Volume 3 Datasheets – Chemical and physical determinands

Part 2.4: Cyanotoxins

2019

# Part 2.4: Cyanotoxins

### Note

Cyanobacteria are discussed in Part 1: Datasheets for micro-organisms,
Part 1.3: Cyanobacteria.

The USEPA concluded on 22 September 2009 that cyanotoxins are known or anticipated to occur in PWSs and may require regulation. Therefore they added cyanotoxins to their CCL 3 (Drinking Water Contaminant Candidate List 3, USEPA 2009). See:

* USEPA. 2009. *Contaminant Information Sheets for the Final CCL 3 Chemicals*. EPA 815-R-09-012. 216 pp. http://water.epa.gov/scitech/drinkingwater/dws/ccl/upload/Final-CCL-3-Contaminant-Information-Sheets.pdf.

WHO Guidelines (2017) note that many practical considerations for the abatement of microcystins apply similarly to the other cyanotoxins (ie, cylindrospermopsins, saxitoxins, anatoxin-a and anatoxin-a(s)), with one key difference that is relevant to the efficacy of their removal in drinking-water treatment: microcystins are usually cell-bound, and substantial amounts are released to the surrounding water only in situations of cell rupture (ie, lysis), whereas the other cyanotoxins may occur to a larger extent dissolved in water.

Microcystins have received the most attention internationally. As a consequence, the microcystin datasheet contains some material that is also relevant to other cyanotoxins.

Contents

Introduction 1

Anatoxin-a 3

Anatoxin-a(S) 10

BMAA 14

Cylindrospermopsin 17

Endotoxins 25

Homoanatoxin-a 29

Microcystins 33

Nodularin 49

Saxitoxins 55

# Introduction

The following general comments have been copied from IARC (2010 – meeting was in 2006) *Ingested Nitrates and Nitrites, and Cyanobacterial Peptide Toxins* 94. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 464 pp. See: http://monographs.iarc.fr/.

Cyanobacterial metabolites can be lethally toxic to wildlife, domestic livestock and even humans. Cyanotoxins fall into three broad groups of chemical structure:

* cyclic peptides
* alkaloids
* lipopolysaccharides.

The table below gives an overview of the specific toxic substances within these broad groups that are produced by different genera of cyanobacteria together, with their primary target organs in mammals. However, not all cyanobacterial blooms are toxic and neither are all strains within one species. Toxic and non-toxic strains show no predictable difference in appearance and, therefore, physicochemical, biochemical and biological methods are essential for the detection of cyanobacterial toxins.

The most frequently reported cyanobacterial toxins are cyclic heptapeptide toxins known as microcystins which can be isolated from several species of the freshwater genera *Microcystis*, *Planktothrix* (*Oscillatoria*), *Anabaena* and *Nostoc*. More than 70 structural variants of microcystins are known. A structurally very similar class of cyanobacterial toxins is nodularins (*<* 10 structural variants), which are cyclic pentapeptide hepatotoxins that are found in the brackish-water cyanobacterium *Nodularia*.

### General features of the cyanotoxins

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

# Anatoxin-a

### Maximum Acceptable Value (Provisional)

Based on health considerations, the concentration of anatoxin-a (ATX) in drinking-water should not exceed 0.006 mg/L.

WHO (2004 and 2011) does not have a guideline value for anatoxin-a.

Drinking water advisory thresholds have been established for anatoxin-a in Quebec Province (3.7 µg/L), Ohio State (20 µg/L) and Oregon State (3 µg/L) – see <http://www.waterrf.org/PublicReportLibrary/4548a.pdf>.

USEPA (2015) developed a Health Effects Support Document for anatoxin-a to be considered in developing a Health Advisory (HA). The available data on toxicity were not adequate to derive a health-based value for anatoxin-a at that time.

### Sources to drinking-water

#### 1 To source waters

Anatoxin-a (CAS No. 64285-06-9) has a semi-rigid bicyclic secondary amine structure. Six structural analogs have been described: homoanatoxin-a (HTX), 2,3-epoxy-anatoxin-a, 4-hydroxy- and 4-oxo-derivatives, dihydroanatoxin-a (dhATX), dihydrohomoanatoxin-a (dhHTX) and 11-carboxyanatoxin-a. The majority of environmental samples collected in New Zealand from *Phormidium*-dominated mats contain low concentrations of ATX, and are dominated by dhATX, HTX and dhHTX (Cawthron 2015).

It is a cyanobacterial neurotoxin produced by species in at least five genera of both benthic and planktonic cyanobacteria. Anatoxin-a is primarily an intracellular compound that is released into water when cells lyse. Anatoxin-a is highly soluble in water and has been found in surface waters around the world.

A screening programme of 80 water bodies in Germany detected anatoxin-a in 22 percent of all analysed samples (Bumke-Vogt et al 1999). The highest concentration found was 0.0131 mg/L (the sum of intracellular and dissolved toxins).

Samples from 12 reservoirs in Nebraska between 2009 and 2010 were analysed. Anatoxin-a was detected in 31 of the 67 samples at concentrations ranging from 0.00005 (detection limit) to 0.035 mg/L (from USEPA 2015).

For current knowledge of sources of anatoxin-a worldwide and in New Zealand, see Tables 9.1 and 9.2 in the Guidelines.

General features associated with cyanobacteria are discussed in the datasheet for cyanobacteria. The toxin anatoxin-a is discussed here. The Alert Levels Framework for management of toxic cyanobacteria detailed in Chapter 9 should be followed if there is a possibility of cyanobacteria in source water.

### About anatoxin-a

There are three types of cyanobacterial neurotoxins, anatoxin-a, anatoxin-a(s) and the saxitoxins. The anatoxins seem unique to cyanobacteria, while saxitoxins are also produced by various dinoflagellates under the name of paralytic shellfish poisons. Homoanatoxin-a is an analog of anatoxin-a.

Anatoxin-a, or 2-acetyl-9-azbicyclo[4:2:1]non-2-ene, is a bicyclic secondary amine with a molecular weight of 165 Da. It is also a homologue of homoanatoxin-a. An *Oscillatoria*-like species of benthic cyanobacteria has been implicated in the deaths of at least 12 dogs in New Zealand between 1998 and 1999. Although only breakdown products of anatoxin-a were discovered, it is likely that anatoxin-a was the parent compound and therefore responsible for the poisonings (Hamill 2001).

### Forms and fate in the environment

Anatoxin-a is highly soluble, is relatively stable in the dark, but in pure solution in the absence of pigments it undergoes rapid photochemical degradation in sunlight with a half-life for photochemical breakdown of one to two hours (Sivonen and Jones 1999); unlike other the cyanotoxins, this occurs even in the absence of cell pigments. Breakdown is further accelerated by alkaline conditions (Stevens and Krieger 1991).

Anatoxin-a is weakly sorbed to sandy sediments and sorbs most strongly to clay-rich and organic-rich sediment. Organic matter promotes sorption of the anatoxin-a molecule due to the availability of negatively charged sites. A half-life of one to two hours at pH 8 to 9 has been reported. In the absence of sunlight, half-lifes of anatoxin-a can range from several days to several months. Anatoxin-a can be degraded by bacteria.

### Typical concentrations in drinking-water

No Ministry of Health surveillance programmes have investigated the concentration of anatoxin-a in drinking-water supplies. Typical concentrations in New Zealand source waters are therefore unknown.

USEPA (2015) reports that anatoxin-a was detected in three samples of finished water in Florida ranging from below the detection limit to 0.0085 mg/L.

### Removal methods

It should be noted that treatment of water containing cyanobacterial cells with oxidants such as chlorine or ozone, while killing cells, will result in the release of free toxin. Therefore, the practice of prechlorination or pre-ozonation is not recommended without a subsequent step to remove dissolved toxins.

Management of raw water abstraction is effective in reducing the amount of cyanobacteria in raw water supplied for treatment. Removal of cyanobacterial cells by processes including bank filtration, flocculation, coagulation, precipitation and dissolved air flotation prior to chemical treatment that may cause cell lysis during water treatment is an effective method to remove toxins. Details of suggested methods are included in Hrudey et al (1999).

Anatoxin-a is photo-labile, being destroyed by strong sunlight with a half-life of between one and two hours. Granular activated carbon, especially biological activated carbon, removes anatoxin-a, but it is believed that microbial activity within the bed degrades the anatoxin-a (UK WIR 1995).

Oxidation by ozone is effective in removing both intracellular and dissolved anatoxin-a provided a residual of 1.0 mg/L can be maintained (Hall et al 2000; Rositano et al 1998). Potassium permanganate is effective for extracellular but not for intracellular toxins, but chlorination is not effective (Hart et al 1998; Hall et al 2000).

Chlorine, chloramines and chlorine dioxide have been shown to be ineffective.

Use of a combination of treatments is considered to be the best management approach, and the complexity of management necessitates consultation with the relevant health authority. Removal of cyanobacterial blooms and their associated toxins is briefly discussed in the datasheet for cyanobacteria.

### Recommended analytical techniques

#### Referee method

LC-MS: Namikoshi et al 2003; Dell’Aversano et al 2004; Furey et al 2003; Rao and Powell 2003; Quilliam et al 2001.

#### Some alternative methods

HPLC-FLD (James et al 1998).

HPLC–UV (Wong and Hindin 1982).

### Health considerations

Anatoxin-a is a neurotoxin. On acute exposure, anatoxin-a can produce observable adverse health effects including death in less than five minutes to a few hours, depending on the species, the amount of toxin ingested, and the amount of food in the stomach (Carmichael 1992). See Cawthron (2015) for some case studies and discussion on toxicity.

#### Acute effects

Anatoxin-a is a nicotinic (cholinergic) agonist that binds to neuronal nicotinic acetylcholine receptors. Its mode of action leads to blocking of electrical transmission between nerve cells. In sufficiently high doses this can lead to paralysis, asphyxiation and death (Kuiper-Goodman et al 1999).

The mouse LD50 toxicity of anatoxin-a is 0.375 mg/kg (body weight) by I.P. injection, the intranasal LD50 is 2 mg/kg (body weight), and the oral LD50 is greater than 5 mg/kg (Fitzgeorge et al 1994).

#### Chronic effects

Exposures are usually not chronic; however, they can be repeated in regions where cyanobacterial blooms are more extensive or persistent.

Several studies have administered anatoxin-a orally to mice and rats over an extended time span, but they provide no conclusive evidence that allows a formal TDI to be established (adapted from Kuiper-Goodman et al 1999). Only acute effects have been shown in mammals and risk assessment is therefore limited to acute exposure at this stage (Kuiper-Goodman et al 1999).

Cyanobacteria have not been fully reviewed by the International Agency for Research on Cancer (IARC). Further investigation in the area of chronic health risk is required. (See microcystin and nodularin.)

Short-term oral toxicity of anatoxin-a in five-day and 28-day systemic toxicity studies in mice, and a developmental toxicity study in mice produced a NOAEL (No Observed Adverse Effect Level) of 0.1 mg/kg-day, derived from the 28-day study that tested groups of 10 mice per sex at dose levels of 0, 0.1, 0.5 and 2.5 mg/kg-day (from USEPA 2015).

### Recreational exposure

Where sources of water are used for contact recreation, recreational exposure to cyanotoxins may be a health issue. There have been repeated descriptions of adverse health consequences for swimmers exposed to cyanobacterial blooms. Even minor contact with cyanobacteria in bathing water can lead to skin irritation and increased likelihood of gastrointestinal symptoms (Pilotto et al 1997). There are three potential routes of exposure to cyanotoxins: direct contact of exposed parts of the body (including sensitive areas such as the ears, eyes, mouth and throat), accidental swallowing, and inhalation of water.

Individual sensitivity to cyanobacteria in bathing waters varies greatly, because there can be both allergic reactions and direct responses to toxins.

### Derivation of the Maximum Acceptable Value

The provisional MAV for anatoxin-a in drinking-water was derived as follows:

0.2 mg/kg per day x 70 kg x 0.8 = 0.0056 mg/L (rounded to 0.006 mg/L)

 2 L x 1000

where:

* NOAEL = 0.2 mg/kg (body weight) per day was adopted by the Ministry of Health
* average weight of an adult in New Zealand = 70 kg (WHO uses 60 kg)
* the adult *per capita* daily water intake in New Zealand = 2 L
* proportion of TDI allocated to drinking water = 0.8
* uncertainty factor = 1000 (10 for intra-species variation; 10 for inter-species variation; 10 for uncertainties in the database.

### References

APHA. 2005. *Standard Methods for the Examination of Water and Wastewater* (21st edition). Washington: American Public Health Association, American Water Works Association, Water Environment Federation.

Bumke-Vogt C, Mailahn W, Chorus I. 1999. Anatoxin-a and neurotoxic cyanobacteria in German lakes and reservoirs. *Environ Toxicol* 14: 117–25.

Carmichael WW. 1992. A review: cyanobacteria secondary metabolites – the cyanotoxins. *Journal of Applied Bacteriology* 72: 445–59.

Cawthron Institute. 2015. Advice to inform the development of a benthic cyanobacteria attribute. Prepared for Ministry for the Environment. *Cawthron Report* No 2752: 91 pp. <http://www.mfe.govt.nz/sites/default/files/media/Fresh%20water/development-of-a-benthic-cyanobacteria-attribute.pdf>.

Dell’Aversano C, Eaglesham GK, Quilliam MA. 2004. Analysis of cyanobacterial toxins by hydrophilic interaction liquid chromatography-mass spectrometry. *Journal of Chromatography* A1028: 155–64.

Fawell JK, Mitchell RE, Hill RE, et al. 1999. The toxicity of cyanobacterial toxins in the mouse: II Anatoxin-a. *Hum Exp Toxicol* 18(3): 168–73.

Fitzgeorge RB, Clark SA, Keevil CW. 1994. Routes of intoxication. In: Codd GA, Jeffries TM, Keevil CW, et al (eds) *1st International Symposium on Detection Methods for Cyanobacterial (Blue-Green Algal) Toxins*. Cambridge, UK: Royal Society of Chemistry, pp 69–74.

Furey A, Crowley J, Lehane M, et al. 2003. Liquid chromatography with electrospray ion-trap mass spectrometry for the determination of anatoxins in cyanobacteria and drinking water. *Rapid Communications in Mass Spectrometry* 17: 583–8.

Hall T, Hart J, Croll B, et al. 2000. Laboratory-scale investigations of algal toxin removal by water treatment. *J Inst Water Environ Management* 14(2): 143–9.

Hamill K. 2001. Toxicity in benthic freshwater cyanobacteria (blue-green algae): first observations in New Zealand. *New Zealand Journal of Marine and Freshwater Research* 35: 1057–9.

Hart J, Fawell J, Croll B. 1998. Fate of both intra- and extracellular toxins during drinking-water treatment. *Water Supply* 16: 611–16.

Hrudey S, Burch M, Drikas M, et al. 1999. Remedial Measures. In: Chorus I, Bartrum J (eds). *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management*. 416 pp. Published on behalf of the World Health Organization by E&FN Spon, London.

James KJ, Furey A, Sherlock IR, et al. 1998. Sensitive determination of anatoxin-a, homoanatoxin-a and their degradation products by liquid chromatography with fluorimetric detection. *Journal of Chromatography* A798: 147–57.

Kuiper-Goodman T, Falconer I, Fitzgerald J. 1999. Safe levels and safe practices. In: Chorus I, Bartrum J (eds). *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management*. 416 pp. Published on behalf of the World Health Organization by E&FN Spon, London.

Namikoshi M, Murakami T, Watanabe MF, et al. 2003. Simultaneous production of homoanatoxin-a, anatoxin-a, and a new non-toxic 4-hydroxyhomoanatoxin-a by the cyanobacterium *Raphidiopsis mediterranea* Skuja. *Toxicon* 42: 533–8.

NHMRC, ARMCANZ. 2000. National water quality management strategy. Revision of the Australian drinking water guidelines. *Cyanobacteria: Public consultation document* June.

Pilotto L, Douglas R, Burch M, et al. 1997. Health effects of recreational exposure to cyanobacteria (blue-green algae) during recreational water-related activities. In: *Aust NZ J Public Health* 21: 562–6.

Quilliam MA, Hess P, Dell’Aversano C. 2001. Recent developments in the analysis of phycotoxins by liquid chromatography-mass spectrometry. In: WJ de Koe, RA Samson, HP van Egmond, et al (eds). *Proceedings of the 10th International IUPAC Symposium on Mycotoxins and Phycotoxins* 21–25 May 2000, Brazil.

