	Carlile (1994)
10	2.0	-	10	Yes (5.1-5.7 mg L^{-1})	7.2-7.7	60	Hart and Stott (1993)
10	2.0	-	10	Yes (2.7-4.2 mg L^{-1})	6.9-7.9	98	Hart and Stott (1993)
Unknown quantity	2.0	-	-	Yes	-	100	Hart et al. (1998)
Nodularin μg L^{-1}							
88	0.05	-	15 sec	No	-	100	Rositano et al. (1998)
Anatoxin-a μg L^{-1}							
24	-	0.11	1	No	-	92	Rositano et al. (1998)
20	1.1	0.06	5	Yes (5.3 mg L^{-1})	7.8	100	Rositano et al. (2001)
20	1.7	0.06	5	Yes (4.6 mg L^{-1})	7.5	100	Rositano et al. (2001)
20	1.5	0.05	5	Yes (5.7 mg L^{-1})	7.8	100	Rositano et al. (2001)
20	>2.2	>0.03	5	Yes (15.5 mg L^{-1})	7.1	100	Rositano et al. (2001)
Unknown quantity	2.0	-	-	Yes	-	>90	Hart et al. (1998)
2.4	4.5	<0.01	5	Yes (raw water)	-	75	Carlile (1994)
3.6	2.2	0.01	5	Yes (treated water)	-	>91	Carlile (1994)

Table 10 Ozonation conditions employed in the literature for cyanotoxin destruction (continued)

Cyanotoxin (concentration)	O_3 Dose (mg L^{-1})	O_3 Residual (mg L^{-1})	Contact Time (min)	NOM Present	pH	Destruction (%)	Reference
Saxitoxin μg L^{-1}							
GTX2 11	6.0	-	5	Yes (treated water)	6.5	100	Kaeding et al. (1999)
GTX3 58	6.0	-	5	Yes (treated water)	6.5	97	Kaeding et al. (1999)
C1 34	6.0	-	5	Yes (treated water)	6.5	100	Kaeding et al. (1999)
C2 167	6.0	-	5	Yes (treated water)	6.5	100	Kaeding et al. (1999)
STX 40	6.0	-	5	Yes (treated water)	6.5	100	Kaeding et al. (1999)
Unknown quantity	6.9 mg min L^{-1}	-	5	Yes (treated water)	-	0	Newcombe and Nicholson, 2002

3.2.3 Other oxidants

Chloramine
Chloramine has been found to be ineffective at oxidising microcystin (Nicholson et al., 1994; Hart et al., 1998) although a more recent study by Karner et al. (2001) indicated that chloramine achieved some oxidation of microcystins after a reaction time of approximately 10 hours.

Potassium permanganate
Petrusevski et al. (1996) showed that preoxidation with permanganate followed by coagulation was effective for the removal of particles and algal cells and also reduced manganese problems. However, the use of permanganate causes algal cells to lyse (if preoxidation is practiced) releasing additional toxin as well as manganese problems (Lam et al., 1995; Pietsch et al., 2002). One study has shown that preoxidation with permanganate resulted in some oxidation of microcystins (Karner et al., 2001). The authors reported 52 and 54 % removals of microcystin at two different WTPs employing permanganate as a preoxidant.

Carlile (1994) showed that permanganate was effective for removing m-LR with a removal of 76 % when a low dose of 0.7 mg L^{-1} was employed. A dose of 1 mg L^{-1} removed 88 % of m-LR. Doses greater than 1 mg L^{-1} removed m-LR to below detection limit. In addition the same author showed that permanganate was effective for removing anatoxin-a with removals in excess of 85 % when a low dose of 0.5 mg L^{-1} was employed. A dose of 1 mg L^{-1} removed the toxin to below the limit of detection.

Fawell et al. (1993) also reported efficient removals of microcystins using permanganate in both raw and clarified water. They showed that a dose of 2 mg L^{-1} resulted in removing microcystin to below detection limit. Similarly, Rositano et al. (1998) showed that a 2 mg L^{-1} dose of permanganate was capable of oxidising more than 90 % of m-LR in a solution of 1 mg L^{-1}.

