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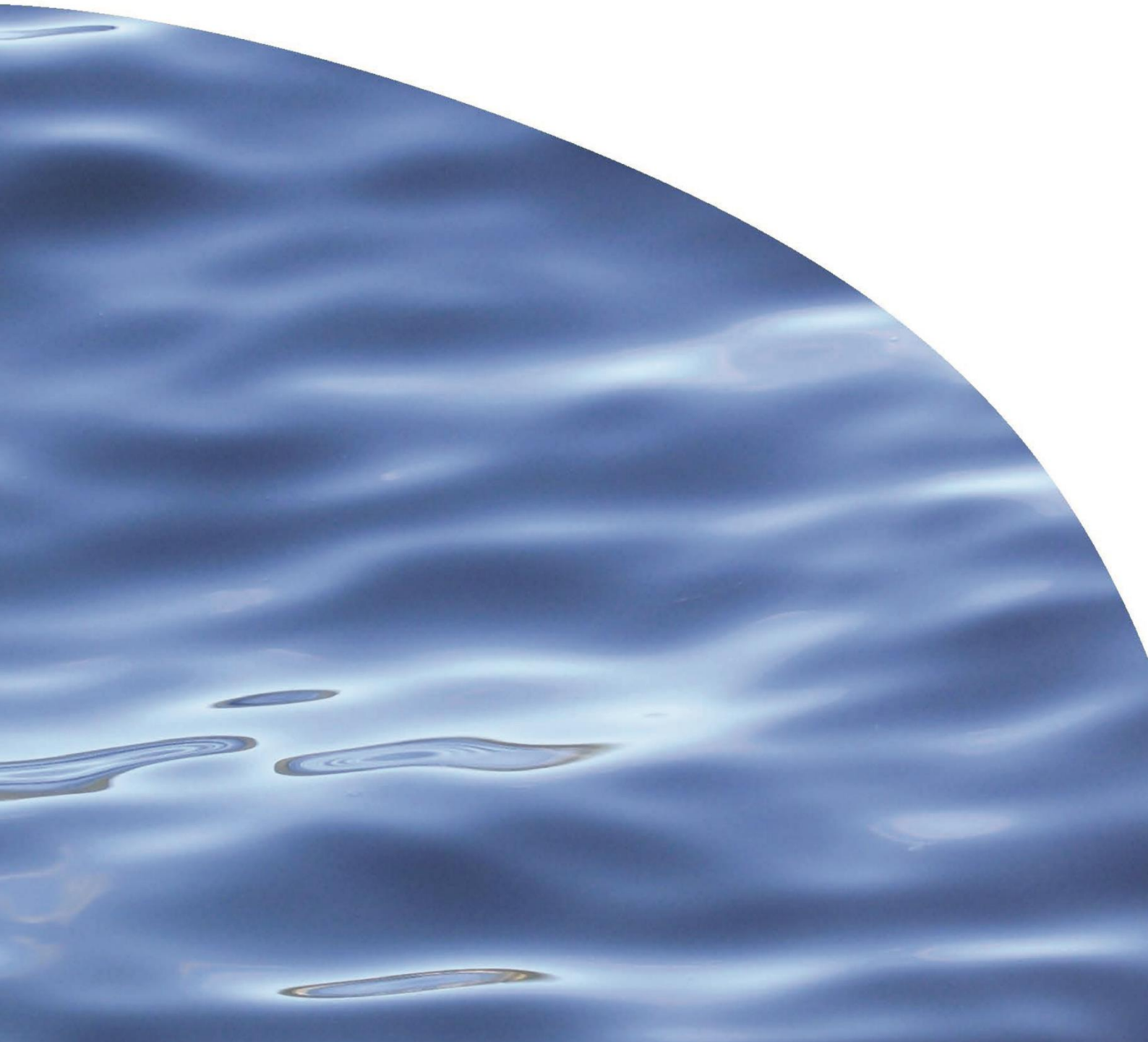
REVIEW OF THE 'NEW ZEALAND GUIDELINES FOR CYANOBACTERIA IN RECREATIONAL FRESH WATERS' - 2018



REVIEW OF THE 'NEW ZEALAND GUIDELINES FOR CYANOBACTERIA IN RECREATIONAL FRESH WATERS' - 2018

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EXECUTIVE SUMMARY

The 'Interim New Zealand Guidelines for Cyanobacteria in Recreational Fresh Waters' were released by the Ministry for the Environment (MfE) and the Ministry of Health (MoH) in 2009. The aim of the present project was to review the 2009 interim guidelines, update and finalise sections (where feasible), and to identify what further knowledge or work is required to finalise the guidelines. The project comprised five stages:

Stage 1 – An end-user workshop to gather feedback on the 2009 interim guidelines and identify knowledge gaps.
Stage 2 – A review of New Zealand cyanobacteria and cyanotoxin literature undertaken during 2009-2017.
Stage 3 – An update of microcystin quota values (amount of toxin produced per cell) based on new data.
Stage 4 – Identification of the next steps required to develop thresholds for benthic cyanobacteria in rivers.
Stage 5 – A final report (this document), which recommends updates for sections of the interim guidelines (where feasible) and identifies further work that is required to finalise updates.

Following completion of the above stages the following five recommendations were made:

1. Additional work is required to include recommendations into the guidelines that were identified during the end-user workshop but were outside the scope of this project. Key areas requiring further work include: updating the planktonic cyanobacterial biovolume table, reviewing picocyanobacteria identification and enumeration methods, developing new education and communication material, and including further information on emerging technologies and how these could be integrated into monitoring programmes.

2. The next version of the guidelines should be hosted by a web-based platform that allows updates to be made to individual sections and increases the functionality of the document including access to specific information.

3. The planktonic alert level framework should be revised to include cell concentration thresholds for known toxin producers and biovolumes for total cyanobacteria. The revision requires additional work to determine anatoxin-a thresholds for *Cuspidothrix issatschenkoi* and nodularin quotas for *Nodularia spumigena.* New *Microcystis* sp. cell concentrations thresholds were determined based on updated microcystin quota data and a revision of the toxicology calculations. This information needs to be incorporated in to the revised guidelines.

4. Further research should be conducted on benthic cyanobacteria in lakes to:
   - Identify toxin producers.
   - Determine whether benthic mats pose a health risk.
   - Develop methods for monitoring.
   - Consider whether thresholds can be developed to assist with risk management.

5. Further refinement of a Quantitative Microbial Risk Assessment using a second-order Monte Carlo approach to determine the risk posed by exposure to anatoxins in river water should be explored. The application of this (or other) risk-modelling approaches is limited by the following knowledge gaps:
   - A lack of toxicological data for the anatoxin congeners dihydrohomoanatoxin-a and homoanatoxin-a.
   - Only acute toxicological data is available for anatoxin-a and dihydroanatoxin-a.
   - The risk of *Microcoleus* mat consumption needs to be incorporated into risk models.
   - There is insufficient information on anatoxin concentrations in river water.
   - There is uncertainty as to how anatoxin thresholds (if they can be calculated) will be translated into metrics that can be measured by water managers (i.e., percent cover of *Microcoleus* mats).

   We recommend a workshop be convened to bring together experts in freshwater ecology, water quality, chemistry, risk modelling, toxicology and policy making to determine how to address these knowledge gaps.

This review has collated the latest information on toxic and bloom-forming cyanobacteria in New Zealand and gathered valuable input from stakeholders. Further updates and research are required to finalise the guidelines. There is an urgent need for these guidelines to be finalised, based on the strength of support for implementation at the end-user workshop.

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GLOSSARY

AF:	Allocation factor
ANOVA:	Analysis of variance
BMMA:	β-*N*-methylamino-L-alanine
DIN:	Dissolved inorganic nitrogen
DRP:	Dissolved reactive phosphorus
ELISA:	Enzyme-linked immunosorbent assay
LC-MS:	Liquid chromatography-mass spectrometry
LOAEL:	Lowest observed adverse effect level
MAV:	Maximum allowable value
MCs:	Microcystins
MfE:	Ministry for the Environment
MoH:	Ministry of Health
NIWA:	National Institute of Water and Atmospheric Research
NOAEL:	No observed adverse effect level
PCR:	Polymerase chain reaction
PP2A:	Protein phosphatase 2A
TAF:	Temporal allocation factor
TDI:	Tolerable daily intake

1. INTRODUCTION

Cyanobacteria are photosynthetic prokaryotic organisms that are an integral part of many terrestrial and aquatic ecosystems. Under favorable conditions, cyanobacteria cells can multiply and form blooms in lakes and rivers. An increasing number of cyanobacterial species are now known to have toxin-producing strains. These natural toxins, known as cyanotoxins, are a threat to humans and animals when consumed in drinking water or from contact during recreational activities.