Rao R, Lu L, Powell MW. 2003. Determination of anatoxin-a in drinking water samples by LC/MS. Anonymous. *ThermoQuest LC/MS Application Report*.

Rositano J, Nicholson B, Pieronne P. 1998. Destruction of cyanobacterial toxins by ozone. *Ozone: Science & Engineering* 20: 223–38.

Sivonen K, Jones G. 1999. Cyanobacterial toxins. In: Chorus I, Bartrum J (eds) *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management*. 416 pp. Published on behalf of the World Health Organization by E&FN Spon, London.

Stevens D, Krieger R. 1991. Stability studies on the cyanobacterial nicotinic alkaloid anatoxin-a. *Toxicon* 29: 167–79.

UK WIR. 1995. *GAC Tests to Evaluate Algal Toxin Removal*. Report DW-07/C. London: UK Water Industry Research Ltd.

USEPA. 2015. *Health Effects Support Document for the Cyanobacterial Toxin Anatoxin-A*. EPA 820-R-15104. 58 pp. <http://www.epa.gov/sites/production/files/2015-06/documents/anatoxin-a-report-2015.pdf>.

Wong SH, Hindin E. 1982. Detecting an algal toxin by high pressure liquid chromatography. *American Water Works Association Journal* 74: 528–9.

# Anatoxin-a(S)

### Maximum Acceptable Value (Provisional)

Based on health considerations, the concentration of anatoxin-a(S) in drinking-water should not exceed 0.001 mg/L.

WHO (2004 and 2011) does not have a guideline value for anatoxin-a(S).

### Sources to drinking-water

#### 1 To source waters

Anatoxin-a(S) is a cyanobacterial neurotoxin known to be produced by *Anabaena flos-aquae* (Canada) and *Anabaena lemmermannii* (Denmark). Anatoxin-a(S) has not been detected in New Zealand.

General features associated with cyanobacteria are discussed in the datasheet for cyanobacteria. The toxin anatoxin-a(S) is discussed here.

The Alert Levels Framework for management of toxic cyanobacteria detailed in Chapter 9 of the Guidelines should be followed if there is a possibility of cyanobacteria in source water.

### About anatoxin-a(S)

There are three types of cyanobacterial neurotoxins, anatoxin-a, anatoxin-a(S) and the saxitoxins. The anatoxins seem unique to cyanobacteria, while saxitoxins are also produced by various dinoflagellates under the name of paralytic shellfish poisons.

Anatoxin-a(S) is an organophosphate with a molecular weight of 252 Da, similar in its action to synthetic organophosphate pesticides such as parathion and malathion. It is the only known naturally produced organophosphate (ARNAT).

### Forms and fate in the environment

Anatoxin-a(S) decomposes rapidly in alkaline solutions but is relatively stable under neutral and acidic conditions (Matsunaga et al 1989).

### Typical concentrations in drinking-water

Anatoxin-a(S) has not been detected in New Zealand yet. No Ministry of Health surveillance programmes have investigated the concentration of anatoxin-a(S) in drinking-water supplies.

### Removal methods

It should be noted that treatment of water containing cyanobacterial cells with oxidants such as chlorine or ozone, while killing cells, will result in the release of free toxin. Therefore, the practice of prechlorination or pre-ozonation is not recommended without a subsequent step to remove dissolved toxins.

Management of raw water abstraction is effective in reducing the amount of cyanobacteria in raw water supplied for treatment. Removal of cyanobacterial cells by processes including bank filtration, flocculation, coagulation, precipitation and dissolved air floatation prior to chemical treatment that may cause cell lysis during water treatment is an effective method to remove toxins. Details of suggested methods are included in Hrudey et al (1999).

Little definitive information is available regarding the removal of anatoxin-a(S) from water supplies except that it decomposes rapidly under alkaline conditions.

Use of a combination of treatments is considered to be the best management approach, and the complexity of management necessitates consultation with the relevant health authority. Removal of cyanobacterial blooms and their associated toxins is briefly discussed in the datasheet for cyanobacteria.

### Recommended analytical techniques

#### Referee method

ChE Inhibition Assay: Mahmood and Carmichael 1987; Barros et al 2004.

#### Some alternative methods

Mouse Bioassay: Falconer 1993.

### Health considerations

The mode of action of anatoxin-a(S) is analogous to organophosphate insecticides. To date there have been no oral toxicity studies for anatoxin-a(S).

#### Acute effects

Anatoxin-a(S) binds to acetylcholinesterase and renders it unable to deactivate the neurotransmitter, acetylcholine. This leads to muscle weakness through exhaustion, respiratory distress (dyspnea) and convulsions (effect on seizure threshold) preceding death. The mouse LD50 by I.P. injection is 0.02 mg/kg body weight. There are no oral toxicity studies for this toxin (Mahmood and Carmichael 1986; Matsunaga et al 1989).

#### Chronic effects

Unknown. There are no oral toxicity studies for this toxin.

### Derivation of Maximum Acceptable Value

The provisional MAV for anatoxin-a(S) in drinking-water was derived as follows:

0.04 mg/kg per day x 70 kg x 0.8 = 0.000224 mg/L

 2 L x 5000

(the analytical limit of detection is 0.001 mg/L so this was adapted as the provisional MAV)

where:

* NOAEL = 0.04 mg/kg (body weight) per day was adopted by the Ministry of Health based on the LD50 in mice (Chorus 2001 personal communication to P Truman, ESR)
* average weight of an adult in New Zealand = 70 kg (WHO uses 60 kg)
* the adult per capita daily water intake in New Zealand = 2 L
* proportion of TDI allocated to drinking water = 0.8
* uncertainty factor = 5,000 (10 for intra-species variation; 10 for inter-species variation; 50 for uncertainties in the database.

### References

ARNAT. 2001. Anatoxins. *Australian Research Network for Algal Toxins*. <http://www.aims.gov.au/arnat/arnat-0002.htm>.

APHA. 2005. *Standard Methods for the Examination of Water and Wastewater* (21st Edition). Washington: American Public Health Association, American Water Works Association, Water Environment Federation.

Barros LPC, Monserrat JM, Yunes JS. 2004. Determining optimised protocols for the extraction of anticholinesterasic compounds in environmental samples containing cyanobacterial species. *Environmental Toxicology and Chemistry* 23: 883–9.

Falconer IR. 1993. Measurement of toxins from blue-green algae in water and foodstuffs. In: Falconer IR (ed) *Algal Toxins in Seafood and Drinking Water*. London: Academic Press, pp 165–75.

Hrudey S, Burch M, Drikas M, et al. 1999. Remedial measures. In: Chorus I, Bartrum J (eds). *Toxic Cyanobacteria in Water. A guide to their public health consequences, monitoring and management*. 416 pp. Published on behalf of the World Health Organization by E&FN Spon, London.

Mahmood N, Carmichael W. 1986. The pharmacology of anatoxin-a(S), a neurotoxin produced by the freshwater cyanobacterium Anabaena flos-aquae NRC 525-17. *Toxicon* 24: 425–34.

Mahmood NA, Carmichael WW. 1987. Anatoxin-a(s), an anticholinesterase from the cyanobacterium *Anabaena flos-aquae* NRC-525-17. *Toxicon* 25: 1221–7.

Matsunaga S, Moore R, Niemczura W, et al. 1989. Anatoxin-a(S), a potent anticholinesterase from *Anabaena flos-aquae*. *J Am Chem Soc* 111: 8021–3.

# BMAA

### Maximum Acceptable Value

Neither the DWSNZ nor the WHO Guidelines have a MAV or guideline value for BMAA.

### Sources to drinking-water

#### 1 To source waters

The finding of the non-protein amino acid, β-methylamino-l-alanine (BMAA) with neurotoxic properties in the brain of patients with degenerative disorders and its reported presence in cyanobacteria found commonly in water sources used to provide drinking water has led to concern over its potential widespread effects on human health.

BMAA has been reported as being present in a wide-range of cyanobacteria species including both free-living and symbiotic strains. BMAA occurs widely amongst free-living cyanobacteria from all the major taxonomic groups. It was present in 95 percent of the genera and 97 percent of the strains tested. So far, many species of cyanobacteria have been shown to produce BMAA under laboratory conditions and to be present in isolates of cyanobacteria from natural blooms. However, there have been some contradictory results and more data are required to confirm that BMAA might be present in natural waters.

### About BMAA

Β-N-methylamino-l-alanine (BMAA) is an uncommon, non-essential amino acid. It has a similar structure to the essential amino acid, glutamate, which is the neurotransmitter in the brain responsible for most excitatory pathways. Dysfunction in glutamate pathways is thought to be involved in the development of a number of degenerative disorders including Parkinson’s Disease, Motor Neuron Disease and, recently, Alzheimer’s Disease. The neurotoxicity of BMAA is thought to involve interaction with glutamate pathways, possibly through a link with bicarbonate. There has more recently been some suggestion that the presence of BMAA in the brain may be associated with Alzheimer’s Disease.

At present, there are insufficient data to confirm an association between the presence of BMAA in the brain and degenerative diseases and this remains a hypothesis.

### Forms and fate in the environment

BMAA is very soluble in water. The Henry’s Law Constant of 3.37 x 10-13 atm-m-3/mole indicates that it is not volatile. Log Octanol-Water Partition Coefficient, Log Kow estimate was -4.00 suggesting that BMAA is hydrophilic with a low soil/sediment coefficient and low capacity to bioconcentrate. Log Kow values of less than +1 usually indicate that a compound is unlikely to be removed by Granular Activated Carbon (GAC).

The suite of biodegradation models suggests that it is readily biodegradable and this will proceed rapidly under normal environmental conditions. The soil adsorption coefficient, Koc value of 2.863 indicates a low tendency to partition to soil and sediment.

### Typical concentrations in drinking-water

No Ministry of Health surveillance programmes have investigated the concentration of BMAA in drinking-water supplies. Typical concentrations in New Zealand source waters are therefore unknown.

### Removal methods

Intact cyanobacteria have been shown to be removed by the mechanical processes of drinking water treatment; however, lysis of cells may release cyanotoxins including BMAA. This may be less for BMAA than for other toxins because at least a portion of BMAA may be protein-bound and potentially less likely to be released from the cell.

While drinking water treatment methods such as chlorination, ozonation and granular activated carbon (GAC) may remove other cyanotoxins, the simpler structure of BMAA may make it less susceptible to these types of treatment, particularly breakdown by oxidants. However, there is no evidence for this at present.

It is also important to note that the effects of water treatment will be different for cell bound and extracellular toxins – it is often the case that what is effective for one is ineffective for the other.

### Recommended analytical techniques

#### Referee method

No MAV.

#### Some alternative methods

See DWI (2008).

### Health considerations

No risk assessment can be made because of the extremely limited current state of knowledge on BMAA.

There is a lack of toxicological information based on standard tests using the oral route of exposure which is more relevant to environmental exposure upon which to base a health-based value for use in a risk assessment.

The toxicity of BMAA cannot be determined at present, in terms of a No Observable Adverse Effect Level (NOAEL) or Lowest Observable Adverse Effect Level (LOAEL) for an animal or human toxicological endpoint.

### Derivation of Maximum Acceptable Value

No MAV.

### References

DWI. 2008. *Risk assessment of BMAA*. Report No: Defra/DWI 7669. 47 pp. http://dwi.defra.gov.uk/research/completed-research/reports/DWI70\_2\_226%20BMAA.pdf.

# Cylindrospermopsin

### Maximum Acceptable Value (Provisional)

Based on health considerations, the concentration of anatoxin-a(S) in drinking-water should not exceed 0.001 mg/L.

WHO (2004 and 2011) does not have a guideline value for cylindrospermopsin. Cylindrospermopsin is included in the [plan of work of the rolling revision](http://www.who.int/entity/water_sanitation_health/dwq/en/index.html) of the WHO Guidelines for Drinking-water Quality.

The Australian Guidelines state: “Due to the lack of adequate data, no guideline value is set for concentrations of cylindrospermopsin. However given the known toxicity, the relevant health authority should be notified immediately if blooms of *Cylindrospermopsis raciborskii* or other producers of cylindrospermopsin are detected in sources of drinking water”. They have established an initial health alert of 0.001 mg/L.

Drinking water advisory thresholds have been established for cylindrospermopsin in Ohio and Oregon States (1 µg/L) – see <http://www.waterrf.org/PublicReportLibrary/4548a.pdf>.

### Sources to drinking-water

#### 1 To source waters

Cylindrospermopsin is a tricyclic guanidine alkaloid liver cyanotoxin known to be produced by *Cylindrospermopsis raciborskii*, *Aphanizomenon ovalisporum, Anabaena bergii, Raphidiopsis curvata* and *Umezakia natans* (see Table 9.1 of the Guidelines). *C. raciborskii* is a tropical/sub-tropical bloom-forming species but does not form visible surface scums and appears to be the major risk source for cylindrospermopsin in water bodies. *U. natans* is a saline species found only in Japan so far and is unlikely to be a potential contaminant source for drinking water. Cylindrospermopsin has been found in New Zealand (see Table 9.2 of the Guidelines). Cylindrospermopsin may be retained within the cell, but most of the time it is found in the water (extracellular) or attached to particulates present in the water (USEPA 2015).

Cylindrospermopsin has been detected unambiguously in *C. raciborskii* in New Zealand (Wood and Stirling 2003).

Concentrations of cylindrospermopsin have been reported at concentrations between 0.05 and 0.2 mg/L in Florida since 1999 by The Harmful Algal Bloom Task Force (from USEPA 2015b).

Benthic *Phormidium* has been shown to synthesise cylindrospermopsin and deoxy-cylindrospermopsin in water supply catchments in Australia (Gaget et al 2017).

The Alert Levels Framework for management of toxic cyanobacteria detailed in Chapter 9 should be followed if there is a possibility of cyanobacteria in source water.

### About cylindrospermopsin

Cylindrospermopsin (CAS No. 143545-90-8) is a tricyclic guanidine alkaloid cytotoxin with a molecular weight of 415 Da, originally isolated from a culture of *C. raciborskii* obtained from a water supply reservoir in tropical North Queensland. Cylindrospermopsin is believed to have been the causative agent in the Palm Island ‘mystery disease’ poisoning incident in Queensland in 1979, in which 148 people were hospitalised. At least one structural variant of cylindrospermopsin (deoxycylindrospermopsin) has been found in cultures of *C. raciborskii* in Australia (Norris et al 1999), and in natural populations of *R. curvata* isolated from a lake in China (Li et al 2001). Analogs have been discovered recently (USEPA 2015b).

### Forms and fate in the environment

Information on the natural breakdown of cylindrospermopsin in natural waters is limited. Chiswell et al (1999) showed that in contrast to other cyanotoxins, a high proportion of cylindrospermopsin in actively growing *C. raciborskii* blooms may be found free in the water and the half-life was between 11 and 15 days for two reservoirs in Queensland.

In sunlight, and in the presence of cell pigments, breakdown occurs quite rapidly becoming more than 90 percent complete within two to three days (Chiswell et al 1999) with a half-life of between 0.6 and 0.9 days; however, pure cylindrospermopsin is relatively stable in sunlight (Sivonen and Jones 1999).

Cylindrospermopsin generally exists in a zwitterionic state (with both positive and negative ions) and is highly soluble in water.

Cylindrospermopsin is relatively stable in the dark and at temperatures from 4°C to 50°C for up to five weeks. Cylindrospermopsin is also resistant to changes in pH and remains stable for up to eight weeks at pH 4, 7 and 10. In the absence of cell pigments, cylindrospermopsin tends to be relatively stable in sunlight, with a half-life of 11 to 15 days in surface waters. Cylindrospermopsin remains a potent toxin even after boiling for 15 minutes. When exposed to both sunlight and cell pigments, cylindrospermopsin breaks down rapidly, more than 90 percent within two to three hours. Taken from USEPA (2015; 2015b).

Groundwater is generally not expected to be at risk of cyanotoxin contamination; however, groundwater under the direct influence of surface water can be vulnerable. Microcystin and cylindrospermopsin do not sorb in sandy aquifers and are transported along with groundwater (USEPA 2015a).

### Typical concentrations in drinking-water

No Ministry of Health surveillance programmes have investigated the concentration of cylindrospermopsin in drinking-water supplies. Typical concentrations in New Zealand source waters are therefore unknown.

In USEPA (2015) it is reported that cylindrospermopsin was detected at concentrations ranging from 8 μg/L to 97 μg/L (0.008 to 0.10 mg/L) in nine finished drinking water samples in a survey in Florida in 2000.