3.3 Adsorption: GAC, PAC

The majority of work relating to the removal of cyanotoxins by activated carbon (AC) has been conducted on the microcystin toxins (Donati et al., 1993, 1994; Fawell et al., 1993; Craig and Bailey, 1995; Lambert et al., 1996; Schumann et al., 1997; Cook and Newcombe, 2002). In addition, most of these studies have suggested that coal and wood based ACs are the best for microcystin adsorption due to their large mesopore volume. Two forms of ACs can be used to remove cyanotoxins: powdered activated carbon (PAC) and granular activated carbon (GAC). The major difference between the two forms is the particle size and the mode of use. Granular activated carbon has a larger particle size and is employed as a filter medium, while PAC is dosed as a powder at various points in the WTP.

3.3.1 Powdered activated carbon

Fawell et al. (1993) and Hart et al. (1998) showed that 20 mg L^{-1} of a wood based PAC was capable of removing 85 % of microcystins while Bruchet et al. (1998) showed that a coal based PAC was effective for the removal of m-LR from Seine River water. They showed that a dose of 12 mg L^{-1} removed >95 % of m-LR at an initial concentration of 50 μg L^{-1}.

Cook and Newcombe (2002) conducted PAC adsorption experiments on 4 variants of microcystin: m-LR, m-LA, m-YR and m-RR. They discovered that the removal of the variants was quite different with the ease of removal for the variants in the order m-RR>m-YR>m-LR>m-LA. These results were confirmed using a range of PACs with different starting materials and activation methods. The authors concluded that while PAC was an effective method for the removal of m-LR,

-RR and -YR, it would not be a viable method for m-LA, due to the high doses required for its complete removal. The only other study which compared AC removal of multiple analogues was by Lawton et al. (1998). They showed that m-LR was 0-10 % less adsorbed than the more hydrophobic analogues (m-LF, -LW and -LY) using domestic jug filters that incorporated a mixture of GAC and ion exchange resin.

Cook et al. (2000) showed that PAC doses greater than 30 mg L^{-1} with long contact times (180 minutes) were required to remove all saxitoxin variants. The authors also showed that the removal of the variants followed the trend, GTX4>STX>GTX2≅GTX3>C1≅C2. This was a significant finding since the most toxic variants, STX and GTX are more easily removed than their less toxic counterparts, the C-toxins. The study also reported the variants in terms of STX-equivalents (STX-eq) and found that 3 steam activated carbons of different starting material reduced toxin concentrations to below the 3 mg L^{-1} STX-eq L^{-1} health alert level proposed by Fitzgerald et al. (1999).

Table 11 lists the PAC conditions found in the literature for the removal of several classes of cyanotoxins.