The 'Interim New Zealand Guidelines for Cyanobacteria in Recreational Fresh Waters' were released by the Ministry for the Environment (MfE) and the Ministry of Health (MoH) in 2009 (Ministry for the Environment and Ministry of Health 2009). Knowledge on cyanotoxin-producing species, cyanotoxin production and toxicity, and methods for detection and monitoring have advanced markedly in the nine years since publication of the guidelines. New issues related to potentially toxic cyanobacteria have emerged that were not covered by the 2009 guidelines. Some parts of the 2009 guidelines have been adopted nationwide, and others need refining based on feedback from key stakeholders. The Cawthron Institute (Cawthron), the University of Waikato, the National Institute of Water and Atmospheric Research (NIWA) and Griffith University (Brisbane, Australia) were contracted by MfE to undertake a review of the 2009 interim guidelines, to update and finalise sections where feasible, and to identify what further knowledge is required and how it can be obtained.

The cyanobacteria guidelines review project involved five objectives addressed in different project stages:

- Stage 1 – Hold an end-user workshop to gather feedback on aspects of the guidelines that are and are not working, and to identify additional areas that need to be addressed.
- Stage 2 – Conduct a review of cyanobacteria and cyanotoxin literature and data from New Zealand undertaken during 2009-2017.
- Stage 3 – Update microcystin quota values based on new data, and if appropriate, use these to revise values in the planktonic thresholds.
- Stage 4 – Identify knowledge gaps and plan the next steps required to develop alert level thresholds for benthic cyanobacteria in rivers.
- Stage 5 – Produce a final report, providing recommendations for further work, and update sections of the interim guidelines (where feasible).

This report (Stage 5) summarises the work conducted during the cyanobacteria guidelines review project and provides recommendations on further work that is required to finalise the cyanobacteria guidelines and to develop alert level thresholds for benthic cyanobacteria.

2. SUMMARY OF END-USER WORKSHOP

Stage 1 of the cyanobacteria guidelines review project involved conducting an end-user workshop in order to gather feedback on how the guidelines are being practically implemented or used, and to identify additional areas that need to be addressed. A detailed summary of the workshop results are provided in Cawthron Report 3181 (Wood et al. 2018).

The workshop was held on Monday 7 May 2018 at Environment House in Wellington. The workshop was facilitated by the authors of this report and was attended by sixteen regional, district or city council scientists, eight District Health Board (DHB) staff and two Ministry for the Environment (MfE) staff. Prior to the workshop, an online survey was conducted to identify key areas of concern for discussion at the workshop. The survey was customised for participants from either council or DHB staff. Based on the survey results, the following six topics were prioritised for discussion at the workshop:

1. Planktonic cyanobacteria
2. Benthic cyanobacteria
3. Communicating risk
4. Emerging issues
5. New technologies
6. Appearance and functionality of guidelines.

The workshop identified gaps in the guidelines (such as benthic cyanobacteria in lakes) and areas that needed improvement (such as the functionality of the alert-level framework for planktonic cyanobacteria). Following on from the workshop, some of the recommendations were incorporated as a part of the guidelines review project (see the recommendations list below). However, many recommendations were outside of the scope of the review project and require further resourcing to implement. High-priority recommendations that were not part of the current project are in the second list below.

Workshop recommendations included as part of the guideline reviews project

- Restructuring the planktonic threshold table (Section 4.1)
- Review sections related to sampling planktonic cyanobacteria
- Identify the ‘next-steps’ for risk modelling related to benthic cyanobacteria in rivers (Section 5)
- Review the communication section of the guidelines
- Include a section on benthic cyanobacteria in lakes

- Include a section on the possibility of freshwater toxins affecting marine environments, strain variability and the possible impact of climate change on cyanobacterial blooms
- Incorporate toxin gene screening as an option to guide the selection of 'Situation' in the alert framework
- Provide a recommendation on the layout/appearance of the new guidelines in the final report.

Workshop recommendations not included as part of the guidelines review project

- Update biovolume table using available data and develop an 'online biovolume calculator'
- Review of methods for picocyanobacteria identification and enumeration
- Develop new education and communication material
- Include a section on emerging technologies and how these could be integrated into monitoring programmes
- Support further research related to benthic cyanobacteria in lakes.

3. LITERATURE REVIEW ON TOXIC CYANOBACTERIA IN NEW ZEALAND

Stage 2 of the guidelines review project involved a review of new literature on toxic cyanobacteria in New Zealand published during 2009-2017. New literature was acquired through the personal knowledge of the research team and by searching Google Scholar using the keywords 'New Zealand' AND 'cyanotoxins', 'toxic cyanobacteria', 'anatoxin', '*Phormidium*', 'cylindrospermopsin', 'nodularin', 'β-N-methylamino-l-alanine', 'BMMA', 'anatoxin-a(S)' or 'microcystin'. With saxitoxins, the keywords 'New Zealand' AND 'saxitoxin' AND 'cyanobacteria' were used for the search, due to the high number of publications for saxitoxins produced by marine phytoplankton and the contamination of shellfish. At least the first 100 results (10 web pages) were screened for relevant literature published in 2009 or later. When relevant results were still present after screening the first 100 results, searching continued until the search results were no longer relevant.

Only studies that related to aquatic freshwater cyanobacteria were retained, so several studies on marine and terrestrial cyanobacteria were not included. Studies that focussed on the ecology of cyanobacteria not known to produce toxins were not included.

A mixture of peer-reviewed research articles and review articles, technical reports, student theses and book chapters were identified during the search (Appendix 1). The 107 pieces of new literature were organised into categories based on toxin type or cyanobacterial genus, and a brief summary of the knowledge advances in each category is provided in Sections 3.1-3.10 and listed in Table 1. The knowledge advances identified through this process were incorporated into the relevant sections of the guidelines (see Section 6).

Table 1. Review of new literature on toxic cyanobacteria in New Zealand published during 2009-2017.

Category	Number of Literary References Identified	Principle Findings
Anatoxin-a(*S*)	0	No new work on anatoxin-a(S) in New Zealand was identified.
β-N-methylamino-L-alanine (BMAA)	0	No new work on BMAA in New Zealand was identified.
Cylindrospermopsin	2	During a 2012/13 survey of 143 New Zealand lakes, cylindrospermopsin was not detected.
Saxitoxins	6	Production of saxitoxin was identified in cyanobacteria from the Canterbury region; New toxicity information was determined.
Anatoxins and *Microcoleus autumnalis*	34	The taxonomy of *Phormidium autumnale* was revised to *Microcoleus autumnalis*; The dihydro-anatoxin congeners produced by *M. autumnalis* were shown to not be degradation products; Extracts of *M. autumnalis* were shown to negatively impact mayflies; The release of anatoxins from *M. autumnalis* mats was established; Anatoxin concentrations in *M. autumnalis* was shown to be highly variable; The environmental parameters leading to *M. autumnalis* proliferations were partially characterised.
Nodularins	5	Nodularin-R was confirmed in Wairewa / Lake Forsyth during a 2012/13 survey of 143 New Zealand lakes and in Lake Tikitapu in 2012; The aerosolisation of nodularin at a lake was demonstrated; Nodularin-R was shown to accumulate in shortfin eels.
Microcystins	25	A benthic *Planktothrix* sp. was shown to produce microcystins; During a 2012/13 survey of 143 New Zealand lakes, microcystin was detected in 21 samples and microcystin production genes were detected in 66 samples; New microcystin congeners were identified in New Zealand cyanobacteria; Studies to assess the triggers for microcystin production were conducted; Improvements in the analysis of microcystins were made by New Zealand researchers; The aerosolisation of microcystins was demonstrated; The accumulation of microcystins was shown in Pacific oysters, koura and kākahi.
New Technologies	10	Solid-phase adsorption toxin tracking sampling was used to assess anatoxins in river water; Polymerase chain reaction assays for toxin production genes were developed; The use of phycocyanin sensors for assessing cyanobacterial blooms was assessed.
Management of Freshwater Cyanobacteria	19	A range of lake management techniques have progressed and been tested in New Zealand lakes.

3.1. Anatoxin-a(*S*)

No new research on the anatoxin-a(*S*) was identified in New Zealand cyanobacteria.

3.2. β-*N*-methylamino-L-alanine (BMAA)

To our knowledge, no research on the presence of BMAA in New Zealand cyanobacteria has been conducted to date. Internationally, BMAA has been identified in a number of different countries since 2009; however, no assessment of the reliability of analytical methodologies was conducted during this literature review.

3.3. Cylindrospermopsin

No new literature on cylindrospermopsins in New Zealand was identified in the literature review. Wood et al. (2014) tested cultures of *Raphidiopsis raciborskii* (previously *Cylindrospermopsis raciborskii*) but no toxin genes were present. This study focused on identifying the origin of *Raphidiopsis raciborskii* in New Zealand (Wood et al. 2014). During a survey of cyanobacteria in 143 New Zealand lakes conducted in 2012/13, the cylindrospermopsin production gene (*cyrJ*) was not detected in any of the 189 samples assessed (Wood et al. 2017c).