### Removal methods

It should be noted that treatment of water containing cyanobacterial cells with oxidants such as chlorine or ozone, while killing cells, will result in the release of free toxin. Therefore, the practice of prechlorination or pre-ozonation is not recommended without a subsequent step to remove dissolved toxins.

Management of raw water abstraction is effective in reducing the amount of cyanobacteria in raw water supplied for treatment. Removal of cyanobacterial cells by processes including bank filtration, flocculation, coagulation, precipitation and dissolved air floatation prior to chemical treatment that may cause cell lysis during water treatment is an effective method to remove toxins. Details of suggested methods are included in Hrudey et al (1999).

Cylindrospermopsin is oxidised readily by a range of oxidants including ozone and chlorine. Chlorine doses of <1 mg/L have been found to be sufficient for degradation of cylindrospermopsin, when the dissolved organic carbon content is low. However, if organic matter other than cylindrospermopsin is present in the solution, the effectiveness of chlorine is reduced as other organic matter present consumes the chlorine (Senogles et al 2000).

Cylindrospermopsin is also adsorbed from solution by both GAC and PAC. Boiling is not effective for destruction of cylindrospermopsin so boiling of water containing whole cells would also lead to cell lysis and release, but not destruction, of free toxin (NHMRC and NRMMC 2004).

Biological filtration for the removal of cylindrospermopsin is possible as this toxin is susceptible to biodegradation under suitable conditions.

For recent information, see Newcombe et al (2010). In the absence of cell damage, conventional treatment employing coagulation, flocculation, clarification (sedimentation or dissolved air flotation) and rapid granular filtration can be effective at removing intact cells and the majority of intracellular toxins (cell bound). However, if toxins are released into solution, a combination of conventional treatment processes with oxidation, adsorption and/or advanced treatment needs to be considered to treat both intracellular and extracellular cyanotoxins. Rapid sand filtration without pre-treatment (ie, direct filtration, without coagulation/clarification) is not effective for cyanobacterial cell removal. From USEPA (2015).

### Recommended analytical techniques

#### Referee method

LC-MS: Eaglesham et al 1999; Dell’Aversano et al 2004.

#### Some alternative methods

HPLC-PDA: Torokne et al 2004. See also EPA Method 545 which is a LC-ESI/MS/MS method for the determination of cylindrospermopsin and anatoxin-a in drinking water.

### Health considerations

Cylindrospermopsin is a potent hepatotoxin but recent studies suggest there are chronic effects due to exposure to sub-lethal doses. Cylindrospermopsin is thought to be the cause of Palm Island Mystery Disease in 1979 where, following algicide treatment of a cyanobacterial bloom in Solomon Dam on Palm Island, North Queensland, 140 children and 10 adults were hospitalised with acute hepatoenteritis along with a range of other ailments (Byth 1980). Differences in response by mice to cell free extracts and pure toxin suggest that other as yet unidentified toxins may be present in cell free extracts of *C. raciborskii*.

Mouse bioassay using dried cells of *R. curvata* containing deoxycylindrospermopsin at a concentration of 1.3 mg/g did not show 1ethal toxicity at doses as high as 2 mg/kg deoxycylindrospermopsin (Li et al 2001), thereby suggesting that deoxycylindrospermopsin is far less toxic than cylindrospermopsin.

#### Acute effects

Acute cylindrospermopsin intoxication results in liver and kidney damage, as well as damage to the gastro-intestinal tract and blood vessels (Falconer et al 1999; Seawright et al 1999). It is a slow acting toxin with time to death dependant on the dose. Due to the seasonality of cyanobacterial blooms, exposures are not expected to be chronic (USEPA 2015). Symptoms reported include fever, headache, vomiting, bloody diarrhoea, hepatomegaly, and kidney damage with loss of water, electrolytes and protein. No reliable data are available on the exposure levels of cylindrospermopsin that induced these effects.

#### Chronic effects

Exposure studies suggest there are observable health effects due to chronic exposure to sub-lethal doses of cylindrospermopsin. Recent research on chronic exposure to albino mice (Humpage and Falconer 2002) are to be considered by the WHO Chemicals Working Group in May 2005 and may result in the release of a formal guideline concentration for cylindrospermopsin in drinking-water.

The NOAEL (No Observed Adverse Effect Level) was determined to be 0.03 mg/kg/day based on kidney toxicity. The total uncertainty factor (UF) applied to the NOAEL was 300. This was based on a UF of 10 for intraspecies variability, a UF of 10 for interspecies variability, and a UF of 3 (10½) to account for deficiencies in the database. The RfD is 0.0001 mg/kg/d. These data would produce a PMAV of 0.003 mg/L (USEPA 2015a; 2015b, based on Humpage and Falconer).

### Derivation of Maximum Acceptable Value

The provisional MAV for cylindrospermopsin in drinking-water was derived as follows:

0.2 mg/kg per day x 70 kg x 0.8 = 0.00112 mg/L (rounded to 0.001 mg/L = 1 µg/L)

 2 L x 5000

where:

* NOAEL = 0.2 mg/kg (body weight) per day was adopted by the Ministry of Health based on the LD50 of the pure toxin by the I.P. route in mice (Chorus 2001 personal communication to P Truman, ESR)
* average weight of an adult in New Zealand = 70 kg (WHO uses 60 kg)
* the adult per capita daily water intake in New Zealand = 2 L
* proportion of TDI allocated to drinking water = 0.8
* uncertainty factor = 5,000 (10 for intra-species variation; 10 for inter-species variation; 50 for uncertainties in the database.

State health authorities in Queensland, Australia have informally applied a Health Alert Value of 0.003 mg/L (3 µg/L) for cylindrospermopsin in drinking water. An unconfirmed report (Fabbro personal communication) indicated that a field sample (water plus *C. raciborskii* cells) taken from central Queensland would have exceeded the Queensland HAV at a cell concentration of 1,500 cells/mL.

New data from Humpage and Falconer (2002) for chronic exposure to cylindrospermopsin are to be presented to the WHO Chemicals Working Group in May 2005. These data may form the basis for adoption of a Tolerable Daily Intake similar to that for microcystin-LR.

The same PMAV was derived by Humpage and Falconer (2003):

0.03 mg/kg per day x 70 kg x 0.8 = 0.00084 mg/L (rounded to 0.001 mg/L)

 2 L x 1000

where:

* NOAEL = 0.03 mg/kg (body weight) per day based on exposing mice to drinking-water containing cylindrospermopsin from a cyanobacterial extract for 10 weeks, and to purified cylindrospermopsin for 11 weeks
* average weight of an adult in New Zealand = 70 kg (WHO uses 60 kg)
* the adult per capita daily water intake in New Zealand = 2 L
* proportion of TDI allocated to drinking water = 0.8
* uncertainty factor = 1,000.

USEPA developed the non-regulatory Health Advisory (HA) Program in 1978 to provide information for public health officials or other interested groups on pollutants associated with short-term contamination incidents or spills for contaminants that can affect drinking water quality. HAs identify the concentration of a contaminant in drinking water at which adverse health effects are not anticipated to occur over specific exposure durations (eg, one day, 10 days, and a lifetime).

The 10-day HA for bottle-fed infants and young children of pre-school age is 0.7 µg/L.

The 10-day HA for school-age children through to adults is 3.0 µg/L.

### References

APHA. 2005. *Standard Methods for the Examination of Water and Wastewater* (21st Edition). Washington: American Public Health Association, American Water Works Association, Water Environment Federation.

Bourke ATC, et al. 1983. An outbreak of hepato-enteritis (the Palm Island mystery disease) possibly caused by algal intoxication. In: *Toxicon* (Suppl 3): 45–8.

Byth S. 1980. Palm Island mystery disease. *Med J Aust* 2: 40–2.

Chiswell R, Shaw G, Eaglesman G, et al. 1999. Stability of cylindrospermopsin, the toxin from the cyanobacterium, *Cylindrospermopsis raciborskii*: effect of pH, temperature and sunlight on decomposition. *Environ Toxicol* 14: 155–61.

Dell’Aversano C, Eaglesham GK, Quilliam MA. 2004. Analysis of cyanobacterial toxins by hydrophilic interaction liquid chromatography-mass spectrometry. *Journal of Chromatography* A1028: 155–64.

Eaglesham GK, Norris RL, Shaw GR, et al. 1999. Use of HPLC-MS/MS to monitor cylindrospermopsin, a blue-green algal toxin, for public health purposes. *Environmental Toxicology* 14: 151–4.

Falconer IR, Hardy SJ, Humpage AR, et al. 1999. Hepatic and renal toxicity of the blue-green alga (cyanobacterium) *Cylindrospermopsis raciborskii* in male Swiss albino mice. *Environ Toxicol* 14: 143–50.

Gaget V, Humpage AR, Huang Q, et al. 2017. Benthic cyanobacteria: a source of cylindrospermopsin and microcystin in Australian drinking water reservoirs. *Water Research* 124: 454–64.

Harada K, Ohtani I, Iwamoto K, et al. 1994. Isolation of cylindrospermopsin from a cyanobacterium *Umezakia natans* and its screening method. *Toxicon* 32: 73–84.

Hrudey S, Burch M, Drikas M, et al. 1999. Remedial Measures. In: Chorus I, Bartrum J (eds) *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management*. 416 pp. Published on behalf of the World Health Organization by E&FN Spon, London.

Humpage A, Falconer I. 2002. Oral toxicity to Swiss albino mice of the cyanobacterial toxin cylindrospermopsin administered daily over 11 weeks. Canadian Research Centre for Water Quality and Treatment. *Research Report* No. 13.

Humpage A, Falconer I. 2003. Oral toxicity of the cyanobacterial toxin cylindrospermopsin in male Swiss albino mice: determination of no observed adverse effect level for deriving a drinking-water guideline value. *Environ Toxicol* 18: 94–103.

Li R, Carmichael WW, Brittain S, et al. 2001. First report of the cyanotoxins cylindrospermopsin and deoxycylindrospermopsin from *Raphidiopsis curvata* (Cyanobacteria). *J Phycol* 37: 1121.

Newcombe G, House J, Ho L, et al. 2010. Management strategies for cyanobacteria (blue-green algae): a guide for water utilities. Adelaide, Australia: Water Quality Research Australia. *Research report* 74 (112 pages). [http://www.researchgate.net/profile/Lionel\_Ho/publication/242740698\_Management\_Strategies\_for\_Cyanobacteria\_(Blue-Green\_Algae)\_A\_Guide\_for\_Water\_Utilities/links/02e7e52d62273e8f70000000.pdf](http://www.researchgate.net/profile/Lionel_Ho/publication/242740698_Management_Strategies_for_Cyanobacteria_%28Blue-Green_Algae%29_A_Guide_for_Water_Utilities/links/02e7e52d62273e8f70000000.pdf).

Newcombe G, Dreyfus J, Monrolin Y, et al. 2015. *Optimizing Conventional Treatment for the Removal of Cyanobacteria and Toxins*. Denver, CO: Water Research Foundation.

NHMRC, NRMMC. 2004. *Australian Drinking Water Guidelines*. Published by the National Health and Medical Research Council and the Natural Resource Management Ministerial Council, Canberra.

NHMRC, NRMMC. 2011. *Australian Drinking Water Guidelines Paper 6 National Water Quality Management Strategy*. Canberra: National Health and Medical Research Council, National Resource Management Ministerial Council, Commonwealth of Australia. 1244 pp. <http://www.nhmrc.gov.au/guidelines/publications/eh52>.

Norris R, Eaglesham G, Pierens G, et al. 1999. Deoxycylindrospermopsin, an analog of cylindrospermopsin from *Cylindrospermopsis raciborskii*. *Environ Toxicol* 14: 163–5.

Seawright A, Nolan C, Shaw G, et al. 1999. The oral toxicity for mice of the tropical cyanobacterium *Cylindrospermopsis raciborskii* (Woloszynska). *Env Toxicol Water Qual* 14: 135–42.

Senogles P, Shaw G, Smith M, et al. 2000. Degradation of the cyanobacterial toxin cylindrospermopsin, from *Cylindrospermopsis raciborskii*, by chlorination. *Toxicon* 38: 1203–13.

Sivonen K, Jones G. 1999. Cyanobacterial Toxins. In: Chorus I, Bartrum J (eds). *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management.* 416 pp. Published on behalf of the World Health Organization by E&FN Spon, London.

Torokne A, Asztalos M, Bankine M, et al. 2004. Interlaboratory comparison trial on cylindrospermopsin measurement. *Analytical Biochemistry* 332: 280–4.

USEPA. 2015. *Drinking Water Health Advisory for the Cyanobacterial Toxin Cylindrospermopsin*. EPA 820-R-15101. 52 pp. http://www.epa.gov/sites/production/files/2015-06/documents/cylindrospermopsin-report-2015.pdf.

USEPA. 2015a. *Drinking Water Health Advisory for the Cyanobacterial Microcystin Toxins*. EPA-820-R-15100. 75 pp. <http://www.epa.gov/sites/production/files/2015-06/documents/microcystins-report-2015.pdf>.

USEPA. 2015b. *Health Effects Support Document for the Cyanobacterial Toxin Cylindrospermopsin*. EPA 820-R-15103. 76 pp. <http://www.epa.gov/sites/production/files/2015-06/documents/cylindrospermopsin-support-report-2015.pdf>.

WHO. 2004. *Guidelines for Drinking-water Quality 2004* (3rd edition). Geneva: World Health Organization. Available at: [www.who.int/water\_sanitation\_health/dwq/gdwq3/en/print.html](http://www.who.int/water_sanitation_health/dwq/gdwq3/en/print.html) see also the addenda.

WHO (to come). *Background Document for Preparation of WHO* *Guidelines for Drinking-water Quality*. Geneva: World Health Organization. For progress, see: <http://www.who.int/water_sanitation_health/dwq/chemicals/cylindrospermopsin/en/index.html>.

Wood SA, Stirling DJ. 2003. First identification of the cylindrospermopsin-producing cyanobacterium *Cylindrospermopsis raciborskii* in New Zealand. *New Zealand Journal of Marine and Freshwater Research* 37: 821–8.

# Endotoxins

### Maximum Acceptable Value

Due to the lack of adequate data, no MAV is set for concentrations of lipopolysaccharide endotoxins in drinking water.

WHO (2004 and 2011) does not have a guideline value for endotoxins.

### Sources to drinking-water

#### 1 To source waters

Endotoxins are a group of lipopolysaccharides (LPS) produced by cyanobacteria. LPS are generally found in the outer membrane of the cell wall of gram-negative bacteria (Weckesser and Drews 1979), including cyanobacteria, where they form complexes with proteins and phospholipids. All cyanobacteria produce LPS and therefore contribute to the LPS content of drinking water.

General features associated with cyanobacteria are discussed in the datasheet for cyanobacteria. Endotoxins are discussed here.

The Alert Levels Framework for management of toxic cyanobacteria detailed in Chapter 9 should be followed if there is a possibility of cyanobacteria in a source water.

### About lipopolyaccharides

LPS are polymers of complex sugars and fatty acids and are a component of the cell walls of gram-negative bacteria. Little is know about the individual LPS in cyanobacteria.

### Forms and fate in the environment

The chemical stability of cyanobacteria LPS is unknown.

### Typical concentrations in drinking-water

No Ministry of Health surveillance programmes have investigated the concentration of endotoxins in drinking-water supplies. Typical concentrations in New Zealand source waters are therefore unknown.

### Removal methods

It should be noted that treatment of water containing cyanobacterial cells with oxidants such as chlorine or ozone, while killing cells, will result in the release of free toxin. Therefore, the practice of prechlorination or pre-ozonation is not recommended without a subsequent step to remove dissolved toxins.

Management of raw water abstraction is effective in reducing the amount of cyanobacteria in raw water supplied for treatment. Removal of cyanobacterial cells by processes including bank filtration, flocculation, coagulation, precipitation and dissolved air floatation prior to chemical treatment that may cause cell lysis during water treatment is an effective method to remove toxins. Details of suggested methods are included in Hrudey et al (1999).

Specific removal methods for cyanobacterial LPS have not been studied.

Use of a combination of treatments is considered to be the best management approach, and the complexity of management necessitates consultation with the relevant health authority. Removal of cyanobacterial blooms and their associated toxins is briefly discussed in the datasheet for cyanobacteria.

### Analytical methods

#### Referee method

A referee method cannot be selected for LPS endotoxins because a MAV has not been established and therefore the sensitivity required for the referee method is not known.