Table 11 Conditions employed in the literature for cyanotoxin removal by PAC

Cyanotoxin (concentration)	PAC Type	PAC Dose (mg L^{-1})	Contact Time (min)	NOM Present	Percent Removal	Reference
Microcystin μg L^{-1} Unless otherwise stated						
50	coal	1.5	-	No	>99	Bruchet et al. (1998)
50	peat	12	-	Yes (river)	>95	Bruchet et al. (1998)
8.8	coal	59.9	120	Yes (7.7 mg L^{-1})	90	Hart and Stott (1993)
10.2	coconut	108.5	120	Yes (7.7 mg L^{-1})	84	Hart and Stott (1993)
5.7	wood	20	30	Yes (7.7 mg L^{-1})	>82	Hart and Stott (1993)
8.9	wood	40	60	Yes (7.7 mg L^{-1})	>89	Hart and Stott (1993)
12.8	wood	30.5	120	Yes (7.2 mg L^{-1})	>91	Hart and Stott (1993)
20	rice hull	100	30	Yes	90	Craig and Bailey (1995)
20	rice hull	30	60	Yes	60	Craig and Bailey (1995)
20	rice hull	20	30	Yes	35	Craig and Bailey (1995)
20	hardwood	20	30	Yes	40	Craig and Bailey (1995)
20	softwood	20	30	Yes	55	Craig and Bailey (1995)
20	tree seed husks	10	1440 (1 day)	No	~95	Warhurst et al. (1997)
20	tree seed husks	50	1440 (1 day)	No	>99.6	UKWIR (1996)
50	wood	25	30	Yes (river)	>98	Donati et al. (1994)
50	coconut	50	30	Yes (river)	60	Donati et al. (1994)
50	-	20	-	Yes	99	Keijola et al. (1988)
58	-	5	-	Yes	20	Keijola et al. (1988)
8 mg L^{-1}	-	800	2	Yes (tap water)	Mice survived	Hoffman (1976)
10	wood	20	-	Yes (5.7 mg L^{-1})	85	Fawell et al. (1993)
10	coal	20	-	Yes (5.7 mg L^{-1})	40	Fawell et al. (1993)
10	coconut	20	-	Yes (5.7 mg L^{-1})	40	Fawell et al. (1993)
5	-	30	30	Yes (reservoir)	86	Jones et al. (1993)
5	-	30	120	Yes (reservoir)	90	Jones et al. (1993)
10	-	100	30	Yes (reservoir)	93	Jones et al. (1993)
10	-	100	120	Yes (reservoir)	95	Jones et al. (1993)
20	-	100	30	Yes (reservoir)	90	Jones et al. (1993)
20	-	100	120	Yes (reservoir)	95.5	Jones et al. (1993)
50	-	100	30	Yes (reservoir)	95.6	Jones et al. (1993)
50	-	100	120	Yes (reservoir)	98.2	Jones et al. (1993)

Table 11 Conditions employed in the literature for cyanotoxin removal by PAC (continued)

Cyanotoxin (concentration)	PAC Type	PAC Dose (mg L^{-1})	Contact Time (min)	NOM Present	Percent Removal	Reference
Saxitoxin-eq μg L^{-1}						
1.1	wood	30	60	Yes (5.7 mg L^{-1})	66	Cook et al. (2000)
1.1	coconut	30	60	Yes (5.7 mg L^{-1})	65	Cook et al. (2000)
1.1	coal	30	60	Yes (5.7 mg L^{-1})	73	Cook et al. (2000)
1.1	wood	30	180	Yes (5.7 mg L^{-1})	85	Cook et al. (2000)
1.1	coconut	30	180	Yes (5.7 mg L^{-1})	80	Cook et al. (2000)
1.1	coal	30	180	Yes (5.7 mg L^{-1})	72	Cook et al. (2000)
Saxitoxin μg L^{-1}						
4.7	wood	15	30	Yes	100	Newcombe and Nicholson (2002)
Anatoxin-a μg L^{-1} Unless otherwise stated						
7.8	wood	38.4	-	Yes (treated)	68	Carlile (1994)
4.4 mg L^{-1}	-	5	-	Yes	58	Keijola et al. (1988)

3.3.2 Granular activated carbon

Granular activated carbon has been shown to be effective for the removal of cyanotoxins (Falconer et al., 1989; Lahti and Hiisvirta, 1989; Jones et al., 1993; Craig and Bailey, 1995). However, excess NOM loading causes the reduction of the operational lifetime of a GAC and consequently reduces its adsorption capacity for microcystin (Lambert et al., 1996). Craig and Bailey (1995) showed significant breakthrough of microcystin after 5 months operation at an empty bed contact time (EBCT) of 15 minutes. In addition, they showed that an EBCT of 6 minutes resulted in significant microcystin breakthrough after 1 month operation.

Newcombe (2002) conducted pilot plant studies at two different WTPs employing a wood based GAC for the removals of m-LR and m-LA. An EBCT of 15 minutes was employed over a 6 month period at each WTP. Virgin GAC was found to completely remove both analogues at each WTP. Breakthrough of the analogues was dependent upon the water quality conditions. In the water containing a lower DOC and SUVA, breakthrough did not occur until after 6 months operation whilst at the higher DOC and SUVA water breakthrough occurred after 1 month operation. In addition, m-LR was found to be more readily adsorbed than m-LA, similar to PAC studies conducted by Cook and Newcombe (2002).