3.4. Saxitoxins

Production of saxitoxin was identified in *Scytonema* cf. *crispum* from the Canterbury region (Smith et al. 2011; Smith 2012; Smith et al. 2012). During a survey of cyanobacteria conducted in 143 New Zealand lakes in 2012/13, a saxitoxin production gene (*SxtA*) was detected in one sample but was not confirmed using analytical methods (Wood et al. 2017c). New information was determined on toxicity of multiple saxitoxin congeners, including via oral administration (Munday et al. 2013; Selwood et al. 2017).

3.5. Anatoxins and *Microcoleus autumnalis* (previously *Phormidium autumnale*)

During a survey of planktonic cyanobacteria in New Zealand conducted in 2012/13, an anatoxin production gene (*AnaC*) was detected in three samples and was confirmed using analytical methods in one sample (Wood et al. 2017c). Gene sequencing indicated that the toxin-producing organisms were likely to be *Cuspidothrix issatschenkoi* (formerly *Aphanizomenon issatschenkoi*) and *Oscillatoria*, but the authors were unable to confirm the identification. The production of anatoxins by

benthic cyanobacteria (*Microcoleus autumnalis*, previously *Phormidium autumnale*) was reported in many regions of New Zealand including the Bay of Plenty (Wood et al. 2010a), Canterbury (Wood et al. 2012d; McAllister et al. 2018), Kaikoura (Wood et al. 2017a), Southland (Heath & Wood 2010) and Wellington (Wood et al. 2010a; Heath et al. 2011; Wood et al. 2012d). A review article in 2016 documents the New Zealand rivers where *Microcoleus* has been reported and provides a summary of the available anatoxin data (McAllister et al. 2016).

Over the past decade, it has been established that the dihydro-anatoxin congeners (present in high quantities in *Microcoleus*) are not breakdown products of anatoxin-a and homoanatoxin-a, but are produced by cyanobacteria (Heath et al. 2014; Méjean et al. 2014). Preliminary research, funded by MfE, suggests that the dihydro-anatoxin congeners may be more toxic than previously thought (Puddick et al. 2017a) and research in this area is continuing (Puddick et al. 2017b; Puddick et al. 2018). An assessment of the quantity of anatoxins released into river water from *Microcoleus* demonstrated that the level of anatoxin detected in the water was related to the severity of the bloom. *Microcoleus* coverage and anatoxin content at the immediate site were assessed, but the upstream *Microcoleus* load and variable dilution from water flow were also likely to affect the anatoxin levels (Wood & Puddick 2018). A study has also examined the impact of *Microcoleus* extracts on mayflies (*Deleatidium* sp.), to further understand the wider impacts on the aquatic ecosystem (Bridge 2014a).

Investigations into the environmental parameters that might influence anatoxin production have been conducted through culturing studies in the laboratory using *Cuspidothrix issatschenkoi* (Gagnon & Pick 2012) and *Microcoleus* (Smith 2010; Harland et al. 2013; Heath et al. 2014; Heath et al. 2016), and field studies focusing on *Microcoleus* in rivers (Brasell et al. 2015; Wood & Puddick 2017; McAllister et al. 2018). The work on anatoxin production in *Microcoleus* indicates that levels may be highest during the early stages of growth, which may be important for substrate colonisation. A field study using molecular measurements identified that toxin levels in *Microcoleus* blooms were correlated with the abundance of toxin-producing cells (Wood & Puddick 2017).

Numerous studies have investigated the potential drivers of *Microcoleus* blooms in New Zealand rivers (Hart et al. 2013; Bridge 2014b; Blaney 2015; Heath et al. 2015; Wood et al. 2015; Broghammer 2016; Heath et al. 2016; Kilner 2016a, 2016b; McAllister et al. 2016; Aristi et al. 2017; Meijer 2017; Wood et al. 2017b; McAllister 2018; McAllister et al. 2018). The main findings from these studies are that *Microcoleus* is more abundant in rivers with low DRP (dissolved reactive phosphorus; < 0.01 mg L^{-1}), slightly elevated DIN (dissolved inorganic nitrogen; > 0.1 mg L^{-1}) and higher loads of phosphorus-enriched fine sediment. However, there are a number of sites where these generalities do not apply. Research has shown that *Microcoleus* mats trap fine sediment and that they have the potential to liberate bound phosphorus

from the sediment within the micro-environment created by the mat structure. The most suitable river habitats for *Microcoleus* are shallow, low-gradient cobble-bed rivers with stable beds where flows result in moderate velocity and shear stress. The relative importance of these factors varies through the successional phases of mat growth and at different sites or rivers (McAllister et al. 2016; Wood et al. 2017b; McAllister et al. 2018).

Polyphasic studies have demonstrated that *Microcoleus* mats are generally comprised of a mixture of *Microcoleus* morphospecies and strains (Heath et al. 2010; Harland et al. 2014). These studies, along with one other study (Wood et al. 2012d), demonstrated that the different *Microcoleus* strains isolated from the same mat can have vastly different anatoxin production potential. Studies that assessed spatial variability of *Microcoleus* blooms identified that different anatoxin levels may be observed in mats sampled from different sites in the same river (Heath et al. 2011) and from mats within very close proximity (< 1 m) (Wood et al. 2010a; Wood & Puddick 2018). Overall, these findings demonstrate that, (1) without assessing for toxins or toxin-production genes, *Microcoleus* blooms should be assumed to be toxic, and (2) when sampling blooms for toxin analysis, a representative sample from multiple rocks should be collected and homogenised. Wood et al. (2010a) suggested collecting 10 samples from different rocks, which is consistent with the guidelines.

3.6. Nodularins

During a survey of cyanobacteria in New Zealand conducted in 2012/13, a microcystin/nodularin production gene (*McyE*) was detected in 66 samples and nodularin-R was confirmed in one sample using analytical methods (Lake Forsyth/Wairewa, Canterbury) (Wood et al. 2017c). Gene sequencing indicated that the toxin-producing organism was likely to be *Nodularia spumigena*, but identification was not confirmed through isolation and characterisation to strain level.

The potential for exposure to aerosolised nodularins was assessed at Lake Forsyth / Wairewa. Aerosolised toxins were detected but the levels were low and deemed not to pose a human health risk (Wood & Dietrich 2011). Nodularin was detected in cyanobacterial mats in Lake Tikitapu, Bay of Plenty, but the source could not be identified (Wood et al. 2012a). The accumulation of nodularin-R was documented in freshwater crayfish (koura; *Paranephrops planifrons*) grazing on benthic mats in Lake Tikitapu (Wood et al. 2012c). The accumulation of nodularin-R was also documented in New Zealand shortfin eels (*Anguilla australis*) from Lake Forsyth/Wairewa in the presence of *Nodularia spumigena* blooms (Dolamore et al. 2017).

3.7. Microcystins (MCs)

Multiple pieces of work have been conducted on microcystin-producing cyanobacteria in New Zealand since 2009. A large body of literature has also been published internationally on the accumulation, removal and analysis of microcystins in freshwater systems.

Microcystin production has been identified in a benthic *Planktothrix* species from the Waitaki River (South Island) (Wood et al. 2010b). During a 2007 survey of Lakes Rotorua, Rotoiti and Rotoehu, microcystin production genes specific to *Microcystis* and *Dolichospermum* (previously *Anabaena*) were detected, although culturing studies were not conducted to confirm the toxin-producing strains (Weller 2011). During a survey of cyanobacteria in 143 New Zealand lakes conducted in 2012/13, a microcystin/nodularin production gene (*McyE*) was detected in 66 samples and microcystins were confirmed in 21 samples using analytical methods (Wood et al. 2017c). Gene sequencing indicated that the toxin-producing organisms were likely to be *Dolichospermum lemmermannii* and *Microcystis*. Culturing of these species confirmed microcystin production by *Microcystis*, but whilst *Dolichospermum lemmermannii* contained one of the genes involved in microcystin production, toxin production was not detected.

The characterisation of ten new microcystin congeners from *Microcystis* CAWBG11 has been reported ([Asp^3] MC-RA, [Asp^3] MC-RAba, [Asp^3] MC-FA, MC-FA, [Asp^3] MC-WA, MC-WA, MC-FAba, MC-WAba, MC-FL and MC-WL) (Puddick 2013; Puddick et al. 2013b; 2013a; 2014). Oxidation artefacts of tryptophan containing microcystins were also documented, which affects the liquid chromatography-mass spectrometry (LC-MS) analysis of these congeners (Puddick 2013; Puddick et al. 2013b). Microcystin congeners which had not been previously documented in New Zealand ([Asp^3, Dha^7] MC-LR, [Asp^3] MC-LR and [Asp^3, ADMAdda^5] MC-LHar) were also reported (Wood et al. 2010b).