#### Some alternative methods

No alternative methods can be recommended for LPS endotoxins for the above reason. However, the following information copied from DWSNZ 2000 may be useful:

* Liquid/liquid extraction/gas chromatography-mass spectrometer (APHA 2005 6410 B).
* Liquid/liquid extraction/gas chromatography (APHA 2005 6440 B).

### Health considerations

LPS endotoxins from cyanobacteria pose a potential health risk for humans, but little is known about the occurrence of individual LPS components or their toxicology. They can elicit both allergic and toxic responses in humans. An outbreak of gastroenteritis in Sewickley, Pennsylvania, is suspected to have been caused by cyanobacterial LPS (Lippy and Erb 1976; Keleti et al 1979). The few results available indicate that cyanobacterial LPS are less toxic than the LPS from other bacteria, such as *Salmonella* (Keleti and Sykora 1982; Raziuddin et al 1983).

#### Primary contact exposure

Primary contact exposure is direct contact with water (bathing, swimming etc) that results in dermal contact and is likely to result in accidental ingestion. The World Health Organization advises that for protection against adverse health effects not due to cyanotoxin toxicity, a guideline concentration of 20,000 cells (of total cyanobacteria) per mL can be derived from a study by Pilloto et al (1997). For cell concentrations between 20,000 and 100,000 cells (of total cyanobacteria) per mL (corresponding to a chlorophyll a concentration of approximately 10 to 50 µg/L), there is a probability of moderate adverse health effects and above 100,000 cells (total cyanobacteria) per mL, the risk is high (Falconer et al 1999). However, the World Health Organization has provided for both guideline concentrations to be modified to account for differences in cell size between different species.

#### Acute effects

LPS can elicit both toxic and allergenic effects (Weckesser and Drews 1979). These responses are predominantly due to the lipid component of the LPS complex. LPS have been implicated as the causative agent in dermal irritation, colloquially referred to as swimmer’s itch, and is the cause of asthma attacks through irritation of the bronchial tubes following inhalation of aerosols containing cyanobacteria. They are also indirectly the causative agents for symptoms including hypotension, coagulopathy,and injury to several organ systems through stimulation of secretion of inflammatorycytokines that elicit these effects (Beutler and Poltorak 2001).

#### Chronic effects

Unknown. There are no oral toxicity studies for cyanobacteria endotoxin LPS.

### Derivation of the Maximum Acceptable Value

There is no toxicological database for acute or chronic exposure to cyanobacterial endotoxin LPS to enable derivation of a formal TDI. Therefore it is not possible to establish a MAV for cyanobacterial endotoxin LPS in drinking-water.

### References

APHA. 2005. *Standard Methods for the Examination of Water and Wastewater* (21st edition). Washington: American Public Health Association, American Water Works Association, Water Environment Federation.

Beutler B, Poltorak A. 2001. The sole gateway to endotoxin response: how LPS was identified as TLR4, and its role in innate immunity. *Drug Metabolism and Disposition* 29: 474–8.

Falconer I, Bartrum J, Chorus I, et al. 1999. Safe levels and safe practices. In: Chorus I, Bartrum J (ed). *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management*. 416 pp. Published on behalf of the World Health Organization by E&FN Spon, London.

Hrudey S, Burch M, Drikas M, et al. 1999. Remedial measures. In: Chorus I, Bartrum J (eds). *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management*. 416 pp. Published on behalf of the World Health Organization by E&FN Spon, London.

Keleti G, Sykora J. 1982. Production and properties of cyanobacterial endotoxins. *Appl Environ Microbiol* 43: 104–9.

Keleti G, Sykora J, Libby E, et al. 1979. Composition and biological properties of lipopolysaccharides isolated from *Schizothrix calcicola* (Ag.) Gomont (cyanobacteria). *Appl Environ Microbiol* 38: 471–7.

Lippy E, Erb J. 1976. Gastrointestinal illness at Sewickley, PA. *J Am Water Works Assoc* 68: 606–10.

Pilotto L, Douglas R, Burch M, et al. 1997. Health effects of recreational exposure to cyanobacteria (blue-green algae) during recreational water-related activities. In: *Aust New Zealand J Public Health* 21: 562–6.

Raziuddin S, Siegelman H, Tornabene T. 1983. Lipopolysaccharides of the cyanobacterium *Microcystis aeruginosa*. *Eur J Biochem* 137: 333–6.

Weckesser J, Drews G. 1979. Lipopolysaccharides of photosynthetic prokaryotes. *Ann Rev Microbiol* 33: 215–39.

# Homoanatoxin-a

### Maximum Acceptable Value (Provisional)

Based on health considerations, the concentration of homoanatoxin-a in drinking-water should not exceed 0.002 mg/L.

WHO (2004 and 2011) does not have a guideline value for homoanatoxin-a.

### Sources to drinking-water

#### 1 To source waters

Homoanatoxin-a is a cyanobacterial neurotoxin known to be produced by *Phormidium* species,including *P. formosa* (syn. *Oscillatoria formosa*), and has been found in New Zealand recreational waters.

General features associated with cyanobacteria are discussed in the datasheet for cyanobacteria. The toxin homoanatoxin-a is discussed here.

The Alert Levels Framework for management of toxic cyanobacteria detailed in Chapter 9 should be followed if there is a possibility of cyanobacteria in source water.

#### About homoanatoxin-a

Homoanatoxin-a is a bicyclic secondary amine with a molecular weight of 179 Da. It is a homologue of anatoxin-a (Skulberg et al 1992).

### Forms and fate in the environment

There is no data on the stability of homoanatoxin-a. It is reasonable to expect that chemical stability would be similar to anatoxin-a and that homoanatoxin-a would also break down in strong sunlight, but possibly with a different half-life.

No information is available on bioaccumulation or other biological pathways.

### Typical concentrations in drinking-water

Homoanatoxin-a has been detected in New Zealand. No Ministry of Health surveillance programmes have investigated the concentration of homoanatoxin-a in drinking-water supplies.

### Removal methods

It should be noted that treatment of water containing cyanobacterial cells with oxidants such as chlorine or ozone, while killing cells, will result in the release of free toxin. Therefore, the practice of prechlorination or pre-ozonation is not recommended without a subsequent step to remove dissolved toxins.

Management of raw water abstraction is effective in reducing the amount of cyanobacteria in raw water supplied for treatment. Removal of cyanobacterial cells by processes including bank filtration, flocculation, coagulation, precipitation and dissolved air flotation prior to chemical treatment that may cause cell lysis during water treatment is an effective method to remove toxins. Details of suggested methods are included in Hrudey et al (1999).

Specific information on the removal of homoanatoxin-a is not available. It is not unreasonable to expect that it exhibits similar stability properties to anatoxin-a.

Use of a combination of treatments is considered to be the best management approach, and the complexity of management necessitates consultation with the relevant health authority. Removal of cyanobacterial blooms and their associated toxins is briefly discussed in the datasheet for cyanobacteria.

### Recommended analytical techniques

#### Referee method

LC-MS: Namikoshi et al 2003; Dell’Aversano et al 2004; Furey et al 2003; Rao and Powell 2003; Quilliam et al 2001.

#### Some alternative methods

HPLC-FLD (James et al 1998).

HPLC–UV (Wong and Hindin 1982).

### Health considerations

Homoanatoxin-a is a postsynaptic depolarising neuromuscular blocking agent that binds strongly to the nicotinic acetylcholine receptor. It is a potent neurotoxin that can cause rapid death in mammals by respiratory arrest in 2 to 12 minutes. Health considerations for homoanatoxin-a are similar to those of anatoxin-a.

While Fawell et al (1993) and AWWA (1995) have suggested that neurotoxins are not considered widespread in water supplies, without appearing to pose the same degree of risk from chronic exposure as microcystins (hepatotoxins), there is accumulating evidence that recreational waters in New Zealand are experiencing the presence of anatoxin-a and homoanatoxin-a (Wood et al 2007). These data suggest that other New Zealand freshwaters, which may also be drinking-water sources, may also experience cyanobacterial blooms with neurotoxins, especially those that are river-fed where the benthic cyanobacteria may dominate the cyanobacterial populations.

#### Acute effects

Acute toxic effects have been observed in mice. The mouse LD50 by I.P. injection is in the range 0.29 to 0.58 mg/kg (body weight) and by oral administration (by gavage) 2.9 to 5.8 mg/kg (body weight) (Lilleheil et al 1997).

#### Chronic effects

Unknown. There are no long-term oral toxicity studies for this toxin.

### Derivation of the Maximum Acceptable Value

The provisional MAV for homoanatoxin-a in drinking-water was derived as follows:

0.25 mg/kg per day x 70 kg x 0.8 = 0.0014 mg/L (rounded to 0.002 mg/L)

 2 L x 5000

where:

* NOAEL = 0.25 mg/kg (body weight) per day was adopted by the Ministry of Health based on the LD50 of the pure toxin by the I.P. route in mice (Chorus 2001 personal. communication to P Truman, ESR)
* average weight of an adult in New Zealand = 70 kg (WHO uses 60 kg)
* the adult *per capita* daily water intake in New Zealand = 2 L
* proportion of TDI allocated to drinking water = 0.8
* uncertainty factor = 5,000 (10 for intra-species variation; 10 for inter-species variation; 50 for uncertainties in the database).

### References

APHA. 2005. *Standard Methods for the Examination of Water and Wastewater* (21st edition). Washington: American Public Health Association, American Water Works Association, Water Environment Federation.

AWWA. 1995. *Cyanobacterial (Blue-Green Algal) Toxins: A resource guide*. Denver, CO: AWWA Research Foundation and American Water Works Association.

Dell’Aversano C, Eaglesham GK, Quilliam MA. 2004. Analysis of cyanobacterial toxins by hydrophilic interaction liquid chromatography-mass spectrometry. *Journal of Chromatography* A1028: 155–64.

Fawell JK, et al. 1993. Blue-green algae and their toxins – analysis, toxicity, treatment and environmental control. In: *Water Supply* 11: 109–21.

Furey A, Crowley J, Lehane M, et al. 2003. Liquid chromatography with electrospray ion-trap mass spectrometry for the determination of anatoxins in cyanobacteria and drinking water. *Rapid Communications in Mass Spectrometry* 17: 583–8.

Hrudey S, Burch M, Drikas M, et al. 1999. Remedial measures. In: Chorus I, Bartrum J (eds). *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management*. 416 pp. Published on behalf of the World Health Organization by E&FN Spon, London.

James KJ, Furey A, Sherlock IR, et al. 1998. Sensitive determination of anatoxin-a, homoanatoxin-a and their degradation products by liquid chromatography with fluorimetric detection. *Journal of Chromatography* A798: 147–57.

Lilleheil G, Andersen RA, Skulberg OM, Alexander L. 1997. Effects of a homoanatoxin-a-containing extract from *Oscillatoria formosa* (Cyanophyceae/cyanobacteria) on neuromuscular transmission. *Toxicon* 35: 1275–89.

Namikoshi M, Murakami T, Watanabe MF, et al. 2003. Simultaneous production of homoanatoxin-a, anatoxin-a, and a new non-toxic 4-hydroxyhomoanatoxin-a by the cyanobacterium *Raphidiopsis mediterranea* Skuja. *Toxicon* 42: 533–8.

Quilliam MA, Hess P, Dell’Aversano C. 2001. Recent developments in the analysis of phycotoxins by liquid chromatography-mass spectrometry. In: de Koe WJ, Samson RA, van Egmond HP, et al (eds). *Proceedings of the 10th International IUPAC Symposium on Mycotoxins and Phycotoxins* 21–25 May 2000, Brazil.

Rao R, Lu L, Powell MW. 2003. Determination of anatoxin-a in drinking water samples by LC/MS. Anonymous. *ThermoQuest LC/MS Application Report*.

Skulberg O, Carmichael W, Anderson R, et al. 1992. Investigations of a neurotoxic Oscillatorialean strain (cyanophyceae) and its toxin. Isolation and characterisation of homoanatoxin-a. *Env Toxicol Chem* 11: 321–9.

Wong SH, Hindin E. 1982. Detecting an algal toxin by high pressure liquid chromatography. *American Water Works Association Journal* 74: 528–9.

Wood SA, Rasmussen JP, Holland PT, et al. 2007. First report of the cyanotoxin anatoxin-A from *Aphanizomenon issatschenkoi* (cyanobacteria). *Journal of Phycology* 43: 356–65.

# Microcystins

IARC (2010) reports a CAS No. of 101043-37-2 for microcystin LR, and then lists 15 individual microcystins along with their CAS numbers, with synonyms.

### Maximum Acceptable Value (Provisional)

Based on health considerations, the concentration of microcystins (measured as MC-LR toxicity equivalents) in drinking-water should not exceed 0.001 mg/L.

WHO (2017) has a provisional guideline value of 0.001 mg/L for total microcystin-LR (free plus cell-bound). The provisional status is because it covers only microcystin-LR, the database is limited and new data for the toxicity of cyanobacterial toxins are being generated.

WHO (2017) notes that many practical considerations for the abatement of microcystins apply similarly to the other cyanotoxins (ie, cylindrospermopsins, saxitoxins, anatoxin-a and anatoxin-a(s)), with one key difference that is relevant to the efficacy of their removal in drinking-water treatment: microcystins are usually cell-bound, and substantial amounts are released to the surrounding water only in situations of cell rupture (ie, lysis), whereas the other cyanotoxins may occur to a larger extent dissolved in water.

The Australian Guidelines (NHMRC, NRMMC 2011) state: *Based on health considerations, the concentration of total microcystins in drinking water should not exceed 0.0013 mg/L expressed as microcystin-LR toxicity equivalents (TE).*

In 2002 Health Canada established a maximum acceptable concentration of 0.0015 mg/L for microcystin-LR. This guideline is believed to be protective of human health against exposure to other microcystins (total microcystins) that may also be present. Although the guideline is specific to microcystin-LR, it is important to measure total microcystins.

Drinking water advisory thresholds have been established for microcystin-LR in Quebec Province (1.5 µg/L), Ohio State (1 µg/L), Oregon State (1 µg/L) and Minnesota (0.04 µg/L for the protection of a short-term exposure for bottle-fed infants) – see <http://www.waterrf.org/PublicReportLibrary/4548a.pdf>.

### Sources to drinking-water

#### 1 To source waters

The microcystins are a group of structurally related liver toxins produced by at least seven different genera of cyanobacteria. They are monocyclic heptapeptides (containing seven amino acids) which comprise one unique phenyl deca-dienoic acid, four invariable D-amino acids and two variable L-amino acids. Their molecular weights range from 800–1,100. There are approximately 80 variants of microcystin; USEPA (2015) states that approximately 100 congeners exist. Microcystin-LR is the best characterised, and is one of the seven most toxic, with an LD50 of 50 µg/kg bw. The majority of human and animal microcystin-related intoxications are attributed to *Microcystis*, and in particular *M. aeruginosa*.

Microcystins are the most common cyanotoxin found worldwide. Microcystins are essentially water soluble intracellular compounds that are only released to the water when cells die (Orr and Jones 1998). Microcystins are produced when cells divide and are not produced in response to environmental stressors, so the toxicity of blooms only varies in response to changes in the biomass of toxic strains within a bloom. Toxin levels do not necessarily coincide with maximum algal biomass, however, abstraction of water from areas with lower cell concentrations results in lower abstraction of toxins from the source water.

Current knowledge of sources of microcystins worldwide and in New Zealand is summarised in Tables 9.1 and 9.2 of the Guidelines.

In a study of shallow ponds in Bangladesh, microcystins could be detected in 39 out of 79 pond samples, most of them with macroscopically visible blooms of cyanobacteria but also in some in which cyanobacteria were only sub-dominant. High concentrations of microcystins were linked to blooms of *Microcystis* in many ponds. From the 39 positive samples, 14 showed only trace concentrations, below 0.001 mg/L, ie, below the provisional MAV. In the remaining 25 pond samples concentrations between 0.001 and more than 1 mg/L could be detected. The most abundant structural variants in all samples were microcystins-RR, YR, and LR that are also the major ones in most samples from *Microcystis* dominated water bodies in temperate and subtropical latitudes. Taken from WHO (2004b), which concluded: depending on the water usage habits of the local population the uptake of microcystins occurring in concentrations of >0.01 mg/L for successive days is likely to lead to hazardous exposure.