A UKWIR study showed that pilot scale GAC adsorbers were effective for the removal of anatoxin-a and m-LR using EBCTs of 7.5 and 15 minutes (UKWIR, 1996). Both virgin and used GACs were evaluated with no observed breakthrough of either toxin. The authors attributed the removals to both adsorption and biodegradation. A GAC study by Carlile (1994) showed that anatoxin-a was better adsorbed than m-LR with the authors stating that a contact time of 15 minutes would be sufficient for adequate levels of removal of anatoxin-a.

Newcombe (2002) reported removals of various saxitoxin analogues in laboratory scale column experiments using a coconut based GAC with a treated reservoir water as the influent. They showed that the ease of removals of the analogues followed the trend: STX>GTX>C, similar to the findings of Cook et al. (2000). Removals were found to be dependent upon the bed life of the GAC. Initially 100 % STX-eq removal was observed with virgin GAC. After 1 month operation greater than 90 % removal was evident while after 6 months, approximately 70 % removal was observed.

Activated carbon filtration has been shown to be ineffective for endotoxin removal where in some cases an increase in endotoxin concentration occurred indicative of desorption (Rapala et al., 2002). This indicates that endotoxins may be weakly adsorbing compounds.

Table 12 lists the GAC conditions which have been documented in the literature for the removal of several classes of cyanotoxins.

Table 12 Conditions employed for cyanotoxin removal by GAC in the literature

Cyanotoxin (concentration)	GACType/Scale	EBCT (min)	Bed Volumes/Life	NOM Present	Percent Breakthrough	Reference
Microcystin μg L^{-1}						
7.7	coal/lab	6.1	49100	Yes (4.2 mg L^{-1})	73	Hart and Stott (1993)
7.7	peat/lab	6.7	43900	Yes (4.2 mg L^{-1})	83	Hart and Stott (1993)
10.9	wood/lab	5.8	78300	Yes (4.2 mg L^{-1})	100	Hart and Stott (1993)
10.9	coconut/lab	5.8	79100	Yes (4.2 mg L^{-1})	82	Hart and Stott (1993)
9.4	coal/lab	6.6	51100	Yes (2.7 mg L^{-1})	97	Hart and Stott (1993)
9.4	wood/lab	6.1	55500	Yes (2.7 mg L^{-1})	80	Hart and Stott (1993)
14.2	coal/pilot	15	7 weeks	Yes	<2	Carlile (1994)
14.2	coal/pilot	7.5	7 weeks	Yes	13	Carlile (1994)
20	coal/full	8	7 months	Yes	72	Craig and Bailey (1995)
20	coal/full	6	7 months	Yes	75	Craig and Bailey (1995)
20	coconut/full	8	7 months	Yes	70	Craig and Bailey (1995)
20	coconut/full	6	7 months	Yes	90	Craig and Bailey (1995)
20	coal/pilot	6	1 month	Yes	50	Craig and Bailey (1995)
20	coal/pilot	15	2 month	Yes	25	Craig and Bailey (1995)
30-50	coal/pilot	7.5	7000-12000	Yes (5.0-6.5 mg L^{-1})	<10	Bruchet et al. (1998)
9.6	coal/pilot	7.5	17 days	Yes (2.3 mg L^{-1})	<3	UKWIR (1996)
9.6	coal/pilot	15	17 days	Yes (2.3 mg L^{-1})	<3	UKWIR (1996)
LR ~28	wood/pilot	15	6 months	Yes (treated ~4 mg L^{-1})	~4	Newcombe (2002)
LA ~32	wood/pilot	15	6 months	Yes (treated ~4 mg L^{-1})	~32	Newcombe (2002)
LR ~3	wood/pilot	15	1 month	Yes (treated ~6 mg L^{-1})	~20	Newcombe (2002)
LA ~5	wood/pilot	15	1 month	Yes (treated ~6 mg L^{-1})	~50	Newcombe (2002)
Saxitoxin-eq μg L^{-1}						
~18	coconut/lab	15	1 month	Yes (treated ~4 mg L^{-1})	<10	Newcombe (2002)
~6	coconut/lab	15	6 months	Yes (treated ~4 mg L^{-1})	~30	Newcombe (2002)
Anatoxin-a μg L^{-1}						
8.2	coal/lab	6.2	49600	Yes (treated)	72	Carlile (1994)
3.1	coal/lab	9.3	50200	Yes (treated)	81	Carlile (1994)
4.8	coal/pilot	7.5	65 days	Yes (2.3 mg L^{-1})	<10	UKWIR (1996)
4.8	coal/pilot	15	65 days	Yes (2.3 mg L^{-1})	<10	UKWIR (1996)