Over the past decade (and prior to 2009), a range of microcystin congeners have been detected in New Zealand freshwater environments (Table 2) and we now have better clarity on the microcystin congeners that are most frequently detected. The most frequently encountered microcystin congener profiles in New Zealand cyanobacteria are from a *Microcystis* sp. which produces predominantly [Dha^7] MC LR, with lower levels of [Asp^3,Dha^7] MC-LR and MC-LR, and a *Microcystis* sp. that produces a wide array of toxin congeners (> 27 congeners including MC-RR, MC-YR, MC-LR, MC-FR, MC-WR, MC-RA, MC-RAba, MC-LA, MC-FA, MC-WA, MC-LAba, MC-FAba, MC-WAba; (Puddick et al. 2014)). Those people testing for microcystins should be aware of the wide array of microcystin congeners observed in New Zealand freshwaters, especially when conducting analysis by LC-MS due to the high degree of specificity of this technique and the potential for specific congeners to be overlooked.

Table 2. Microcystin congeners reported in New Zealand studies on environmental samples and cyanobacteria strains.

Sample Type	Microcystin Congeners Detected	Refs.
Cyanobacterial strain isolated from Lake Hakanoa (Huntly; *Microcystis* CAWBG11)	[Asp^3]MC-RR, MC-RR, MC-YR, [Asp^3]MC-LR, MC-LR, [Asp^3]MC-FR, MC-FR, [Asp^3]MC-WR, MC-WR, [Asp^3]MC-RA, MC-RA, [Asp^3]MC-RAba, MC-RAba, MC-RL, MC-YA, [Asp^3]MC-LA, MC-LA, [Asp^3]MC-FA, MC-FA, [Asp^3]MC-WA, MC-WA, MC-LAba, MC-FAba, MC-WAba, MC-LL, MC-FL, MC-WL	(Puddick et al. 2013b; 2013a; 2014)
Cyanobacterial strain isolated from Lake Horowhenua (Levin; *Microcystis* CAWBG16, listed as CYN11 in the publication)	dmMC-LR	(Wood et al. 2008b)
Cyanobacterial strain isolated from Lake Rotorua (Kaikoura; *Microcystis* CAWBG624, listed as Rotorua A in the thesis)	[Dha^7]MC-LR, [Asp^3,Dha^7]MC-LR, [Dha^7]MC-HilR	(Rogers 2014)
Cyanobacterial strain isolated from Lake Rotorua (Kaikoura; *Microcystis* CAWBG617, listed as Rotorua D in the thesis)	[Dha^7]MC-LR, [Asp^3,Dha^7]MC-LR	(Rogers 2014)
Algal mats from the Mataura River	MC-YR	(Hamill 2001)
Environmental samples from lakes, ponds, dams and oxidation ponds	MC-RR, dmMC-RR, MC-YR, MC-LR, dmMC-LR, MC-FR, MC-WR, MC-RA (recorded as MC-AR in the publication), MC-LA, MC-LY	(Wood et al. 2006a)
Bloom material from Lake Hakanoa (Huntly)	MC-RR, MC-LR, MC-FR, MC-WR, dmMC-RR, MC-LY, dmMC-LR, MC-RA (recorded as MC-AR in the publication), MC-LA, MC-YR	(Crush et al. 2008)
Bloom material from Lake Horowhenua	[Dha^7]MC-LR, MC-LR	(Somdee et al. 2013)
Bloom material from Lake Wiritoa (Manawatu)	MC-RR, MC-LR, dmMC-FR, MC-FR, dmMC-WR, MC-WR, MC-RA (recorded as MC-AR in the publication), MC-YR, MC-(H4)YR	(Puddick & Prinsep 2008; Puddick 2013)
Environmental samples from lakes	MC-RR, dmMC-RR, didmMC-RR, MC-YR, MC-LR, dmMC-LR, didmMC-LR, MC-FR, MC-WR, MC-LA, MC-LY, MC-LF	(Wood et al. 2017c)
Environmental samples from Lake Rotorua (Kaikoura)	MC-RR, dmMC-LR, didmMC-LR, MC-FR, MC-WR, MC-LA	(Wood et al. 2017d)
Environmental samples from Lake Waitawa (Wellington region)	dmMC-RR, MC-RR, MC-YR, dmMC-LR, MC-LR, MC-FR, MC-WR, MC-RA, MC-RAba, MC-LA, MC-FA, MC-WA, MC-LAba, MC-FAba, MC-Waba	(Steiner et al. 2017)
Environmental samples from Lake Horowhenua (Levin)	MC-RR, dmMC-RR, MC-YR, MC-LR, dmMC-LR, didmMC-LR, MC-FR, MC-WR, MC-RA, MC-LA, MC-FA, MC-WA, MC-LAba, MC-FAba, MC-WAba	(Puddick et al. 2016b)
Environmental samples from Lake Rotoehu (Rotorua)	MC-LR	(Weller 2011)
Environmental samples from Lakes Rotoehu and Rotoiti (Rotorua region)	MC-RR, MC-LR, MC-FR, MC-WR, MC-RA (recorded as MC-AR in the publication), MC-YR, MC-LA	(Wood et al. 2006b)

Three studies have investigated how microcystin production is triggered in *Microcystis* when dense blooms form on the lake surface (Wood et al. 2011b, 2012b; Puddick et al. 2016b). A seasonal study conducted at Lake Rotorua (Kaikoura) assessed the phytoplankton composition and microcystin levels during 2013-2015 (Borges 2016; Wood et al. 2017d). A study conducted at Lake Waitawa (Wellington) assessed cyanobacterial scums (comprised of *Microcystis* and *Dolichospermum*) as they decomposed but did not find strong links between microcystin production and the other parameters that were assessed (Steiner et al. 2017). A culturing experiment using *Microcystis* CAWBG11 assessed how dissolved nitrogen concentrations affected the microcystin congener composition (Puddick 2013; Puddick et al. 2016c). All of these studies reported microcystin toxin quotas from *Microcystis* which have been used to support Stage 3 of the guidelines review project (see Section 4.2).

Several updates to methodologies for analysing microcystins were made by New Zealand researchers, including: better understanding on how sample handling procedures affect the final result (Rogers et al. 2015); what laboratory surfaces microcystins bind to and how to alleviate these effects (Altaner et al. 2017); and developments in the analysis of microcystins by matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (Puddick et al. 2012, 2016a; Puddick 2013;).

A study to determine if microcystins were aerosolised was conducted at Lake Rotorua (Kaikoura). Aerosolised microcystins were detected although levels were below those deemed to be dangerous to humans (Wood & Dietrich 2011). The presence of microcystins was detected in Pacific oysters (*Magallana gigas*) from Hokianga (Northland) using Adda enzyme-linked immunosorbent assays (ELISA), although when a controlled feeding study was conducted, microcystin accumulation was not detected by LC-MS (Wall 2012; Wall et al. 2014). The accumulation and effects of microcystin exposure on freshwater crayfish (koura; *Paranephrops planifrons*) (Clearwater et al. 2014) and freshwater mussel (kākahi; *Echyridella menziesii*) showed microcystins accumulated in these species, and at high concentrations they affect the organisms' behaviour (Clearwater et al. 2014; Collier et al. 2018).

A microcystin-degrading bacteria was isolated from Lake Rotoiti (Bay of Plenty) and its biodegradation efficiency was tested using [Dha^{7}] MC-LR and MC-LR purified from Lake Horowhenua bloom material (Somdee 2010; Somdee et al. 2013).

3.8. New technologies for assessing cyanobacteria and cyanotoxins

Since 2009, there have been advances in methodologies to assess cyanotoxins and cyanobacteria, and these have been applied in New Zealand. One such advance is a molecular method of quantifying the expression of microcystin production genes (Rueckert & Cary 2009). A review on the use of molecular tools for environmental

monitoring has also been published (Wood et al. 2013). The development of a solid-phase adsorption toxin tracking (SPATT) system for monitoring anatoxins in river water has been described, where sachets of anatoxin-binding material are deployed into river water, from which toxins are re-extracted for analysis in the lab (Wood et al. 2011a). A review article on use of the SPATT sampling system has been published (MacKenzie 2010). The feasibility of using flow cytometry to assess water samples for freshwater algae and cyanobacteria was explored using lake samples from the Rotorua region (Dennis et al. 2011). Validation work was conducted on the use of fluorometric probes that measure phycocyanin, a photosynthetic pigment present in cyanobacteria, for determining the abundance of cyanobacteria (and other phytoplankton) in benthic (Echenique-Subiabre et al. 2016) and planktonic systems (Hodges 2016; Cotterill 2017; Hodges et al. 2018). The studies on planktonic systems have demonstrated that: regular calibration of probes is required using cyanobacteria biovolumes; sensors vary in their response according to the manufacturer, the sensitivity and gain settings of the probe; and, dense colonies or filaments may decouple linear relationships between phycocyanin and cyanobacteria biomass (reviewed in (Bertone et al. 2018)). The potential of phycocyanin probes has been demonstrated in Lake Rotoehu (Bay of Plenty) by providing continuous *in situ* data showing high variability of natural cyanobacteria populations (Hamilton et al. 2015).