In 2006, the USGS conducted a study of 23 lakes in the Midwestern US in which cyanobacterial blooms were sampled to determine the co-occurrence of toxins in cyanobacterial blooms. This study reported that microcystins were detected in 91 percent of the lakes sampled. Mixtures of all the microcystin congeners measured (LA, LF, LR, LW, LY, RR, and YR) were common and all the congeners were present in association with the blooms. Microcystin--LR and –RR were the dominant congeners detected with mean concentrations of 0.10 and 0.91 mg/L respectively. Taken from USEPA (2015).

General features associated with cyanobacteria are discussed in the datasheet for cyanobacteria. The microcystin toxins are discussed here.

The Alert Levels Framework for management of toxic cyanobacteria detailed in Chapter 9 should be followed if there is a possibility of cyanobacteria in source water.

### About microcystins

Microcystin was first identified from *Microcystis aeruginosa* from where it derives its name. There are currently more than 70–80 known variants based on a common structure that consists of a ring of seven amino acids. Five of those amino acids are common to all the microcystins but two are variable. Each variant is named according to the amino acid residues present in positions 2 and 4 of the amino acid ring (Carmichael 1997). Microcystin-LR (leucine (L), arginine(R)) was the first microcystin chemically identified and to-date, is still the most common and one of the most toxic of all the microcystins yet discovered. Microcystin-YA contains tyrosine (Y) and alanine (A). Different microcystins have different toxicities that may be conferred by minor conformational changes or differing fat solubilities that result from the variable amino acids. The toxicity of different microcystins varies by more than 1,000-fold (Bourne et al 1996).

Several microcystins can be produced concurrently by a single strain within a bloom and the mass ratio of the different microcystins within a strain is fixed. The microcystin content of a cell is no indication of toxicity so toxicity is normally expressed as the toxin mass equivalent of microcystin-LR – the mass of microcystin-LR that has equivalent toxicity. For example, microcystin-RR is between 6 and 12 times less toxic than microcystin-LR, so for toxicity, 1 µg microcystin-RR is equivalent to 0.17–0.08 µg microcystin-LR. Where the toxicity of a microcystin is unknown, it is assumed to be equal to microcystin-LR for the purpose of calculating toxicity.

### Health considerations

The major route of human exposure to microcystin is from the consumption of drinking-water. Minor exposure routes include recreational use of contaminated water bodies, consumption of blue-green algal dietary supplements containing small concentrations of cyanotoxins, and by exposure to aerosols containing cyanotoxins (eg, during water skiing or from hydro-electric power stations).

Microcystins are the best-studied of the cyanobacterial toxins. Their toxicology is well-understood and they exhibit both acute and chronic effects. Numerous exposure studies have been carried out and these have enabled the derivation of a recommended Tolerable Daily Intake (TDI) by the World Health Organization (Falconer et al 1999). To date, most of the work on microcystins has been conducted using the LR variant because of its presence in most countries reporting toxic episodes.

Blooms of microcystin-producing cyanobacteria in water bodies can contain more than one cyanobacterial strain or species, and multiple variants of toxins.

#### Acute effects

Microcystin-LR is an extremely potent acute toxin. Microcystin-LR is a potent inhibitor of eukaryotic protein serine/threonine phosphatases 1 and 2A. The primary target for microcystin toxicity is the liver, as microcystins cross cell membranes chiefly through the bile acid transporter.

The LD50 for microcystin-LR by I.P. injection is approximately 0.025–0.150 mg/kg (body weight) in mice. The oral (by gavage) LD50 is 5 mg/kg (body weight) in mice, and higher in rats (Fawell et al 1994). The LD50 by I.P. injection of several other commonly occurring microcystins (microcystin-LA, -YR, and -YM) are similar to microcystin-LR, but the I.P. LD50 for microcystin-RR is between 6 and 12 times higher (Pearson et al 1990). However, it cannot be presumed that the I.P. LD50 will predict toxicity after oral administration (Kuiper-Goodman et al. 1999). Therefore, uncertainty is associated with the acute toxicities of different microcystins.

Symptoms include headache, sore throat, vomiting and nausea, stomach pain, dry cough, diarrhoea, blistering around the mouth, and pneumonia. However, human data on the oral toxicity of microcystins are limited and can be confounded by potential co-exposure to other contaminants.

There is a significant body of reports describing animal poisonings from ingesting water containing *Microcystis*, with some examples confirming hepatotoxicity and the associated presence of microcystins (Ressom et al 1994). In a major incident in Brazil, in excess of 70 fatalities were ascribed to accidental intravenous exposure of kidney dialysis patients to microcystins in dialysis water (Jochimsen et al 1998). Significant human illness has been associated strongly with exposure of microcystins in recreational waters (Turner et al 1990).

In Salisbury, Rhodesia, seasonal acute childhood gastroenteritis during the years
1960–1965 was linked to annual blooms of *Microcystis* in the lake serving as the water supply. An adjacent water supply was not affected similarly and was not associated with this disease (Zilberg 1966).

Acute death results from dissolution of the liver structure and intrahepatic pooling of blood, which lead to overall haemorrhagic shock. Doses that are not immediately lethal can result in death from liver failure in animals and humans several months after the initial exposure to microcystins (IARC 2010).

Fish, shellfish and crustaceans accumulate microcystins and nodularins, which remain stable and unchanged during cooking; this leads to their ingestion by humans (IARC 2010).

The genotoxic properties of microcystin-LR have been studied extensively in a variety of test systems and the results have been contradictory (IARC 2010). In conclusion, no evidence was provided for the mutagenic or clastogenic properties of microcystins or nodularins in non-mammalian or mammalian test systems. However, an increased frequency of polyploid cells as well as centromere-positive micronuclei were observed, which possibly suggests that both microcystins and nodularin are aneugenic.

#### Chronic effects

Due to the seasonality of cyanobacterial blooms, exposures are not expected to be chronic. While acute toxicity is the most obvious problem in cyanobacterial poisoning, a long-term risk may also be present. Short-term exposures to toxins may result in long-term injury, and chronic low-level exposure may cause adverse health effects. The MAV is determined on the basis of protection from adverse health effects from chronic exposure.

An epidemiological survey in Haimen city (Jian-Su province) and Fusui county (Guangxi province) in China found a close relationship between the incidence of primary liver cancer and the use of drinking-water from ponds and ditches (Ueno et al 1996). In 1993 and 1994, microcystin concentrations ranged from 0.058 to 0.460 g/L; the highest concentrations occurred from June to September. A similar survey on 26 drinking-water samples in the Guangxi province showed a high frequency of microcystins in the water of ponds/ditches and rivers, but no microcystins were found in shallow and deep wells. According to Ueno et al (1996), the combined effect of microcystin toxin from the drinking-waters from ponds/ditches and rivers or both, and other carcinogens such as aflatoxin B1 found in food, may be the cause of the high incidence of primary liver cancer in Haimen city and other areas in China (WHO 1998).

Health Canada (2000, edited 2002) reports that a study by the WRc (UK) found a NOAEL of 40 μg/kg bw per day.

Cyanotoxins have only recently started to be reviewed by the International Agency for Research on Cancer (IARC) and use of their standard evaluation procedures on available information led to the conclusion that, at present, the human evidence for microcystin carcinogenicity is inadequate and the animal evidence is limited (Kuiper-Goodman et al 1999). IARC (2010) classified microcystin-LR (CAS No. 101043-37-2) as possibly carcinogenic to humans (Group 2B) based on human epidemiological studies in China showing elevated incidences of hepatocellular carcinoma and colorectal cancer associated with drinking surface water compared with drinking well water, though no clear correlation with exposure to microcystins could be ascertained. *Microcystis* extracts are not classifiable as to carcinogenicity to humans.

USEPA (2015a) estimated a reference dose (RfD) for microcystins of 0.05 μg/kg/day based on increased liver weight, slight to moderate liver lesions with necrosis with haemorrhages, and increased enzyme levels in rats from the study. This study identified a LOAEL of 50 μg/kg/day, based on these effects.

### Forms and fate in the environment

The cyclic structure of microcystins and the presence of unusual amino acids makes microcystins very stable compounds. Jones et al (1995) showed that dried *M. aeruginosa* crusts obtained from the shores of Lake Mokoan were still highly toxic after nine months of exposure. In the laboratory, Wannemacher (1989) demonstrated that microcystins are stable at 300 C. Bourneet al (1996) showed that microcystin-LR was degraded by bacteria following a lag of several days, but that subsequent addition of more microcystin-LR resulted in immediate degradation. Cousins et al (1996) measured a half-life of less than a week for microcystin-LR when added to water from a reservoir in the United Kingdom. Similarly, Codd and Bell (1996) found that microcystin was biodegraded readily in ambient waters, with a half-life of about one week. Microcystins are relatively persistent in the aquatic environment; studies in Australia have shown that microcystin-LR was present up to 21 days following treatment of a Microcystin aeruginosa bloom with an algicide. The half-life of microcystin-LR in natural water has been noted at about five days (reported in Health Canada 2000, edited 2002).

USEPA (2015) reports that microcystins typically have a half-life of four days to 14 days in surface waters, depending on the degree of sunlight, natural organic matter, and the presence of bacteria. They can precipitate out of the water column and reside in sediments for months. Groundwater is generally not expected to be at risk of cyanotoxin contamination, however, groundwater under the direct influence of surface water can be vulnerable; microcystin and cylindrospermopsin do not sorb in sandy aquifers and are transported along with groundwater.

### Typical concentrations in drinking-water

Open water cell *M. aeruginosa* concentrations for depth integrated samples in Australia can be up to 100,000 cells/mL. The highest intracellular toxin concentrations yet found in *M. aeruginosa* is around 200 fg/cell so the typical maximal microcystin concentration could be as high as 0.02 mg/L. Compliance with the WHO guideline level of 0.001 mg/L would nominally equate to 5,000 cells/mL, and for compliance with the current Australian Drinking Water Guidelines, 6,500 cells/mL (NHMRC and NRMMC 2004).

The American Water Works Association Research Foundation (AWWARF) conducted a study on the occurrence of cyanobacterial toxins in source and treated drinking waters from 24 public water systems in the United States and Canada in 1996–1998. Of 677 samples tested, microcystin was found in 80% (539) of the waters sampled, including source and treated waters. Only two samples of finished drinking water were above 1 μg/L. A later survey found a drinking water sample containing 0.0125 mg/L. Taken from USEPA (2015).

No Ministry of Health surveillance programmes have investigated the concentration of microcystins in drinking-water supplies; typical concentrations in New Zealand source waters are therefore unknown.

WHO (2017a) provides the following assessment:

**Box A4.2. Tier 0 exposure assessment**

Concentrations of microcystins in drinking-water can be predicted from those found in untreated sources of drinking-water by applying expected reductions achieved by treatment processes and manipulation of water intakes. In some circumstances the depths of intakes into treatment plants or distribution systems can be varied to reduce toxin concentrations. This can be effective for species such as *Microcystis* and *Dolichospermum* (*Anabaena*) which produce surface blooms but less so for *Planktothrix* which can be more evenly spread in the water. Coagulation and filtration are effective in removing cyanobacterial cells and intracellular toxins while chlorine and other oxidising disinfectants are effective in reducing concentrations of extracellular toxins.

The concentration in drinking-water PECdw can be calculated using the following formula:

PECdw = RW x (100−DR) x (100−TR1) x (100−TR2)

 100 x 100 x 100

where:

* PECdw is the predicted concentration in drinking-water (μg/L)
* RW is the concentration in untreated water
* DR is the reduction in concentration as a percentage by manipulating the intake depth
* TR1 is the reduction in concentration as a percentage by treatment process 1 (eg, filtration)
* TR2 is the reduction in concentration as a percentage by treatment process 2 (eg, chlorination).

To reflect a worst-case situation, conservative assumptions are made to determine maximum risk without variable depth intakes and inadequate or poorly managed treatment. In this example, a concentration of 55 μg/L of microcystin-LR has been used which is at the upper end of concentrations detected in Lake Taihu in China (which has been subject to severe cyanobacterial blooms – Jia et al 2003; Sakai et al 2013).

As there is a provisional WHO drinking water guideline value available for microcystin-LR, this can be utilised for risk characterisation. The estimated concentration of microcystin-LR assuming no removal by treatment processes, 55 μg/L, is clearly in excess of the provisional WHO guideline value for microcystin-LR of 1 μg/L. Therefore, further evaluation considering impacts of treatment on exposure assessment should be undertaken.

**Box A4.5. Refined exposure assessment (tier 1) exposure assessment**

The initial modelling assumptions outlined in Box A4.2 can be revised as follows where surface water is subject to coagulation, filtration and disinfection:

* the reduction achieved by manipulation of the intake depth is 90 percent
* the reduction achieved by coagulation and filtration is 99.5 percent of whole cells (Westrick et al 2010)
* in healthy blooms 90–95 percent of toxin is intracellular; based on this filtration can remove 90 percent of toxins
* in the latter stages of a bloom 50 percent of the toxin may be intracellular; based on this filtration can remove 50 percent of toxins
* provided a chlorine contact time of at least 30 mg.min/L is achieved, chlorination can remove at least 98% of toxins remaining after filtration (Ho et al 2006).

Using a deterministic approach and based on a conservative estimate of 50 percent of the toxin being intracellular the predicted concentration PECdw can be calculated as:

PECdw = 55 x (100−90) x (100−50) x (100−98) = 0.06 μg/L

 100 x 100 x 100

**Hazard assessment.** As for tier 0, the microcystins are assumed to have the same mode of action and the most toxic is microcystin-LR which has a provisional WHO guideline value of 1 μg/L.

**Risk characterisation.** The estimated drinking-water concentration of 0.06 μg/L is well below the provisional WHO guideline value. Even if intake depth data was not available, further assessment would not be necessary as the estimated concentration of microcystins-LR in that case would be 0.6 μg/L, which is still below the WHO provisional guideline value (1 μg/L).

### Removal methods

In the absence of cell damage, conventional treatment employing coagulation, flocculation, clarification (sedimentation or dissolved air flotation) and rapid granular filtration can be effective at removing intact cells and the majority of intracellular toxins (cell bound). However, effective sludge removal from sedimentation/clarification processes is important to minimise the release of intracellular and extracellular microcystins into the surrounding waters, as significant cell numbers can accumulate within the sludge, and cells contained within the sludge can lyse rapidly. Recycling washwater may need to be reconsidered (Newcombe 2015).

It should be noted that treatment of water containing cyanobacterial cells with oxidants such as chlorine or ozone (chlorine dioxide is not effective), while killing cells, will result in the release of free toxin. Therefore, the practice of prechlorination or pre-ozonation is not recommended without a subsequent step to remove dissolved toxins. Once the cells have been removed, oxidation may be effective. For example, microcystins are efficiently oxidised if pH is maintained below 8, the chlorine dose is greater than 3, and 0.5 to 1.5 of free chlorine residual is present after 30 minutes of contact time (Newcombe 2010). A 90 percent oxidation of microcystin-LR at a dose of 1.0 mg/L potassium permanganate with a contact time of 60 minutes, pH of 8, at 20°C is reported in USEPA (2015).

Management of raw water abstraction is effective in reducing the amount of cyanobacteria in raw water supplied for treatment. Removal of cyanobacterial cells by processes including bank filtration, flocculation, coagulation, precipitation and dissolved air floatation prior to chemical treatment that may cause cell lysis during water treatment is an effective method to remove toxins. Details of suggested methods are included in Hrudey et al (1999).

Microcystins are readily oxidised by ozone and chlorine. Nicholson et al (1994) showed that chlorine was effective provided a chlorine residual of 0.5 mg/L could be maintained for longer than 30 minutes and the pH was less than 8. Similarly, ozone is very effective (Rositano et al 1998) provided the dose is sufficiently high to oxidise other organic material. It must also be recognised that while oxidation techniques may destroy the parent toxin, they may also give rise to by-products that may be more or less toxic than the parent compound.

The microcystins are readily biodegradable and biological filtration has been shown to be extremely effective at the laboratory scale, but often after a lag phase during which there is no removal.

Microcystins are adsorbed readily from solution by both granular activated carbon and powdered activated carbon, but the efficacy of different carbons varies widely (Jones et al 1993). Calculated octanol-water partition coefficients (which measure solubility and interaction with water molecules) for the majority of microcystin variants suggest that their adsorption by activated carbon would be similar to, or greater than, that of microcystin-LR. According to Newcombe et al (2010), a PAC dose of 20 mg/L and a contact time of at least 45 minutes should be considered for removal of most extracellular microcystins (with the exception of microcystin-LA).