3.4 Biological Filtration

Cyanotoxins, in particular the microcystin toxins, are susceptible to biological degradation (Watanabe et al., 1992; Jones and Orr, 1994; Rapala et al., 1994; Lam et al., 1995; Cousins et al., 1996; Tsuji et al., 1996; Christoffersen et al., 2002). With the exception of Watanabe et al. (1992) and Lam et al. (1995), these studies described the degradation of microcystins in batch reactors containing natural waters from lakes and reservoirs. Watanabe et al. (1992) employed a specific growth medium in their batch experiments, whereas Lam et al. (1995) conducted their experiments using sewage effluent from a wastewater treatment plant as their growth medium. In addition, James et al. (1993) has shown that anatoxin-a could be biologically degraded in a natural water body.

Only Bourne et al. (1996, 2001) and Takenaka and Watanabe (1997) have identified specific microorganisms for the degradation of microcystin. Bourne et al. (1996, 2001) identified enzymes, produced by a *Sphingomonas* species, which could degrade m-LR. Similarly, Takenaka and Watanabe (1997) isolated an alkaline protease enzyme from *Pseudomonas aeruginosa* that was shown to be the enzyme responsible for the degradation m-LR.

However, biodegradation of microcystin has been documented not to occur in some studies (Kiviranta et al., 1991; Lambert et al., 1996; Newcombe, 2002). This indicates that specific conditions may be required for degradation to occur. In addition, the presence and perhaps numbers of specific bacteria may be required for degradation to occur.

Perhaps the best way to utilise bacteria for the biological degradation of cyanotoxins is through biological filtration processes. Of the biological filtration studies conducted on cyanotoxins, a majority have been via sand media. Lahti and Hiisvirta (1989) found promising results using a pilot scale slow sand filter for the removal of microcystin. Up to 86 % removal of a microcystin analogue from *Microcystis aeruginosa* was achieved. Studies by Sherman et al. (1995) and Grützmacher et al. (2002) also implicated biological degradation of microcystin in slow sand filters. Grützmacher et al. (2002) showed that more than 90 % of microcystin was biologically degraded inside the filter bed. In addition, sand filters have the propensity to remove cyanobacterial cells with Mouchet and Bonnélye (1998) reporting up to 99 % removal.

Miller and Hallowfield (2001) reported biological degradation of microcystin via batch scale bank filtration experiments using soil/water matrices. They showed complete microcystin removal within 10-16 days in soils containing a high organic and low sand content compared with soils with a low organic, high sand content. The authors attributed the differences to a lack of degrading organisms and/or a lack of nutrients to support the growth of the degrading organisms in the low organic, high sand soil. However, some removal of microcystins via adsorption cannot be disregarded. Miller et al. (2001) showed that soils containing high organic and clay content were capable of adsorbing both nodularin and m-LR.

A recent study by Saitou et al. (2002) showed that a biofilm grown on a non adsorbing honeycomb tube made of vinyl chloride was capable of degrading m-LR, -RR and -YR. In their study, the biofilm was scraped off the surface of the tube (located at the biological treatment facility at the Kasumigara Water Works) and used as a bacterial inoculum in laboratory batch experiments.

GAC filters have also been used as a biological process for the removal of cyanotoxins. Studies by Hart and Stott (1993), Carlile (1994) the UKWIR (1996), Newcombe et al. (2003 a,b), have all implicated biodegradation of cyanotoxins in GAC filters.