3.9. Management of freshwater cyanobacteria

Multiple pieces of work investigating how the management of New Zealand water bodies influences the occurrence of cyanobacteria blooms have been published. Some topics include managing nutrient loads in inflows and outflows (Hickey & Gibbs 2009; Paul et al. 2012; Abell et al. 2015; Hamilton et al. 2016b), the effect aquatic animals (e.g., fish and freshwater mussels) have on phytoplankton communities (Burns et al. 2014; Duggan et al. 2015), lake restoration practices (Sukias et al. 2010; McBride et al. 2015; Douglas et al. 2016; Hamilton & Dada 2016; Hamilton et al. 2016a, 2018; Eager 2017; Lehmann et al. 2017; Zhang et al. 2018) and the effect of river flows on periphyton populations (Bergey et al. 2010; Lessard et al. 2013). Because international and New Zealand knowledge on the management and restoration of lakes has reached a point where it can be adequately summarised, an appendix on this topic was produced for the guidelines.

3.10. Other areas

Ten pieces of new literature (listed in Appendix 1) did not fall into the categories above. Whilst they relate to work on toxic cyanobacteria from New Zealand, the information they provide is either outside of the scope of information required for the review of the Guidelines, providing information already captured above, or summarising information already present in the 2009 Interim Guidelines.

4. UPDATED MICROCYSTIN QUOTA VALUES AND A REVIEW OF TOXICOLOGY CALCULATIONS

Stage 3 of the guidelines review project comprised three components:

1. **Revision of the planktonic cyanobacteria alert-level framework:** Based on discussions at the end-user workshop held as part of Stage 1 of the guidelines review project (Wood et al. 2018), we developed a new planktonic cyanobacteria alert-level framework based on cell concentrations for known toxic species.
2. **Revision of microcystin quota values:** The current microcystin quotas, which form the basis of the threshold calculations for the planktonic alert-level framework, were determined in 2009 from five strains of *Microcystis* isolated from one lake. We have collated more extensive datasets to provide a microcystin quota value for use in the toxicology calculation review stage.
3. **Review of the microcystin toxicity calculations:** The microcystin toxicity calculations, which form the basis of the threshold values, were reviewed by Prof. Daniel Dietrich (an international expert in microcystin/cyanotoxin toxicology). With his guidance, we updated the toxicity calculations and suggest a new cell concentration threshold for *Microcystis* sp.

4.1. Suggested revision of planktonic alert-level framework

In view of new knowledge gained since the interim guidelines were issued in 2009, and based on discussions at the end-user workshop held as part of Stage 1 of the guidelines review project (Wood et al. 2018), we identified how the planktonic alert-level framework could be restructured. We suggest that species-specific cell concentration thresholds replace the current potentially toxic species biovolumes.

Research undertaken in New Zealand over the past 10 years has established that at least three planktonic cyanobacteria taxa are toxin producers; *Microcystis* sp., *Nodularia spumigena* and *Cuspidothrix issatschenkoi*. Cell count thresholds could be developed for each of these taxa based on measured cell toxin quotas (i.e., the amount of toxin produced per cell), although further work is required to implement this strategy for *Nodularia spumigena* and *Cuspidothrix issatschenkoi*. A mock alert-level framework for planktonic cyanobacteria is provided below (Table 3). There is a need to stress that it is highly likely that there are other planktonic toxin-producing species that have not yet been identified or tested. A strong recommendation in the new guidelines is to continue toxin gene screening, or toxin testing, in parallel with cell counts, until more comprehensive knowledge of the toxin-producing capabilities of taxa within a specific lake system has been obtained.

The microcystin threshold (12 µg L^{-1}) in the Action mode has been removed. To our knowledge this has never been used, and end user advice was that it was impractical

to implement due to costs associated with testing. If this value is retained, it will need to be revised based on the new microcystin toxicity calculations (Section 4.2).

Table 3. Mock-up of possible modifications to the alert-level framework for planktonic cyanobacteria.[a].

Alert level
Surveillance (green mode) ***Situation 1:*** The cell concentration of total cyanobacteria **< 500 *cells mL*$^{-1}$** ***Situation 2:*** The biovolume equivalent for the combined total of all cyanobacteria **< 0.5 mm^3 L^{-1}**
Alert (amber mode) ***Situation 1:*** *Microcystis* spp. **500 *cells mL*$^{-1}$ to < *12,100 cells mL*$^{-1}$*** *Nodularia spumigena* **< *XX,XXX cells mL*$^{-1}$*** *Cuspidothrix issatschenkoi* **< *XX,XXX cells mL*$^{-1}$*** ***Situation 2:*** **0.5 to < 10 mm^3 L^{-1}** total biovolume of all cyanobacteria.
Action (red mode) ***Situation 1:*** *Microcystis* spp. **≥ *12,100 cells mL*$^{-1}$** *Nodularia spumigena* **≥ *XX,XXX cells mL*$^{-1}$** *Cuspidothrix issatschenkoi* **≥ *XX,XXX cells mL*$^{-1}$** ***Situation 2:*** **≥ 10 mm^3 L^{-1}** total biovolume of all cyanobacteria; **or** ***Situation 3***: Cyanobacterial scums consistently present.
Note: These calculations are based on one month of recreational activity in an affected lake per year. For a small portion of the population, e.g., those involved in sport training (rowers, sailors, etc.) or lakeside resident who may swim daily, usage may be much greater and these values are not applicable. * Further assessment is required. Consideration also needs to be given to a scenario where these taxa co-occur. [a] The rationale for biovolumes of non-toxic cyanobacteria is described in the 2009 interim cyanobacteria guidelines. The use of < 500 cell mL^{-1} in the surveillance mode is to prevent biovolumes needing to be calculated (which can be time-consuming and expensive) when cell concentrations are very low.

In Sections 4.2–4.3 we review the data available for microcystins and propose that the Action Level Threshold—Situation 1 for *Microcystis* be set at 12,100 cells mL^{-1} (the previous Action Level Threshold was based on a cell concentration of 19,000 cells mL^{-1}; Appendix 2 of the 2009 interim cyanobacteria guidelines (Ministry for the Environment and Ministry of Health 2009)).

There are currently no data on nodularin quotas from *Nodularia spumigena* in New Zealand. There are five cultures of this species in the Cawthron Institute Culture Collection of Microalgae, and blooms occur annually in several Canterbury and Hawke's Bay lakes. The current cultures could be used to determine nodularin quota values, new cultures could be established during summer, and quotas could also be determined from environmental samples.

Anatoxin quotas are available for *Cuspidothrix issatschenkoi*, albeit only from one strain. This species does not bloom often and determining more accurate quotas might be challenging. Additionally, the microcystin toxicological calculation cannot be applied to anatoxins as this toxin has a different mode of action and toxicity. New data on the oral toxicity of anatoxin-a have recently been obtained in the MfE-funded '*Phormidium* Toxicity' project (MfE Contract 21508), which may enable toxicology calculations to be undertaken for planktonic environments.

Consideration also needs to be made for situations where different toxin-producing taxa of cyanobacteria co-occur.

In summary, we recommend that:

- The planktonic thresholds are restructured to include a combination of cell counts (for known toxic species) and biovolumes for all other taxa (see Table 3 as an example).
- Further resources are provided to determine toxin quotas for nodularin in New Zealand *Nodularia spumigena*. The same toxicological values as used for microcystins could then be applied, as nodularin has a similar mode of action and toxicity. The toxicological values for nodularins may need to be revisited within 5 years' time if new research suggests that different toxicological values are more applicable than those for microcystins.
- A toxicologist is commissioned to review the data available for anatoxin-a toxicity and advise on whether an appropriate cell concentration threshold for C*uspidothrix issatschenkoi* can be determined.

4.2. Revision of microcystin quota values

Data on microcystin cell quota values were collated from six studies that have been undertaken either in New Zealand lakes or using *Microcystis* sp. cultures isolated from New Zealand lakes (Table 4 and Figure 1). Data from the 2009 interim guidelines were also included in the analysis. A brief description of each study is provided below.