Health Canada (2000, edited 2002) reports:

Lambert et al examined the removal of microcystins from drinking water at two full-scale treatment plants in Alberta that employed coagulation-sedimentation, dual-media filtration and chlorination combined with either granular activated carbon (GAC) or powdered activated carbon (PAC) filtration. The two processes generally removed more than 80 percent of the microcystins from raw water, particularly when the raw water concentrations were high; however, a residual concentration of 0.05 to 2 μg microcystin-LR equivalents/L was observed at both treatment facilities. Because the toxins are non-volatile, neither aeration nor air stripping would be effective in removing the soluble toxins.

With regard to oxidation, the residual oxidant level is important. Below pH 8, aqueous chlorine (largely present in the form of hypochlorous acid) at a concentration of 15 mg/L will destroy microcystins; at neutral pH values, chlorination is effective provided a chlorine residual concentration of at least 0.5 mg/L is present after a 30-minute contact time. Destruction is significantly reduced above pH 8 due to the rapid decrease in the concentration of hypochlorous acid with increasing pH.

Ozone pretreatment at 1 mg/L can remove microcystins as long as a residual ozone level of 0.05 to 0.1 mg/L is maintained; the residual ozone level is significant because the effectiveness of ozone is affected by total organic carbon concentration.

With regard to other oxidation treatments, potassium permanganate at 1 to 2 mg/L was found to be effective, but further work is required; hydrogen peroxide, chloramine and chlorine dioxide were not effective; and UV radiation as a point-of-use treatment was not potent enough.

Microcystin-LR has a molecular weight of about 1000 daltons so its concentration should be reduced by nanofiltration and RO. Removal rates exceeding 80 percent have been reported.

Use of a combination of treatments is considered to be the best management approach, and the complexity of management necessitates consultation with the relevant health authority. Removal of cyanobacterial blooms and their associated toxins is discussed briefly in the datasheet for cyanobacteria.

The WHO Guidelines for Drinking-water Quality Working Group meeting (in Geneva 2005) reviewed and approved the text for the second addendum and agreed to include a treatment section in the second addendum, which has already been prepared. The draft document is now available for review. This summarises the efficacy of several treatment processes, and can be downloaded from <http://www.who.int/water_sanitation_health/dwq/chemicals/microcystin/en/index.html>. This draft document also discusses sampling and testing procedures.

### Recommended analytical techniques

#### Referee method

HPLC-UV/PDA: Lawton et al 1994; Meriluoto 1997.

#### Some alternative methods

LC-MS: Zweigenbaum et al 2000; Barco et al 2002; Spoof et al 2003.

ADDA-ELISA: Fisher et al 2001.

PP2A: An and Carmichael 1994; Meriluoto 1997; Ward et al 1997.

USEPA (2015) discusses a new liquid chromatography/tandem mass spectrometry (LC/MS/MS) method for microcystins and nodularin (combined intracellular and extracellular) in drinking water (EPA Method 544).

Purified standards are commercially available for microcystin-LR, -RR, -YR, -LW and -LF toxins. Results should state which microcystins are included in the test, and whether the result refers to free microcystin-LR, or free plus cell bound microcystin-LR. Also, see IARC 2010.

NHMRC, NRMMC (2011) states:

When quantitative standards are available, the most precise technique in this regard is liquid chromatography with confirmation by mass spectrometry
(LC–MS/MS); although this technique still involves estimation of the concentration and therefore toxicity of some microcystins in a sample against microcystin-LR as the analytical standard, which may result in a slight overestimate of total microcystins (as microcystin-LR, toxicity equivalents).

WHO (2017a) states:

There is no single technique that provides an accurate measure of the toxin concentration in microcystin-LR toxicity equivalents where complex mixtures of microcystins occur in a water sample. Assays that measure overall toxicity exist but the precision of these methods is variable. The most precise method currently in use is that of liquid chromatography (to separate mixtures of microcystins) combined with photodiode array detection (HPLC-PDA). Confirmation of variant identity is usually possible by subsequent mass spectrometry. The HPLC-PDA method can be used to calculate microcystin-LR equivalents from detected microcystin variants based on the characteristic UV absorbance peak at 238 nm (WHO 2003). This method will typically result in an overestimation of toxicity as most variants are less toxic than microcystin-LR.

### Recreational exposure

Exposure to cyanotoxins may be a health issue where sources of water are used for contact recreation. There have been repeated descriptions of adverse health consequences for swimmers exposed to cyanobacterial blooms. Even minor contact with cyanobacteria in bathing water can lead to skin irritation and increased likelihood of gastrointestinal symptoms (Pilotto et al 1997). There are three potential routes of exposure to cyanotoxins: direct contact of exposed parts of the body (including sensitive areas such as the ears, eyes, mouth and throat), accidental swallowing, and inhalation of water. Individual sensitivity to cyanobacteria in bathing waters varies greatly, because there can be both allergic reactions and direct responses to toxins.

Microcystins have been detected unambiguously in water samples collected from a recreational lake in the Wellington region. Calculation of the amount of microcystin-LR and RR in the lake water samples was carried out and gave concentrations of 8.1 μg/L and 5.6 μg/L for LR and RR respectively (Stirling and Quilliam 2001).

### Derivation of the Maximum Acceptable Value

A 13-week study in mice with microcystin-LR (Fawell et al 1994) is considered the most suitable for the derivation of a TDI. In this study, a NOAEL of 40 g/kg (body weight) per day was determined for liver pathology. The value is supported by a 44-day study in which pigs were exposed in their drinking-water to an extract from *M. aeruginosa* containing microcystin-LR (Falconer et al 1994).

The TDI applies to all microcystins, through conversion of concentrations to microcystin-LR toxicity equivalents is described above. The TDI has been used to derive the MAV for microcystins in drinking-water, as follows:

0.04 mg/kg per day x 70 kg x 0.8 = 0.0011 mg/L (rounded to 0.001 mg/L)

 2 L x 1000

where:

* NOAEL = 0.04 mg/kg (body weight) per day from a 13-week study in mice with microcystin-LR, which is considered the most suitable study for the derivation of a guideline value for microcystin. It is supported by a 44-day study in which pigs were exposed, in their drinking water, to an extract from *M. aeruginosa* containing microcystin-LR
* the MAV is for total microcystins (free plus cell-bound) measured as microcystin-LR toxic mass equivalents
* average weight of an adult in New Zealand = 70 kg (WHO uses 60 kg)
* the adult per capita daily water intake in New Zealand = 2 L
* proportion of TDI allocated to drinking water = 0.8
* uncertainty factor = 1,000 (10 for intra-species variation – between juveniles and adults and for within species genetic differences; 10 for inter-species variation – between mice, pigs, rats and humans;10 for uncertainties in the database, in particular lack of chronic toxicity data and carcinogenicity in humans).

This calculated PMAV of 0.0011 mg/L differs slightly from the value adopted by the *Australian Drinking Water Guidelines* of 0.0013 mg/L. The slight difference relates to use of a TDI allocation to drinking water of 0.9 in the calculation of the Australian value. Canada has adopted a value of 0.0015 mg/L.

Based on a LOAEL of 0.05 mg/kg/d, USEPA (2015) established 10-day Health Advisories for microcystins:

* for bottle-fed infants and young children of pre-school age: 0.3 μg/L (0.0003 mg/L)
* for school-age children through adults: 1.6 μg/L (0.0016 mg/L).

They added that populations such as pregnant women and nursing mothers, the elderly, immune-compromised individuals, or those receiving dialysis treatment, may be more susceptible than the general population to the health effects of microcystins. As a precautionary measure, individuals that fall into these susceptible groups may want to consider following the recommendations for children pre-school age and younger.

Applying the USEPA Guidelines for Carcinogen Risk Assessment, there is *inadequate information to assess carcinogenic potential* of microcystins.

### References

An J, Carmichael WW. 1994. Use of a colorimetric protein phosphatase inhibition assay and enzyme linked immunosorbent assay for the study of microcystins and nodularins. *Toxicon* 32: 1495–507.

APHA. 2005. *Standard Methods for the Examination of Water and Wastewater* (21st Edition). Washington: American Public Health Association, American Water Works Association, Water Environment Federation.

Barco M, Rivera J, Caixach J. 2002. Analysis of cyanobacterial hepatotoxins in water samples by microbore reversed-phase liquid chromatography-electrospray ionisation mass spectrometry. *Journal of Chromatography* A959: 103–11.

Bourne DG, Jones GJ, Blakeley RL, et al. 1996. Enzymatic pathways for the bacterial degradation of the cyanobacterial cyclic peptide toxin, microcystin-LR. *Appl Environ Microbiol* 62: 4086–94.

Carmichael WW. 1997. The cyanotoxins. *Advances in Botanical Research* 27: 211–56.

Codd GA, Bell SG. 1996. The occurrence and fate of blue-green algal toxins in freshwaters. *National Rivers Authority R&D Report* 29. London, Her Majesty’s Stationary Office.

Cousins IT, Bealing DJ, James HA, et al. 1996. Biodegradation of microcystin-LR by indigenous mixed bacterial populations. *Water Research* 30: 481–5.

Falconer IR, Burch MD, Steffensen DA, et al. 1994. Toxicity of the blue-green algae (cyanobacterium) *Microcystis aeruginosa* in drinking water to growing pigs, as an animal model for human injury and risk assessment. *J Environ Toxicol Water Qual* 9: 131–9.

Falconer I, Bartrum J, Chorus I, et al. 1999. Safe levels and safe practices. In: Chorus I, Bartrum J (eds). *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management*. 416 pp. Published on behalf of the World Health Organization by E&FN Spon, London.

Fawell JK, James CP, James HA. 1994. *Toxins from Blue-Green Algae: Toxicological assessment of microcystin-LR and a method for its determination in water*. Medmenham, Marlow, Bucks, Water Research Centre, pp 1–46.

Fischer WJ, Garthwaite I, Miles CO, et al. 2001. Congener-independent immunoassay for microcystins and nodularins. *Environmental Science & Technology* 35: 4849–56.

Health Canada (2000, edited 2002). Cyanobacterial toxins – microcystin-LR. *Guidelines for Canadian Drinking Water Quality: Supporting Documentation*. 22 pp. <http://hc-sc.gc.ca/ewh-semt/alt_formats/hecs-sesc/pdf/pubs/water-eau/cyanobacterial_toxins/cyanobacterial_toxins-eng.pdf>.

Hrudey S, Burch M, Drikas M, et al. 1999. Remedial measures. In: Chorus I, Bartrum J (eds). *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management*. 416 pp. Published on behalf of the World Health Organization by E&FN Spon, London.

IARC. 2010 – meeting was in 2006. Ingested nitrates and nitrites, and cyanobacterial peptide toxins. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* 94: 464 pp. See: http://monographs.iarc.fr/.

Jochimsen E, Carmichael W, An J, et al. 1998. Liver failure and death after exposure to microcystins at a hemodialysis centre in Brazil. *The New England Journal of Medicine* 338: 873–8.

Jones GJ, Falconer I, Wilkins RM. 1995. Persistence of cyclic peptide toxins in dried cyanobacterial crusts from Lake Mokoan, Australia. *Environmental Toxicology and Water Quality* 10: 19–24.

Jones GJ, Minato W, Craig K, et al. 1993. The removal of low level cyanobacterial peptide toxins from drinking water using powdered and granular activated carbon and chlorine – results of laboratory and pilot plant studies. In: *Proc. 15th AWWA Federal Convention*, Gold Coast, pp 339–46.

Kuiper-Goodman T, Falconer I, Fitzgerald J. 1999. Safe levels and safe practices. In: Chorus I, Bartrum J (eds). Toxic cyanobacteria in water. *A Guide to their Public Health Consequences, Monitoring and Management*. 416 pp. Published on behalf of the World Health Organization by E&FN Spon, London.

Lawton LA, Edwards C, Codd GA. 1994. Extraction and high-performance liquid chromatographic method for the determination of microcystins in raw and treated waters. *Analyst* 119: 1525–30.

Meriluoto J. 1997. Chromatography of microcystins. *Analytica Chimica Acta*352: 277–98.

Newcombe G, House J, Ho L, et al. 2010. Management strategies for cyanobacteria (blue-green algae): a guide for water utilities. *Water Quality Research Australia. Research Report* 74: 112 pp. Adelaide, Australia. [http://www.researchgate.net/profile/Lionel\_Ho/publication/242740698\_Management\_Strategies\_for\_Cyanobacteria\_(Blue-Green\_Algae)\_A\_Guide\_for\_Water\_Utilities/links/02e7e52d62273e8f70000000.pdf](http://www.researchgate.net/profile/Lionel_Ho/publication/242740698_Management_Strategies_for_Cyanobacteria_%28Blue-Green_Algae%29_A_Guide_for_Water_Utilities/links/02e7e52d62273e8f70000000.pdf).

Newcombe G, Dreyfus J, Monrolin Y, et al. 2015. *Optimizing Conventional Treatment for the Removal of Cyanobacteria and Toxins*. Denver, CO: Water Research Foundation.

NHMRC, NRMMC. 2011. *Australian Drinking Water Guidelines Paper 6 National Water Quality Management Strategy*. Canberra: National Health and Medical Research Council, National Resource Management Ministerial Council, Commonwealth of Australia. 1244 pp. <http://www.nhmrc.gov.au/guidelines/publications/eh52>.

Nicholson BC, Rositano J, Burch MD. 1994. Destruction of cyanobacterial peptide hepatotoxins by chlorine and chloramine. *Wat Res* 28: 1297–303.

Orr PT, Jones GJ. 1998. Relationship between microcystin production and cell division rates in nitrogen-limited *Microcystis aeruginosa* cultures. *Limnol Oceangr* 43: 1604–14.

Pearson MJ, Fergusson ADJ, Codd GA, et al. 1990. Toxic blue-green algae: the report to the National Rivers Authority. *Water Quality Series* 2. Petersborough, UK: NRA.

Pilotto L, Douglas R, Burch M, et al. 1997. Health effects of recreational exposure to cyanobacteria (blue-green algae) during recreational water-related activities. In: *Aust New Zealand J Public Health* 21: 562–6.

Ressom R, Soong F, Fitzgerald J, et al. 1994. *Health Effects of Toxic Cyanobacteria (Blue Green Algae)*. Canberra: National Health and Medical Research Council.

Rositano J, Nicholson B, Pieronne P. 1998. Destruction of cyanobacterial toxins by ozone. *Ozone: Science and Engineering* 20: 223–38.

Sivonen K, Jones G. 1999. Cyanobacterial toxins. In: Chorus I, Bartrum J (eds) *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management*. 416 pp. Published on behalf of the World Health Organization by E&FN Spon, London.

Spoof L, Vesterkvist P, Lindholm T, et al. 2003. Screening for cyanobacterial hepatotoxins, microcystins and nodularin in environmental water samples by reversed-phase liquid chromatography-electrospray ionisation mass spectrometry. *Journal of Chromatography* A1020: 101–15.

Stirling DJ, Quilliam MA. 2001. First report of the cyanobacterial toxin cylindrospermopsin in New Zealand. In: *Toxicon* 39: 1219–22.

Turner P, Gammie A, Hollinrake K, et al. 1990. Pneumonia associated with contact with cyanobacteria. *British Medical Journal* 300: 1440–1.

Ueno Y, Nagata S, Tsutsumi T, et al. 1996. Detection of microcystins, a blue-green algal hepatotoxin, in drinking-water sampled in Haimen and Fusui, endemic areas of primary liver cancer in China, by highly sensitive immunoassay. *Carcinogenesis* 17: 1317–21.

USEPA. 2015. *Drinking Water Health Advisory for the Cyanobacterial Microcystin Toxins*. EPA-820-R-15100. 75 pp. <http://www.epa.gov/sites/production/files/2015-06/documents/microcystins-report-2015.pdf>.

USEPA. 2015a. *Health Effects Support Document for the Cyanobacterial Toxin Microcystins*. EPA 820-R-15102. 138 pp. <http://www.epa.gov/sites/production/files/2015-06/documents/microcystins-support-report-2015.pdf>.

Wannemacher RW. 1989. *Chemical Stability and Laboratory Safety of Naturally Occurring Toxins*. Fort Detrick, Frederick, MD: US Army Medical Research, Institute of Infectious Disease.

Ward CJ, Beattie KA, Lee EYC, et al. 1997. Colorimetric protein phosphatase inhibition assay of laboratory strains and natural blooms of cyanobacteria: comparisons with high performance liquid chromatographic analysis for microcystins. FEMS *Microbiology Letters* 153: 465–73.