The operational conditions for biological filtration are important for the successful removal of cyanotoxins. The type and concentration of bacteria, water quality, filter media, filter contact time and hydraulic loading are all expected to have a major impact on biological filtration processes. Perhaps the most difficult aspect of biological filtration processes is the delay for biological degradation to occur. This is often referred to as the lag period or lag phase. Studies have reported lag periods of days to months before organic compounds are completely degraded. The lag period is a major hindrance for the application of biological filtration processes, particularly for the removal of transient contaminants such as cyanotoxins.

In some cases lag periods can be reduced upon re-addition of the target compound. Senogles et al. (2002) showed that CYN could be degraded by natural aquatic bacteria in a surface water. They found that after 30 days, CYN was degraded to below detection limit with a lag period of approximately 15 days. Upon re-addition of CYN to the sample they observed no lag period. Similarly, Rapala et al. (1994), Christoffersen et al. (2002), Newcombe et al. (2003b) and Holst et al. (2003) have shown that lag periods, in microcystin biological degradation studies, could be substantially reduced, in some circumstances removed, when bacteria were pre-exposed to microcystin.

3.5 UV, Membranes

3.5.1 UV treatment

Microcystins are chemically and physically stable compounds (Tsuji et al., 1994). However, Welker and Steinberg (1999) have showed that natural sunlight (through indirect photolysis) can degrade m-LR, -YR and -RR, in the presence of humic substances. Removals of 45-56 % of the toxins were achieved after 8 hours of irradiation (maximum cumulative UV-radiation was about 30 Wm^2).

Carlile (1994) showed that anatoxin-a was slightly less well removed by UV irradiation than m-LR. They showed that the dose required for 90 % removal of anatoxin-a was 22.5 Ws cm^{-2}, which is two orders of magnitude greater than that required for disinfection. Likewise, for m-LR, a dose of 24 Ws cm^{-2} achieved 91 % destruction.

Advanced Oxidation Processes
Senogles et al. (2001) found that cylindrospermopsin was efficiently degraded using titanium dioxide under UV photolysis. The authors also found that optimum cylindrospermopsin oxidation occurred at pH 9, where there would be greater production of hydroxyl radicals. However, the efficiency of the photocatalytic degradation was reduced at elevated DOC concentrations (15-32.5 mg L^{-1}). The authors attributed this to a reduction in UV transmission with the elevated DOC concentrations. In addition, the authors showed that the presence of other inorganic matter in natural waters greatly assisted the photocatalytic process.

Feitz et al. (1999) showed that the photocatalytic degradation of m-LR using a TiO_2 catalyst was highly dependent upon the pH with rapid degradation occurring at low pH (3.5), while at higher pH, a distinct lag was observed before toxin degradation commenced. This was attributed to the adsorption of m-LR to TiO_2 at this high pH resulting in degradation by long-lived organic radicals. In contrast, Shephard et al. (2002) showed that adsorption of m-LR to TiO_2 occurs between pH 3 and 5, which corresponded to the maximum m-LR degradation rates. Liu et al. (2003) showed that the TiO_2 induced photocatalytic oxidation of m-LR resulted in no toxic byproducts as they determined that the major mechanism of photocatalysis was to isomerize, substitute and cleave the Adda conjugated diene which is generally associated with the toxicity of microcystin.

The photocatalytic oxidation of m-LR by TiO_2 and UV was enhanced with the addition of H_2O_2 (Cornish et al., 2000; Liu et al., 2002). The authors also showed that H_2O_2 with UV illumination in the absence of TiO_2 is capable of degrading m-LR. The combination of H_2O_2/TiO_2/UV resulted in no detectable byproducts with a bioassay indicating that the toxicity of the treated water had been removed. The authors showed that the concentration of H_2O_2 was a limiting factor with maximum m-LR degradation when the concentration was between 0.005 and 0.1 % (33.28 and 27.80 μg min^{-1}, respectively).

Gajdek et al. (2001) showed that the generation of OH radicals via Fenton oxidation was effective for the degradation of m-LR. In their study the combination of H_2O_2 and Fe^{2+} at concentrations of 15 mM and 1.5 mM, respectively, completely degraded m-LR after 30 minutes. A slower reaction was observed when Fe^{2+} was substituted with Fe^{3+}.