The studies use two different methods to quantify microcystins. These provide slightly different information and consideration of this diversity of source data is required when interpreting the results. The enzyme-linked immunosorbent assay (ELISA) used in the

studies below has antibodies raised against Adda (an amino acid unique to microcystins/nodularin) and should detect over 80% of all known microcystin variants and nodularin (Fischer et al. 2001). 'Free' Adda may also be detected in some instances, potentially overestimating total microcystin load in a sample (e.g., Wood et al. 2008a). This method cannot distinguish between microcystins and nodularin, although nodularin is not likely to be present in the examples provided below. Liquid chromatography-mass spectrometry (LC-MS) detects the specific mass of individual toxins in a sample and thus provides information on which congeners are present (Puddick et al. 2014). This is particularly relevant for microcystins, where over 250 congeners exist (Meriluoto et al. 2016), and structural differences can affect their toxicity. In the studies detailed below, the LC-MS methods used were able to detect the 18 most commonly observed microcystin congeners found in New Zealand *Microcystis* (see Section 3.7), and the sum of the concentration of the individual microcystin congeners was used.

- In the 2009 interim guidelines (Ministry for the Environment and Ministry of Health 2009), five *Microcystis* strains isolated from Lake Horowhenua were assessed. Microcystin concentrations were determined using LC-MS, cells were enumerated using light microscopy and an assumption was made that all cells are toxin-producers.
- Puddick et al. (2016b), undertook a study using cyanobacterial material from Lake Horowhenua to develop a new sampling device that allows fine-scale sampling of cyanobacterial scums. Total microcystins were determined using LC-MS (no compensation for dissolved microcystins was possible) and toxic *Microcystis* cells were determined using a quantitative-polymerase chain reaction (PCR) assay that targeted the *mcyE* gene.
- A culturing experiment using *Microcystis* CAWBG11 isolated from Lake Hakanoa assessed how dissolved nitrogen concentrations affected the microcystin congener composition (Puddick et al. 2016c). Microcystins were determined using LC-MS, and the assumption was made that all cells were toxic. Cells were enumerated using light microscopy.
- Two studies were undertaken at Lake Rotorua (Kaikoura) to investigate how biotic and abiotic variables influenced microcystin production (Wood et al. 2011b, 2012b). Total and dissolved microcystins were determined using ELISA and intracellular values were determined by subtracting the dissolved microcystin from the total microcystin. All cells were assumed to be toxic and were enumerated using microscopy.
- Wood et al. (2017d) undertook a seasonal study at Lake Rotorua (Kaikoura) and assessed the phytoplankton composition and microcystin levels during 2013-2015. Total and dissolved microcystin concentrations were determined using LC-MS and intracellular values were calculated as the difference between the total and dissolved microcystin concentrations. The number of toxic *Microcystis* cells was determined using a quantitative-PCR targeting the *mcyE* gene.

- Steiner et al. (2017) conducted a study at Lake Waitawa (Wellington) which assessed cyanobacterial scums (comprised of *Microcystis* and *Dolichospermum*). Microcystin concentrations were determined as per Wood et al. (2017d). The number of toxic *Microcystis* cells was determined using a quantitative-PCR assay that targeted the *mcyE* gene. Molecular tests showed that the *Dolichospermum* cells present in the samples did not produce microcystins.

Table 4. Collated microcystin cell quota values from studies on New Zealand *Microcystis*.

Study	Sample Description	n	Microcystin Cell Quotas (pg cell^{-1})[a]
Ministry for the Environment and Ministry of Health (2009)	Cultured strains from Lake Horowhenua (Levin)	5	0.27, 0.21, 0.81, 0.57, 1.27
Puddick et al. (2016b)	Lake Horowhenua - 2015	32	5.445, 2.600, 6.609, 5.268, 8.706, 7.522, 9.724, 8.159, 8.564, 7.050, 8.916, 5.230, 8.224, 13.384, 4.590, 1.837, 5.318, 1.176 2.844, 4.517, 8.148, 6.644, 5.332, 5.728, 7.706, 6.175, 6.566, 2.789, 5.823, 7.849, 4.330, 5.938
Puddick et al. (2016c).	Cultured Strain from Lake Hakanoa (Huntly; CAWBG11)	78	2.637, 3.714, 1.843, 1.571, 0.921, 0.425, 0.468, 0.456, 0.150, 0.124, 0.131, 0.226, 0.151, 4.404, 5.189, 3.183, 2.776, 1.102, 1.075, 0.978, 1.001, 0.327, 0.304, 0.203, 0.364, 0.258, 1.619, 5.949, 2.307, 1.738, 0.838, 0.542, 0.504, 0.413, 0.125, 0.192, 0.150, 0.135, 0.204, 1.829, 3.385, 1.019, 0.523, 0.399, 0.261, 0.356, 0.392, 0.338, 0.284, 0.290, 0.203, 0.113, 4.390, 5.703, 1.325, 1.452, 0.409, 0.529, 0.387, 0.380, 0.337, 0.172, 0.469, 0.173, 0.195, 2.289, 3.306, 1.689, 1.408, 0.560, 0.563, 0.481, 0.514, 0.784, 0.866, 0.466, 0.472, 0.434
Wood et al. (2011b)	Lake Rotorua (Kaikoura) - 2009	14	0.038, 0.047, 0.035, 0.214, 0.127, 0.019, 0.080, 0.539, 0.644, 0.022, 0.015, 0.032, 0.021, 0.029
Wood et al. (2012b)	Lake Rotorua (Kaikoura) Mesocosm Experiment- 2010	18	0.096, 0.079, 0.058, 0.019, 0.040, 0.109, 0.115, 0.072, 0.073, 0.051, 0.060, 0.063, 0.126, 0.077, 0.100, 0.594, 0.504, 1.379
Wood et al. (2017d)	Lake Rotorua (Kaikoura) - 2014-2015	32	2.144, 1.634, 1.144, 0.539, 0.306, 0.279, 0.266, 0.122, 0.070, 0.066, 0.065, 0.062, 0.059, 0.058, 0.058, 0.055, 0.037, 0.034, 0.032, 0.030, 0.028, 0.024, 0.024, 0.024, 0.022, 0.020, 0.014, 0.010, 0.008, 0.007, 0.007, 0.006
Steiner et al. (2017)	Lake Waitawa - 2015	8	2.471, 3.034, 0.608, 0.271, 0.340, 0.115, 4.223, 1.052

[a] Values are rounded to three decimal places for all studies except for Ministry for the Environment and Ministry of Health (2009) which were calculated to two decimal places.

A total of 187 microcystin quota values were collated during this exercise (Table 4). The highest microcystin quota detected among the seven studies was 13.384 pg cell^{-1} in the Puddick et al. (2016b) study (Figure 1). The median microcystin quota in this study was also higher than all other studies (6.056 pg cell^{-1}; Figure 1). This experiment was conducted to stimulate microcystin production by encouraging scum formation, although such an event might occur in the natural environment when cyanobacterial accumulate on the edge of lakes. As noted above, a further limitation with the techniques deployed in this study was that the researchers were unable to determine the proportion of extracellular microcystins. Given that this can account for greater than 50% of total microcystins (Wood et al. 2011b), we have decided to exclude these values in the toxicological calculation review (see Section 4.3). The decision to exclude this dataset was further supported by an ANOVA with Tukey's post-hoc test which showed the microcystin quotas reported in the Puddick et al. (2016b) study were significantly higher than those of all of the other studies ($p < 0.05$; Figure 1).

When the data were assessed excluding the Puddick et al. (2016b) study, the median microcystin cell quota from the combined data sets was 0.277 pg cell^{-1}, the lowest quota value was 0.006 pg cell^{-1} (Wood et al. 2017d), and the highest was 5.949 pg cell^{-1} (Puddick et al. 2016c). The median values from the individual studies ranged from 0.036 pg cell^{-1} (Wood et al. 2011b) to 0.830 pg cell^{-1} (Steiner et al. 2017). Whilst there were statistically significant differences between the studies (ANOVA $F_{5,149} = 27.02$, $p < 0.001$), the differences were likely to be an outcome of the different methodologies used. The highest microcystin cell quota value (5.949 pg cell^{-1}) was used for the toxicology calculations described in Section 4.3 as this value represents the highest known risk from microcystins in New Zealand water bodies and will provide an additional level of safety (i.e., the worst-case scenario).