WHO. 1998. *Guidelines for Drinking-water Quality* (2nd edition). Addendum to Volume 2. Health Criteria and other Supporting Information. World Health Organization, Geneva, pp 95–110.

WHO. 2003. Cyanobacterial toxins: microcystin-LR in drinking-water. *Background Document for Development of WHO* *Guidelines for Drinking-water Quality*. Geneva: World Health Organization. WHO/SDE/WSH/03.04/57. <http://www.who.int/water_sanitation_health/dwq/chemicals/microcystin/en/index.html>.

WHO. 2004a. *Guidelines for Drinking-water Quality 2004* (3rd edition). Geneva: World Health Organization. Available at: [www.who.int/water\_sanitation\_health/dwq/gdwq3/en/print.html](http://www.who.int/water_sanitation_health/dwq/gdwq3/en/print.html) see also the addenda.

WHO. 2004b. *Occurrence of Cyanobacterial Toxins (Microcystins) in Surface Water of Rural Bangladesh: Pilot study*. WHO/SDE/WSH/04.06. By Welker M, Chorus I, Fastner J. Available at: <http://www.who.int/water_sanitation_health/emerging/wsh0406.pdf>.

WHO. 2017. *Guidelines for Drinking-water Quality: Fourth edition incorporating the first Addendum*. Geneva: World Health Organization. 631 pp. [http://www.who.int/water\_sanitation\_health/publications/drinking-water-quality-guidelines-4-including-1st-addendum/en/](file:///C%3A/Users/sgilbert/AppData/Local/Microsoft/Windows/INetCache/Content.Word/www.who.int/water_sanitation_health/publications/2011/dwq_guidelines/en/).

WHO. 2017a. *Chemical Mixtures in Source Water and Drinking-water*. Geneva: World Health Organization. Licence: CC BY-NC-SA 3.0 IGO. 104 pp. <http://www.who.int/water_sanitation_health/publications/chemical-mixtures-in-water/en/>.

Zilberg B. 1966. Gastroenteritis in Salisbury European children – a five-year study. *Central African Journal of Medicine* 12: 164–8.

Zweigenbaum JA, Henion JD, Beattie KA, et al. 2000. Direct analysis of microcystins by microbore liquid chromatography electrospray ionisation ion-trap tandem mass spectrometry. *J Pharm Biomed Anal* 23: 723–33.

# Nodularin

IARC (2010) reports a CAS no. of 118399–22–7 for nodularin, and gives its official chemical name, plus synonym. There are several variants.

### Maximum Acceptable Value (provisional)

Based on health considerations, the concentration of nodularin in drinking-water should not exceed 0.001 mg/L.

WHO (2004 and 2011) does not have a guideline value for nodularin.

### Sources to drinking-water

#### 1 To source waters

*Nodularia spumigena* is currently the only species known to produce nodularin. While *N. spumigena* is predominantly found in brackish and saline environments, adaptation to freshwater has been observed in Australia, where it has been found growing in Lake Alexandrina (South Australia). The contribution of nodularin to human drinking-water supplies is therefore less problematic than for toxins derived from obligate freshwater species. Currently, nodularin has been recorded in *N. spumigena* strains in Australia, the Baltic Sea and New Zealand (Wood et al 2006).

The Alert Levels Framework for management of toxic cyanobacteria detailed in Chapter 9 should be followed if there is a possibility of cyanobacteria in source water.

### About nodularin

Nodularins are cyclic peptide cyanotoxins, structurally similar to the microcystins, but with only five amino acids in the ring, comprising a unique phenyl deca-dienoic acid and four invariable D-amino acids. There are several variants of nodularin. It has a similar mode of action and similar toxicity to microcystin-LR. There have been no confirmed reported incidents of human intoxication from nodularin but there have been numerous reports of livestock deaths, including the first ever record in the scientific literature of a cyanobacterial poisoning (Francis 1878). A few structural variants of nodularin exist but little is know about them including their toxicity (Sivonen and Jones 1999).

### Forms and fate in the environment

Nodularin is water soluble. In aquatic environments, nodularin usually remains contained within the cyanobacterial cells and is only released in substantial amounts on cell lysis.

The most likely exposure route for human intoxication is through recreational and occupational use of water contaminated by nodularin. A minor route is the consumption of drinking-water because *N. spumigena* does not usually grow in freshwater.

In addition, nodularin has been shown to bioaccumulate in mussels in estuaries. The consumption of contaminated shellfish therefore represents a potential alternative route of human exposure (Falconer et al 1992).

### Typical concentrations in drinking-water

Nodularin is not normally found in human drinking-water but has been recorded in water supplies used for stock watering and human recreation. Nodularin has been isolated and purified from a water sample from Lake Ellesmere, a saline lagoon near Canterbury, New Zealand (Carmichael et al 1988). The concentration of nodularin was not determined.

No Ministry of Health surveillance programmes have investigated the concentration of nodularin in drinking-water supplies. Typical concentrations in New Zealand source waters are therefore unknown.

### Removal methods

It should be noted that treatment of water containing cyanobacterial cells with oxidants such as chlorine or ozone, while killing cells, will result in the release of free toxin. Therefore, the practice of prechlorination or pre-ozonation is not recommended without a subsequent step to remove dissolved toxins.

Management of raw water abstraction is effective in reducing the amount of cyanobacteria in raw water supplied for treatment. Removal of cyanobacterial cells by processes including bank filtration, flocculation, coagulation, precipitation and dissolved air floatation prior to chemical treatment that may cause cell lysis during water treatment is an effective method to remove toxins. Details of suggested methods are included in Hrudey et al (1999).

Water treatment techniques can be highly effective for removal of both cyanobacterial cells and nodularin with the combination of the appropriate technology. As with other cyanotoxins a high proportion of nodularin remains intracellular unless cells are lysed or damaged, and can therefore be removed by coagulation and filtration in a conventional treatment plant (Hrudey et al 1999). It should be noted that treatment of water containing cyanobacterial cells with oxidants such as chlorine or ozone, while killing cells, would result in the release of free toxin. Boiling is not effective for destruction of nodularin. Therefore the practice of pre-chlorination or pre-ozonation is not recommended without a subsequent step to remove dissolved toxins.

Nodularin is oxidised readily by chlorine but has not been evaluated with ozone. Adequate contact time and pH control needs to be achieved to ensure optimum removal of these compounds, and this will be more difficult to achieve in the presence of whole cells (Hrudey et al 1999). It must also be recognised that while oxidation techniques may destroy the parent toxin, they may also give rise to by-products that may be more or less toxic than the parent compound. The potential for by-products to present additional health hazards is largely unknown.

Nodularin is also adsorbed from solution by powdered activated carbon. Boiling is not effective for destruction of nodularin. Boiling of water containing whole cells would also lead to the release of free toxin. The effectiveness of the treatment process needs to be determined by monitoring toxin in the product water.

Use of a combination of treatments is considered to be the best management approach, and the complexity of management necessitates consultation with the relevant health authority. Removal of cyanobacterial blooms and their associated toxins is briefly discussed in the datasheet for cyanobacteria.

### Recommended analytical techniques

#### Referee method

HPLC-UV/PDA: Lawton et al 1994; Meriluoto 1997.

#### Some alternative methods

LC-MS: Zweigenbaum et al 2000; Barco et al 2002; Spoof et al 2003.

ADDA-ELISA: Fisher et al 2001.

PP2A: An and Carmichael 1994; Meriluoto 1997; Ward et al 1997.

Also, see IARC (2010).

### Health considerations

There are no reports of human health effects from consumption of water containing nodularin and/or *N. spumigena*. In addition there are no human or animal studies of toxicity by oral exposure to nodularin.

Fish, shellfish and crustaceans accumulate microcystins and nodularins, which remain stable and unchanged during cooking; this leads to their ingestion by humans (IARC 2010).

Stock losses have been associated with blooms of *Nodularia* sp (Francis 1878; Rinehart et al 1988). Nodularin is at least as hepatotoxic as microcystin for intraperitoneal exposure in experimental animals, and given its identical mode of action, can be regarded as presenting at least the same risk to human health as microcystin if ingested via drinking water. In addition, nodularin is known to accumulate in mussels in estuaries, and the consumption of contaminated shellfish therefore represents a potential alternative route of human exposure (Falconer et al 1992).

#### Acute effects

The toxicity and liver pathology induced by nodularin is similar to that caused by microcystins, with hepatocyte necrosis and haemorrhagic diathesis (Runnegar et al 1988). Nodularin inhibits protein phosphatases 1 and 2A with the same potency as microcystin-LR (Yoshizawa et al 1990). Acute death results from dissolution of the liver structure and intrahepatic pooling of blood, which lead to overall haemorrhagic shock.

#### Chronic effects

In view of nodularin’s similar toxicity to microcystin-LR in rodent intraperitoneal studies, it is reasonable to assume that nodularin represents at least the same human health risk as microcystin-LR.

No evidence was provided for the mutagenic or clastogenic properties of microcystins or nodularins in non-mammalian or mammalian test systems. However, an increased frequency of polyploid cells as well as centromere-positive micronuclei were observed, which possibly suggests that both microcystins and nodularin are aneugenic (IARC 2010).

IARC (2010) considers nodularins are not classifiable as to their carcinogenicity to humans (Group 3). In reaching the overall evaluation of nodularins, the IARC Working Group noted that although a tumour promoter mechanism was plausible, few studies had been conducted to demonstrate this.

### Derivation of the Maximum Acceptable Value

There is no toxicological database for chronic exposure to nodularin to enable derivation of a formal TDI.

As there are some similarities between the toxicity of nodularin and microcystins, the guideline for microcystins has been used to derive a provisional MAV for nodularin.

Latest advice offered to Australian water suppliers states that as there are some similarities between the toxicity of nodularin and microcystins, the guideline for microcystins could be used to derive a cell concentration for *N. spumigena* that represent a preliminary indication of a potential hazard. The only available monitoring data for nodularin in fresh water indicated that the upper range for cell concentration of *N. spumigena* was 50,000 to 80,000 cells/mL, and this correlated with nodularin concentrations of 1.0–1.7 µg/L (Heresztyn and Nicholson 1997). Based on this limited data, nodularin concentrations (intra plus extracellular) of around 1.3 µg/L (the Australian guideline value for microcystins) would be associated with cell concentrations of between 40,000 to 100,000 cells/mL.

### References

An J, Carmichael WW. 1994. Use of a colorimetric protein phosphatase inhibition assay and enzyme linked immunosorbent assay for the study of microcystins and nodularins. *Toxicon* 32: 1495–507.

APHA. 2005. *Standard Methods for the Examination of Water and Wastewater* (21st Edition). Washington: American Public Health Association, American Water Works Association, Water Environment Federation.

Barco M, Rivera J, Caixach J. 2002. Analysis of cyanobacterial hepatotoxins in water samples by microbore reversed-phase liquid chromatography-electrospray ionisation mass spectrometry. *Journal of Chromatography* A959: 103–11.

Carmichael WW, Eschedor JT, Patterson GML, et al. 1988. Toxicity and partial structure of a hepatptoxic peptide produced by the cyanobacterium *Nodularia spumigena* Mertens emend. L575 from New Zealand. *Applied and Environmental Microbiology* 54: 2257–63.

Falconer IR, Choice A, Hosja W. 1992. Toxicity of the edible mussel (*Mytilus edulis*) growing naturally in an estuary during a water-bloom of the blue-green alga *Nodularia spumigena*. *Environ Toxicol Water Qual* 7: 119–23.

Fischer WJ, Garthwaite I, Miles CO, et al. 2001. Congener-independent immunoassay for microcystins and nodularins. *Environmental Science & Technology* 35: 4849–56.

Francis G. 1878. Poisonous Australian lake. *Nature* 18: 11–12.

Heresztyn T, Nicholson BC. 1997. Nodularin concentrations in Lakes Alexandrina and Albert, South Australia, during a bloom of the cyanobacterium (blue-green alga) *Nodularia spumigena* and degradation of the toxin. *Environmental Toxicology and Water Quality* 12: 273–82.

Hrudey S, Burch M, Drikas M. et al. 1999. Remedial measures. In: Chorus I, Bartrum J (eds). *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management*. 416 pp. Published on behalf of the World Health Organization by E&FN Spon, London.

IARC. 2010 – meeting was in 2006. Ingested nitrates and nitrites, and cyanobacterial peptide toxins. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* 94: 464 pp. See: http://monographs.iarc.fr/.

Lawton LA, Edwards C, Codd GA. 1994. Extraction and high-performance liquid chromatographic method for the determination of microcystins in raw and treated waters. *Analyst* 119: 1525–30.

Meriluoto J. 1997. Chromatography of microcystins. *Analytica Chimica Acta*
352: 277–98.

NHMRC, NRMMC. 2011. *Australian Drinking Water Guidelines Paper 6 National Water Quality Management Strategy*. Canberra: National Health and Medical Research Council, National Resource Management Ministerial Council, Commonwealth of Australia. 1244 pp. <http://www.nhmrc.gov.au/guidelines/publications/eh52>.

Rinehart KL, Harada K, Namikoshi M, et al. 1988. Nodularin, microcystin and the configuration of adda. *J Am Chem Soc* 110: 8557–8.

Runnegar M, Jackson A, Falconer I. 1988. Toxicity of the cyanobacterium *Nodularia spumigena* mertens. *Toxicon* 26: 143–51.

Sivonen K, Jones G. 1999. Cyanobacterial toxins. In: Chorus I, Bartrum J (eds) *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management*. 416 pp. Published on behalf of the World Health Organization by E&FN Spon, London.

Spoof L, Vesterkvist P, Lindholm T, et al. 2003. Screening for cyanobacterial hepatotoxins, microcystins and nodularin in environmental water samples by reversed-phase liquid chromatography-electrospray ionisation mass spectrometry. *Journal of Chromatography* A1020: 101–15.

Ward CJ, Beattie KA, Lee EYC, et al. 1997. Colorimetric protein phosphatase inhibition assay of laboratory strains and natural blooms of cyanobacteria: comparisons with high performance liquid chromatographic analysis for microcystins. *FEMS Microbiology Letters* 153: 465–73.

Wood SA, Rasmussen JP, Holland PT, et al. 2007. First report of the cyanotoxin anatoxin-A from *Aphanizomenon issatschenkoi* (cyanobacteria). *Journal of Phycology* 43: 356–65.

Yoshizawa S, Matsushima R, Watanabe M, et al. 1990. Inhibition of protein phosphatases by microcystin and nodularin associated with hepatotoxicity. *J Cancer Res Clin Oncol* 116: 609–14.

Zweigenbaum JA, Henion JD, Beattie KA, et al. 2000. Direct analysis of microcystins by microbore liquid chromatography electrospray ionisation ion-trap tandem mass spectrometry. *J Pharm Biomed Anal* 23: 723–33.

# Saxitoxins

### Maximum Acceptable Value (Provisional)

Based on health considerations, the concentration of saxitoxins (expressed as STX equivalent) in drinking-water should not exceed 0.003 mg/L.

WHO (2004 and 2011) does not have a guideline value for saxitoxins.

The Australian Guidelines state: Due to the lack of adequate data, no guideline value is set for concentrations of saxitoxins. However given the known toxicity, the relevant health authority should be notified immediately if blooms of Anabaena circinalis (Dolichospermum circinalis) or other producers of saxitoxins are detected in sources of drinking water. Note: a change of nomenclature has been proposed for Anabaena to Dolichospermum. They have derived a health alert value of 3 μg STX-eq/L (0.003 mg/L) in drinking water.

Drinking water advisory thresholds have been established for saxitoxin in Ohio State (0.2 µg/L) and Oregon State (3 µg/L) – see <http://www.waterrf.org/PublicReportLibrary/4548a.pdf>.

### Sources to drinking-water

#### 1 To source waters

Saxitoxins are a group of neurotoxic alkaloids that are also known as paralytic shellfish poisons, or PSPs. They are produced by at least four species of freshwater cyanobacteria along with numerous species of marine dinoflagellates. The term saxitoxins is now used widely to refer to PSPs present in freshwater cyanobacteria.

Saxitoxins are primarily intracellular compounds that are released into the water following cell death (lysis). The occurrence of saxitoxins is summarised in Tables 9.1 and 9.2 of the Guidelines.

Features associated with cyanobacteria are discussed in the datasheet for cyanobacteria. The saxitoxins are discussed here.