3.5.2 Membrane filtration

Membranes have been used successfully to remove intracellular toxins. Chow et al. (1997) used microfiltration (MF) (~0.3 μm pore size) and ultrafiltration (UF) (molecular weight (MW) cut-off of 100000 or pore size of ~0.01 μm) membranes for the removal of *Microcystis aeruginosa* cells using the dead end (DE) and continuous flow (CF) modes. The transmembrane pressure was adjusted to 200 kPa (2 Bar), a worst case scenario pressure since most studies use 30-150 kPa. Both membranes performed better under the CF mode with only small percentages of damaged cells; the highest damage was <17% in the DE mode and this was more pronounced with MF. Only the MF-DE resulted in a small percentage (3 %) of dead cells while the other modes had no dead cells in the backwash water. However, no additional release of microcystin was observed in the permeate. Interestingly, the UF membrane was found to remove some dissolved microcystin, indicating some rejection ability or adsorption capacity for microcystin.

Between 82-99 % removal of dissolved m-LR has been reported by Simpson and MacLeod (2002) and Smith et al. (2002) when using 8 different nanofiltration (NF) membranes. The authors employed NF membranes as a polishing step after a conventionally treated surface water. Similarly, Hart and Stott (1993) evaluated a NF membrane with a nominal MW cut-off of 200 and found that it rejected microcystin with no toxin detected in the permeate. Such performance would be expected for an undamaged NF membrane, as the MW of the microcystins is close to 1000.

Muntisov and Trimboli (1996) used a MEMCOR MF unit followed by a spiralwound Hydranautics PVD1 NF unit for the removal of m-LR and nodularin from River Murray water (Turbidity = 61 NTU; DOC = 9.6 mg L^{-1}). They used a flux of 120 L m^{-2} hr^{-1} and a transmembrane pressure of 60 kPa for MF. NF was operated with a high recycle rate (7.6 times the inflow), a membrane recovery of 7.7 % and an overall recovery of 67 %. Nodularin and m-LR were spiked at 8.4 and 8.0 μg L^{-1}, respectively into the microfiltered water prior to treatment with NF and were found to be removed to below detection by HPLC.

Neumann and Weckesser (1998) showed that three different reverse osmosis (RO) membranes efficiently removed m-LR and m-RR from tap water and tap water spiked with 3000 ppm of NaCl. The initial concentration range of both toxins was 70-130 μg L^{-1}. The membranes were tested for 8 and 48 hours using flow rates of 250 L hr^{-1} at 25 bar for two membranes and 350 L hr^{-1} at 35 bar for one membrane. The average retention rates for m-LR and m-RR were between 96.7 and 99.9 % in the tap water experiments and 98.5 and 99.6 % for the salt water experiments.

Vuori et al. (1997) showed that RO and vacuum distillation, methods based on desalination, effectively removed nodularin from brackish water.

In general, low pressure MF and UF membranes may be used to remove cyanobacterial cells and intracellular cyanotoxins while high pressure membranes such as NF and RO have the ability to remove extracellular dissolved cyanotoxins.

3.6 Multiple Barrier Options

The combination of ozone and granular activated carbon (O_3/BAC) has been shown to be very effective for the removal of CYN (Craig et al., 1998). Craig et al. (1998) showed that the ozone step alone removed all the CYN. Granular activated carbon alone can also be considered a multi-barrier system as it allows for the removal of cyanotoxins via adsorption and biodegradation, as mentioned previously.

Maatouk et al. (2002) showed that the combination of pre-ozonation (0.07 mg L^{-1}) and PAC (20 mg L^{-1}) was found to be effective in completely removing microcystin, while the combination of pre-chlorination (0.42 mg L^{-1}) and PAC (20 mg L^{-1}) only achieved 45 % removal of microcystin. Only when the PAC dose was increased to 40 mg $L^{-1}$for the latter, was complete removal achieved.

A hybrid membrane system which incorporates PAC and UF has been shown to be promising for the removal of cyanobacteria and their metabolites (Mouchet and Bonnélye, 1998).