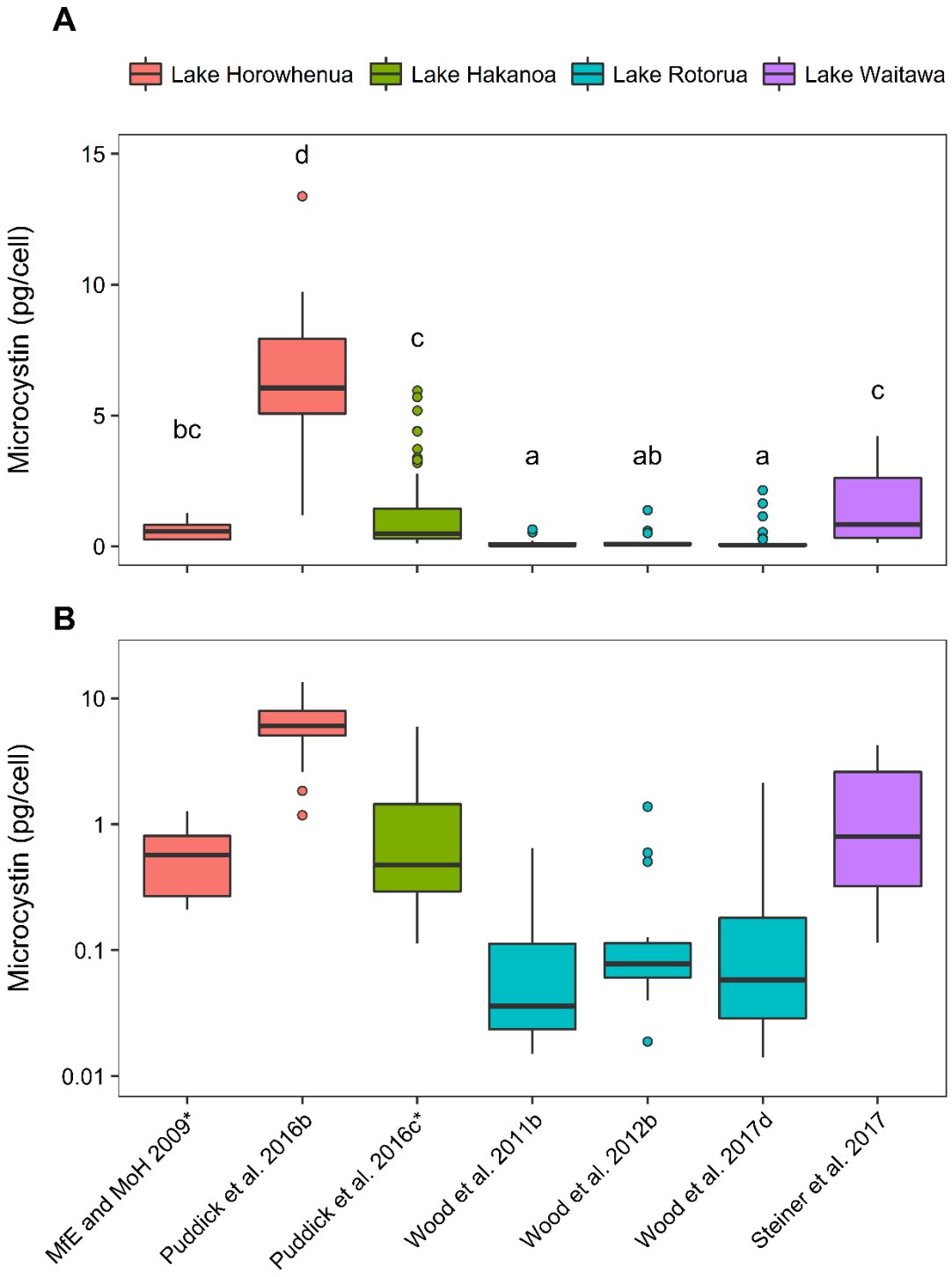

Figure 1. Microcystin cell quotas from studies on New Zealand *Microcystis* sp., sourced from four different lakes (denoted by colour). All studies used environmental samples except for those denoted with *, which used cultured stains of *Microcystis*. Data are displayed on (A) a linear and (B) a logarithmic y-axis. Identical letters above the box indicate no significant difference ($p < 0.05$) between sites (One-way ANOVA, Tukey HSD).

4.3. Review of microcystin toxicity calculations

The microcystin toxicity calculations which form the basis of the threshold values for the planktonic cyanobacteria alert-level framework were reviewed by Professor Daniel Dietrich (University of Konstanz) (an international expert in microcystin/cyanotoxin toxicology). To improve the robustness of the calculations in light of recent knowledge advances in microcystin toxicology, Professor Dietrich suggested several changes to the calculations found in Appendix 2 of the 2009 interim cyanobacteria guidelines (Ministry for the Environment and Ministry of Health 2009):

- That the highest microcystin cell quota value from Section 4.2 is used for the calculations (5.949 pg cell^{-1}; excluding the data from Puddick et al. (2016b)). The rationale is that this value represents the highest known risk from microcystins in New Zealand water bodies and thus will provide for a level of redundancy in the level of safety (i.e., use of the worst-case scenario).
- That an additional safety factor of 2 is included to compensate for the more rapid uptake kinetics observed for certain microcystin congeners (e.g., Fischer et al. (2010)).
- That a temporal allocation factor (TAF) is included to account for people not using recreational waterways for the entire year. This was set to 0.083 or 1 month of the year.
- That an allocation factor (AF) is included in the calculations. This was set to 100%, which assumes that that the only source of exposure to microcystins is from recreational activities (i.e., no additional toxin is consumed from drinking water or food sources).

When these changes were incorporated into the microcystin toxicity calculations, the revised cell concentration threshold for *Microcystis* sp. was 12,100 cells mL^{-1} (reduced from 19,000 cells mL^{-1} in the 2009 interim guidelines). The revised calculations are provided in Appendix 2 of this report and have been included in the revised version of the guidelines document (see Section 6 for more information). This cell concentration has been used for the Action Level Threshold – Situation 1 for *Microcystis* sp. in the proposed restructure of the alert-level framework for planktonic cyanobacteria (Table 3), however, further work needs to be undertaken to develop robust thresholds for *Nodularia spumigena* and *Cuspidothrix issatschenkoi* and to determine how to deal with co-existing toxin-producing cyanobacteria (see Section 4.1).

5. EVALUATION OF A RISK MODELLING APPROACH AND KNOWLEDGE GAPS RELATED TO ANATOXINS PRODUCED BY *MICROCOLEUS* IN RIVERS

The aim of this stage of the project was to (a) explore a possible quantitative risk model approach to assessing anatoxins produced by *Microcoleus* in rivers, and (b) identify knowledge gaps required to further develop such risk models. The ultimate aim of these steps is to progress towards determining if alert level thresholds can be developed for *Microcoleus.*

5.1. Main steps in quantitative risk assessment and progress towards a risk model for anatoxin in river water

There are six sequential steps in a framework for quantitative health risk assessment:

i. Establish the context
ii. Identify the hazard(s)
iii. Assess exposure to the hazard(s)
iv. Review and adopt dose-response functions
v. Calculate risk profiles using Monte Carlo models, identifying knowledge gaps
vi. Communicate the results to resource management agencies and to the public.

In the case of developing a risk assessment for anatoxins produced by *Microcoleus* in rivers, step (i) has been established through the associated work carried out to date (Wood et al. 2015); step (ii) concerns anatoxins; step (iii) has largely been analysed in Wood et al. (2015); steps (iv and v) are outlined below in the context of 'Monte Carlo'[1] models and the knowledge gaps (Section 5.2). Step (vi) should only be contemplated once substantial progress has been made in the risk assessment.

The risk assessment below only considers the risk posed by anatoxin released into water. As discussed in Wood et al. (2015) it will also be necessary to explore soil ingestion (or similar) models to estimate rate and likelihood of *Microcoleus* mat ingestion. Investigating this further was beyond the scope of the present project.

In this section, first use of technical jargon is presented in **bold** text.

Quantitative **risk models** transform **inputs** (such *Microcoleus* cover) into **outputs** (such as human risk attributable to exposure to cyanotoxins). To produce such risks,

[1] Monte Carlo simulation is a computerised mathematical technique that allows the in quantitative analysis and decision making to be accounted for. Monte Carlo simulation performs risk analysis by building models of possible results by substituting a range of values—a probability distribution—for any factor that has inherent uncertainty.

some transforming calculation procedures are necessary, these are known as **algorithms**.

In risk model **calibration**, the algorithm is tuned to data (e.g., from clinical trials) by changing values of its **parameters** (aka **coefficients**). The calibrated parameter values should also be used in model **testing** and **prediction**, comparing outputs against data not used in the calibration exercise. Adjusting these calibrated parameter values to match new outputs is merely a re-calibration, not an independent test. These fundamental features are depicted in Figure 2.