The Alert Levels Framework for management of toxic cyanobacteria detailed in Chapter 9 should be followed if there is a possibility of cyanobacteria in a source water.

### About saxitoxins

There are three types of cyanobacterial neurotoxins, anatoxin-a, anatoxin-a(s) and the saxitoxins. The anatoxins seem unique to cyanobacteria, while saxitoxins are also produced by various dinoflagellates under the name of paralytic shellfish poisons.

The saxitoxins consist of a group of about 20 carbamoyl and decarbamoyl alkaloid neurotoxins that are divided into three groups based around a saxitoxin backbone. The non-sulfated toxins include saxitoxin (STX) and neo-saxitoxin (neo-STX), the singly-sulfated toxins are referred to as gonyautoxins (GTX) and the doubly-sulfated saxitoxins are referred to as C-toxins. There is a 160-fold variation in the molecular toxicity of the saxitoxins (Orr et al 2004) found in *A. circinalis* in Australia with C-toxins having the lowest toxicity and STX having the highest. STX is the most toxic non-protein substance yet discovered and on a per molecule basis, STX is 8,000 times more toxic than sodium cyanide by mouse I.P. injection (Pearson et al 1990).

### Forms and fate in the environment

Only the de-oxy saxitoxins (STX, C-1, C-2, GTX-2, GTX-3 and GTX-5) along with their equivalent decarbomyl derivatives are found in Australian blooms of *A. circinalis*. Of these, the C-toxinsare the least toxic but they can degrade spontaneously in the environment to the more toxic decarbomyl-GTX (Negri et al 1997; Ravn et al 1995). The half-lives for breakdown of saxitoxins found in Australian strains of *A. circinalis* varies from just a few minutes in sterile water at 90°C for C-toxins, to 69 days in sterile water at 25°C for gonyautoxins (Jones and Negri 1997).

The polar nature of saxitoxins allows them to dissolve readily in water when released from the cells. Generally they are stable in solution at neutral and acidic pHs even at high temperatures, but alkaline exposure oxidises and inactivates the toxin (ARNAT 2001).

The major routes of human exposure to saxitoxins is the consumption of drinking-water and the consumption of freshwater shellfish containing these toxins. Freshwater mussels can bioaccumulate saxitoxins to concentrations that exceed standards for PSP toxins in marine shellfish (Negri and Jones 1995). A minor exposure route is the recreational or occupational use of lakes and rivers or water derived from them.

### Typical concentrations in drinking-water

No Ministry of Health surveillance programmes have investigated the concentration of saxitoxins in drinking-water supplies. Typical concentrations in New Zealand source waters are therefore unknown.

### Removal methods

It should be noted that treatment of water containing cyanobacterial cells with oxidants such as chlorine or ozone, while killing cells, will result in the release of free toxin. Therefore, the practice of prechlorination or pre-ozonation is not recommended without a subsequent step to remove dissolved toxins.

Management of raw water abstraction is effective in reducing the amount of cyanobacteria in raw water supplied for treatment. Removal of cyanobacterial cells by processes including bank filtration, flocculation, coagulation, precipitation and dissolved air floatation prior to chemical treatment that may cause cell lysis during water treatment is an effective method to remove toxins. Details of suggested methods are included in Hrudey et al (1999).

Water treatment techniques can be highly effective for removal of both cyanobacterial cells and saxitoxins with the combination of the appropriate technology. As with other cyanotoxins, a high proportion of saxitoxins remain intracellular unless cells are lysed or damaged, and can therefore be removed by coagulation and filtration in a conventional treatment plant (Hrudey et al 1999). It should be noted that treatment of water containing cyanobacterial cells with oxidants such as chlorine or ozone, while killing cells, will result in the release of free toxin. Therefore the practice of pre-chlorination is not recommended without a subsequent step to remove dissolved toxins. Saxitoxins have been found to be resistant to oxidation by ozone alone or in combination with hydrogen peroxide (Orr et al 2004).

Both PAC and GAC can be effective for removal of saxitoxins, but is dependent upon the selection of the correct carbon type. Orr et al (2004) demonstrated complete removal of saxitoxin and gonyautoxins by biologically activated GAC (biological activated carbon) in experimental columns but found that removal of C-toxins was less effective.

The saxitoxins are not readily biodegradable and it is unlikely that biological filtration can be used for their effective removal.

Saxitoxins can degrade spontaneously in natural water at neutral pH. GTX degrade immediately to non-toxic breakdown products but C-toxins degrade to dc-GTXs that are between 6 and 100 times more toxic than the parent C-toxins (Jones and Negri 1997). Orr et al (2004) showed that water which complied initially with the suggested Australian saxitoxin guideline concentration immediately following treatment actually became more toxic for more than 50 days, and exceeded the suggested guideline concentration by up to three-fold during that period.

Boiling may be an effective treatment for destruction of saxitoxins in drinking water because of the enhanced rates of breakdown of parent and daughter compounds (see Table 1 in Jones and Negri 1997), but the effectiveness of the treatment process needs to be determined by monitoring saxitoxins in the product water.

### Recommended analytical techniques

#### Referee method

HPLC-FLD (Lawrence and Niedzwiadek 2001; Oshima et al 1989; Oshima 1995a; Oshima 1995b; Thomas et al 2004).

#### Some alternative methods

LC-MS: Quilliam et al 2001; Dell’Aversano et al 2004.

Mouse Bioassay: Falconer 1993.

Receptor Binding Assay: Powell and Doucette 1999; Doucette et al1997; Ruberu et al 2003.

The Australian Guidelines state: The established method for measuring toxicity due to the presence of saxitoxins/PSPs is the mouse bioassay (Hollingworth and Wekell 1990) which provides a result in terms of equivalence to μg saxitoxin activity (STX-eq). This is the standard method used in association with the shellfish industry and recognised by Foods Standards Australia and New Zealand. Where appropriate standards are available, the analytical technique of high performance liquid chromatography with post-column derivatisation can be used to quantify a range of saxitoxins in both water and cell material (Rositano et al 1998, Chorus and Bartram 1999 Chapter 13). This information can then be used to derive an estimate of total toxins in terms of saxitoxin equivalents (STX-eq) using a conversion based on specific mouse toxicities given by Oshima (1995) (see Rositano et al 1998).

### Health considerations

There is no record of human health effects caused directly by consumption of water contaminated with saxitoxins, cyanobacteria that produce saxitoxins, or dinoflagellates that produce PSPs. There are however, numerous reports of human toxicity associated with consumption of shellfish containing relatively high concentrations of PSPs (Kao 1993). Saxitoxin intoxication is an acute disorder that can lead to paraesthesia of the mouth and throat progressing to the neck and extremities, dizziness, weakness, ataxia and muscular paralysis with associated symptoms including nausea, vomiting, thirst and tachycardia. Symptoms can occur within five minutes and in fatal cases death occurs within 2–12 hours. In non-fatal cases, intoxication generally resolves within
1–6 days. Saxitoxins are cleared rapidly by urinary excretion.

#### Acute effects

Of the various saxitoxins, only STX has been studied in detail for pharmacological effects, partly because the other toxins are usually not available in sufficient quantities for such studies. Nearly all the systemic actions of STX can be explained by its pharmacological effect on nerve axon membranes (ie, it is a neurotoxin). This involves a widespread blockage of sodium ion channels of the excitable membranes of nerves, thereby affecting (partially or completely, depending on dose) impulse generation in peripheral nerves and skeletal muscles (Catterall 1980). This results in generalised nerve dysfunction as measured by electromyography (Easthaugh and Shepherd 1989). In mammals, these effects lead to paralysis, respiratory depression and respiratory failure. Direct cardiac effects are usually minimal.

In the early stages of a saxitoxin poisoning, victims experience tingling and numbness of the mouth, tongue, face and extremities. Nausea and vomiting may accompany the above symptoms. In severe cases, the patient will exhibit advanced neurological dysfunction such as ataxia, weakness, dizziness and a sense of dissociation followed by complete paralysis. The diaphragm may stop working and death can occur after cardio-respiratory failure.

No data are available on saxitoxin absorption, distribution, metabolism and excretion (Kuiper-Goodman et al 1999).

#### Chronic effects

There are no known chronic effects but long-term animal studies are lacking. Only acute effects have been shown in mammals and risk assessment is, therefore, limited to acute exposure at this stage.

### Derivation of the Maximum Acceptable Value

The provisional MAV for saxitoxins in drinking-water was derived as follows:

0.01 mg/kg per day x 70 kg x 0.8 = 0.000093 mg/L (rounded to 0.0001 mg/L or 0.1 μg/L)

 2 L x 3000

(The MoH has adopted the Australian and Brazilian values of 0.003 mg/L instead.)

where:

* NOAEL = 0.01 mg/kg (body weight) per day was adopted by the Ministry of Health based on the LD50 of the pure toxin by the I.P. route in mice (Chorus 2001 personal. communication to P Truman, ESR)
* average weight of an adult in New Zealand = 70 kg (WHO uses 60 kg)
* the adult *per capita* daily water intake in New Zealand = 2 L
* proportion of TDI allocated to drinking water = 0.8
* uncertainty factor = 3,000 (10 for intra-species variation; 10 for inter-species variation; 30 for uncertainties in the database.

The Australian guideline value was based on an analysis of data from reported events of paralytic shellfish poisonings that found that most cases were associated with consumption in excess of 200 μg (STX-eq.). A Health Alert Value of 3 μg/L (STX-eq) in drinking water was calculated for acute exposure associated with occurrence of intermittent blooms of *A. circinalis* in Australia (Fitzgerald et al 1999). Based on Australian monitoring data this would require *A. circinalis* cell concentrations exceeding 20,000 cells/mL (NHMRC and NRMMC 2004). Water associated with cell concentrations of this magnitude would normally be malodorous and unpalatable, with the threshold for off-tastes in water being 1,000 to 2,000 cells/mL.

In Australia it is recommended that notification and further assessment occur where *A. circinalis* cell concentration exceeds 20,000 cells/mL or when *A. flos-aquae* or *C. raciborskii* cells are detected in drinking-water supplies.

### References

AOAC. 1995. Paralytic shellfish poison: biological method. Sec 35.1.37, Method 959.08. In: Cunniff PA (ed) *Official Methods of Analysis of AOAC International* (16th edition), pp 22–23. Gaithersburg, MD: AOAC International.

APHA. 2005. *Standard Methods for the Examination of Water and Wastewater* (21st edition). Washington: American Public Health Association, American Water Works Association, Water Environment Federation.

ARNAT. 2001. Saxitoxins. *Australian Research Network for Algal Toxins*. [http://www.aims.gov.au/arnat/arnat-0008.htm](http://www.aims.gov.au/arnat/arnat-0002.htm).

Catterall W. 1980. Neurotoxins that act on voltage-sensitive sodium channels in excitable membranes. *Ann Rev Pharmacol Toxicol* 20: 15–43.

Dell’Aversano C, Eaglesham GK, Quilliam MA. 2004. Analysis of cyanobacterial toxins by hydrophilic interaction liquid chromatography-mass spectrometry. *Journal of Chromatography* A1028: 155–64.

Doucette GJ, Logan MM, Ramsdell JS, et al. 1997. Development and preliminary validation of a microtiter plate-based receptor binding assay for paralytic shellfish poisoning toxins. *Toxicon* 35: 625–36.

Easthaugh J, Shepherd S. 1989. Infectious and toxic syndromes from fish and shellfish consumption. *Arch Intern Med* 149: 1735–40.

Falconer IR. 1993. Measurement of toxins from blue-green algae in water and foodstuffs. In: Falconer IR (ed) *Algal Toxins in Seafood and Drinking Water*. London: Academic Press, pp 165–75.

Fitzgerald DJ, Cunliffe DA, Burch MD. 1999. Development of health alerts for cyanobacteria and related toxins in drinking water in South Australia. *Environmental Toxicology* 14: 203–7.

Hrudey S, Burch M, Drikas M, et al. 1999. Remedial measures. In: Chorus I, Bartrum J (eds). *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management*. 416 pp. Published on behalf of the World Health Organization by E&FN Spon, London.

Jones G, Negri A. 1997. Persistence and degradation of cyanobacterial paralytic shellfish poisons (PSPs) in freshwaters. *Water Research* 31: 525–33.

Kao CY. 1993. Paralytic shellfish poisoning. In Falconer I (ed) *Algal Toxins in Seafood and Drinking Water* pp 75–86. London: Academic Press.

Kuiper-Goodman T, Falconer I, Fitzgerald J. 1999. Safe levels and safe practices. In Chorus I, Bartrum J (eds) *Toxic Cyanobacteria in Water. A guide to their public health consequences, monitoring and management*. 416 pp. Published on behalf of the World Health Organization by E&FN Spon, London.

Lawrence JF, Niedzwiadek B. 2001. Quantitative determination of paralytic shellfish poisoning toxins in shellfish by using prechromatographic oxidation and liquid chromatography with fluorescence detection. *Journal of AOAC International* 84: 1099‑1108.

Negri AP, Jones GJ. 1995. Bioaccumulation of paralytic shellfish poisoning toxins from the cyanobacterium *Anabaena circinalis* by the freshwater mussel *Alathyria condola*. *Toxicon* 33: 667–78.

Negri A, Jones G, Blackburn S, et al. 1997. Effect of culture and bloom development and of sample storage on paralytic shellfish poisons in the cyanobacterium *Anabaena circinalis*. *Journal of Phycology* 33: 26–35.

NHMRC and NRMMC. 2004. *Australian Drinking Water Guidelines*. Canberra: National Health and Medical Research Council and the Natural Resource Management Ministerial Council.

NHMRC, NRMMC. 2011. *Australian Drinking Water Guidelines Paper 6 National Water Quality Management Strategy*. Canberra: National Health and Medical Research Council, National Resource Management Ministerial Council, Commonwealth of Australia. 1244 pp. <http://www.nhmrc.gov.au/guidelines/publications/eh52>.

Orr PT, Jones GJ, Hamilton GR. 2004. Removal of saxitoxins from drinking water by granular activated carbon, ozone and hydrogen peroxide – implications for compliance with the Australian drinking water guidelines. *Wat Res* 38: 4455–61.

Oshima Y, Sugino K, Yasumoto T. 1989. Latest advances in HPLC analysis of paralytic shellfish toxins. In Natori S, Hashimoto K, Ueno Y (eds) *Mycotoxins and Phycotoxins ‘88 Conference Proceedings* pp 319–42. Amsterdam: Elsevier.

Oshima Y. 1995a. Postcolumn derivatisation liquid chromatographic method for paralytic shellfish toxins. *Journal of AOAC International* 78(2): 528–32.

Oshima Y. 1995b. Post-column derivatisation HPLC methods for paralytic shellfish toxins. In Hallegraeff GM, Anderson DM, Cembella AD (eds) *Manual on Harmful Marine Microalgae* pp 81–94. Paris: Intergovernmental Oceanographic Commission of UNESCO.

Pearson MJ, Fergusson ADJ, Codd GA, et al. 1990. Toxic blue-green algae: the report to the National Rivers Authority. *Water Quality Series* 2. Petersborough, UK: NRA.

Powell CL, Doucette GJ. 1999. A receptor binding assay for paralytic shellfish poisoning toxins: recent advances and applications. *Natural Toxins* 7: 393–400.

Quilliam MA, Hess P, Dell’Aversano C. 2001. Recent developments in the analysis of phycotoxins by liquid chromatography-mass spectrometry. In de Koe WJ, Samson RA, van Egmond HP, et al (ed) *Proceedings of the 2001 Conference: Mycotoxins and phycotoxins in perspective at the turn of the millennium* pp 383–91. Wageningin: de Koe.

Ravn H, Anthoni U, Christophersen C, et al. 1995. Standardised extraction method for paralytic shellfish toxins in phytoplankton. *Journal of Applied Phycology* 7: 589–94.

Ruberu SR, Liu Y, Wong CT, et al. 2003. Receptor binding assay for paralytic shellfish poisoning toxins: optimisation and interlaboratory comparison. *Journal of AOAC International* 86: 737–45.

Sivonen K, Jones G. 1999. Cyanobacterial toxins. In: Chorus I, Bartrum J (eds) *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management*. 416 pp. Published on behalf of the World Health Organization by E&FN Spon, London.

Thomas K, Chung S, Ku J, et al. 2004. Analysis of PSP toxins by liquid chromatography with post column reaction and fluorescence detection. In: Holland P, Rhodes L, Brown L (eds) *HABTech 2003 Workshop Proceedings* pp 143–50. Cawthron Report No 906, Cawthron Institute.