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APPENDICES

Appendix 1. Calculations and Projections for the "Likely Case" scenario.

Calculations															
Scenario Assumptions:	The climatic conditions are favourable for cyanobacterial growth	The proportion of bio-availble TP is:	The proportion of bio-available P converted to chl *a* is:	The proportion of chl *a* that is *Anabaena*:	The proportion of chl *a* that is *Microcystis*:	The chl *a* content of *Anabaena circinalis* is: (pg $cell^{-1}$)	The chl a content per cell of *Microcystis aeruginosa* is: (pg $cell^{-1}$)	The ratio of geosmin to *Anabaena* chl *a* is:	The proportion of extra-cellular geosmin is:	The ratio of microcystin to *Microcystis* chl *a*	The possible production of saxitoxins from Anabaena	The expected proportion of chl *a* that is *Anabaena* with full destratification	The expected proportion of chl *a* that is *Microcystis* with full destratification	The resulting extra-cellular geosmin concentration with full destratification	The ratio of Microcystin to Microcystis chl a with full destratification
Value:	Yes	0.6	0.8	0.5	0.5	0.72	0.36	100	0.1	0.12	0.33	0.2	0.2	0.1	0.12
Comments:	Population size is then determined by carrying capacity of the reservoir	Some total P unavailable due to binding to particles etc	Some bioavailable P will be taken up by other organisms	Will depend upon the degree of dominance achieved	Will depend upon the degree of dominance achieved	Published value (Reynolds, 1984)	Published value (Reynolds, 1984)	Mean of published range (Bowmer et al. 1992)	Published range (Bowmer et al. 1992)	Maximum of published range (Chorus and Bartram, (1999)	Estimated saxitoxin load is 0.33 ug L^{-1} for every 5000 anabaena cells (Humpage and Falconer, 2003, unpublished).	Cyanobacteria will be outcompeted by other algal species due to destratification	Cyanobacteria will be outcompeted by other algal species due to destratification	Lower anabaena numbers per unit chl *a* will produce less geosmin	Lower microcyctis numbers per chl *a* will produce less microcyctin
Justification:	Given Stable conditions phosphorus concentration is likely to determine biomass in freshwaters	Higher proportions of TP are bioavailable in more eutrophic conditions	Most bio-available P is taken up by phytoplankton	Major blooms of cyanobacteria can form practically monospecific dominance	Major blooms of cyanobacteria can form practically monospecific dominance			Depends upon the strain and environmental conditions	Depends upon the strain and environmental conditions	Depends upon the strain and environmental conditions	Figure represents high abundance of saxitoxin producing Anabaena strain. Levels would vary depending on strain	Emperical modelling of Happy Valley shows that anabaena numbers will be reduced	Emperical modelling of Happy Valley shows that microcystis numbers will be reduced	Bioavailable phosphorus taken up by other algal species	Bioavailable phosphorus taken up by other algal species

Appendix 1 contd. Calculations and Projections for the “Likely Case” scenario.

Concentration in Reservoir

	TP	FRP	Chl a	Chl a	Chl a	Anabaena	Microcystis	geosmin	geosmin	microcystin	Saxitoxin	Anabaena	Microcystis	geosmin	microcystin
	ug L^{-1}	ug L^{-1}	ug L^{-1}	ug L^{-1}	ug L^{-1}	cells mL^{-1}	cells mL^{-1}	ng L^{-1}	ng L^{-1}	ug L^{-1}	ug L^{-1}	cells mL^{-1}	cells mL^{-1}	ng L^{-1}	ug L^{1}
Maximum Level	160	96.00	76.80	38.40	38.40	53,333	106,667	3840.00	384.00	4.61	3.52	7680	7680	154	1.84
Current Level	80	48.00	38.40	19.20	19.20	26,667	53,333	1920.00	192.00	2.30	1.76	3840	3840	77	0.92
Minimum Level	40	24.00	19.20	9.60	9.60	13,333	26,667	960.00	96.00	1.15	0.88	1920	1920	38	0.46