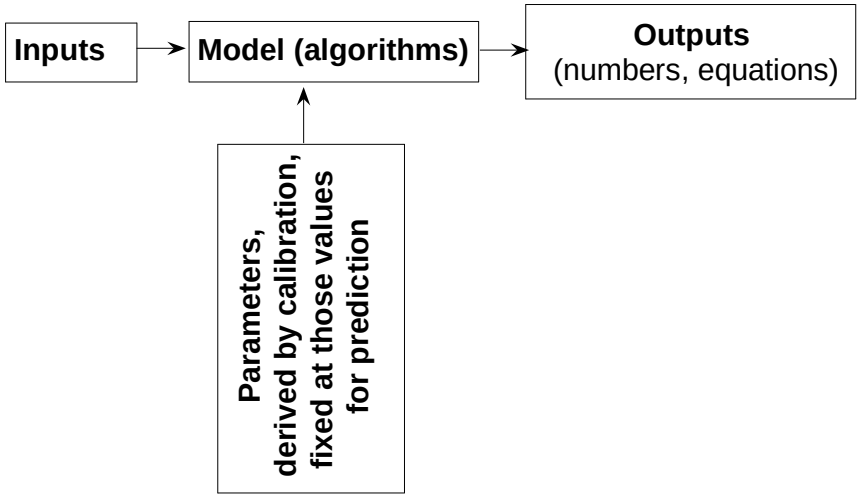

Figure 2. Model inputs transformed to outputs via algorithms informed by inputs and parameters.

The model must have sufficient flexibility to enable model calibration. This flexibility is provided by the algorithm. It can be based on a purely **empirical** approach, typically using some form of **correlation** or **association** analysis, such as statistical **regression**. A less flexible, but more powerful, approach uses **theoretical algorithms**, using **dose-response functions**.[2]

Models facilitate addressing 'what if?' scenarios, by changing any or all inputs and parameters, thereby determining sensitivity to these changes.

Further technical details of the Quantitative Microbial Risk Assessment (QMRA) using a second-order Monte Carlo approach are provided in Appendix 3.

[2] For example, the mathematical form of many viral pathogen *theoretical* dose-response curves is derived from fundamental assumptions, e.g., the 'single-hit' hypothesis—that infection is guaranteed to occur even if only one virus survives the host's defences. Consequently, the mathematical form of the dose-response function is fixed, hence 'less flexible' above. However, it is 'more powerful' because it has an underlying theoretical basis, and so greater confidence is attached to any extrapolation beyond the range of the calibration data—as is often required.

In summary, the approach is based on the QMRA paradigm for pathogens. We suggest this can be extended to anatoxins, using a 'binning' procedure for translating continuous anatoxin concentration data into a series of numbers akin to a pathogen dose. The second-order Monte Carlo approach model explicitly accounts for variability (exposure duration, ingestion rate, anatoxin concentration) and uncertainty (regarding dose-response). Each of these can be changed, thereby assessing the sensitivity of the model to such changes. Using a second-order Monte Carlo approach facilitates the selection of an appropriate burden-of-proof (once all information is available) and this allows results to be discussed with policy development teams. A potential challenge with using this approach is that, unlike pathogens, there is no epidemiological or dose response information available for anatoxins, and as noted above, there is uncertainty as to how the risk posed by consumption of mats can be incorporated into this risk model approach.

There is currently sufficient information available to determine human water ingestion rates during recreational activities. Dufour et al. (2017) and Cressey and Horn (2016a, 2016b) contain reviews of swimming-related ingestion and body surface area for primary and secondary contact with environmental water. This information is essential for determining the risk anatoxins released into river water pose. However, a number of other critical knowledge gaps remain that need further investigation or advice before the approach described above, and other risk modelling approaches, can be developed or tested.

5.2. Critical knowledge gaps

5.2.1. Acute toxicity assessment of dhHTX and HTX

The MfE-funded project '*Phormidium* Toxicity' (contract: 21508) determined voluntary consumption, gavage and intraperitoneal injection toxicity values for two of the four anatoxin variants produced by *Microcoleus* (ATX and dhATX). Considerable technical development and effort was invested in purifying dhHTX for acute toxicity assessments. Due to funding constraints this work was not completed. Further investment is required to complete the final stages of purification and determine the acute toxicity of this compound. Due to the difficulty in producing sufficient HTX source material it is unlikely that sufficient material can be purified for toxicology assessment, unless a new source can be located. We recommend undertaking further assessments to explore whether sufficient material can be located.

Alternatively, if under the scenario that obtaining the above data for dhHTX and HTX is not feasible, a toxicologist should be commissioned to determine the implication of calculating daily tolerable intakes (TDI) only on ATX and dhATX data. A toxicologist is also required to provide advice on how to calculate a TDI for ATX and dhATX. These calculations should include safety factors to account for intraspecies and interspecies

variability, and to determine how to account for differences in toxicity among congeners.

5.2.2. Recreational contact thresholds

The toxicological data generated in the *'Phormidium* Toxicity' project is for acute exposure only. Internationally, only one sub-chronic study on anatoxins has been conducted (reviewed in Wood et al. (2015)), and longer-term chronic studies are lacking. The current end points determined in the *'Phormidium* Toxicity' study are death, and provide no information on lowest observed adverse effect levels (LOAEL) or no observed adverse effect levels (NOAE). It might be necessary to have these data to determine TDIs; i.e., LOAEL and NOAE are used in the toxicology calculations for microcystins (see Appendix 2).

Further discussions with toxicologists are required to determine whether obtaining this information is possible, and whether this is necessary for the purpose of determining recreational contact thresholds. Given that recreational contact is likely to be relatively short-term (as opposed to drinking water consumption) acute toxicity values may be sufficient. We recommend the inclusion of policy makers in these discussions.

5.2.3. Including the risk from consumption of mats

The development of a risk model needs to consider accidental consumption of mats. Aside from ingestion of water while swimming (or other recreational activities), a further exposure route may be from direct ingestion of *Microcoleus* mat material, particularly by younger children playing at the water's edge. We consider that this route of ingestion to be extremely high-risk due to the high concentration of anatoxins within mats. Further consideration should also be given to how soil ingestion (or similar) risk models can be applied to *Microcoleus*. An expert in this field should be consulted.

5.2.4. Further data on anatoxin in river water

There are data from only four rivers on anatoxin concentrations in the water column. Data from a wider number of rivers is required. Previous research indicated there might be a relationship between the anatoxin load present in the river (a combination of the anatoxin content of the *Microcoleus* mats and the abundance of *Microcoleus* mats) and the concentration of anatoxins detected in the water column (Wood et al. 2015). Further data are needed to confirm this, to enable water column anatoxin data to be used in risk models. We suggest that 30–50 rivers should be surveyed. This could be achieved relatively easily by developing a standardised sampling protocol and sampling kit. This kit could be dispatched to regional councils, who could undertake the sampling and would likely involve site surveys, water and mat sampling, and short-term deployments of solid-phase tracking technology (SPATT) samplers.

5.2.5. Further refinement and testing of the Monte Carlo and other approaches

Further refinement and testing of the Monte Carlo approach outlined in this section and detailed in Appendix 3 is required. Consideration should be given to other approaches given that all of the assumptions for pathogens may not be applicable for cyanotoxins. It may be more applicable to follow an approach more in line with that used to determine the planktonic microcystin thresholds (see Appendix 2). Regardless of approach, there remains uncertainty as to how anatoxin thresholds will (or if they can) be converted in to a useable measurement for monitoring purposes (i.e., percent cover or biomass).

We suggest a workshop that brings together experts in a diverse range of fields could address this issue. The workshop should include ecologists/water quality scientists with knowledge on *Microcoleus*, chemists with expertise in cyanotoxins, risk modellers with water and soil expertise, toxicologist and policy makers.

6. UPDATES MADE TO SECTIONS OF THE INTERIM GUIDELINES

In this section, we describe recommend changes to the 'Interim New Zealand Guidelines for Cyanobacteria in Recreational Fresh Waters' (Ministry for the Environment and Ministry of Health 2009). Major changes are identified by yellow highlighting of the relevant text (additions or modification of entire sentences) or 'strikethrough' of the text (recommendations portions of text to delete). Prior to release of a revised version of the guidelines, the text recommended for deletion should be deleted (if acceptable to MfE and MoH) and the yellow highlighting should be removed.

Major recommendations from the end-user workshop are documented in the appropriate locations of the guidelines document as boxes with a red border. Information is provided on whether the recommendation was executed as a part of the Guidelines Review project or if it was outside of the scope and/or resources of the Guidelines Review project. These boxes should be removed prior to the release of a revised version of the guidelines.

A summary of the changes made to each section is documented below.

6.1. Changes throughout the document

The references for the document were compiled using an EndNote library, allowing the formatting of the reference list and in-text citations to be modified more easily. The referencing style was changed to a numbered system to allow for easier compilation of data tables.

Minor editing of the document was conducted. These minor edits were not tracked.

Changes in cyanobacterial taxonomy were updated.

6.2. Preface

The author information and acknowledgements need to be updated to reflect the 2018 (or later) revision of the document.

6.3. Section 1: Introduction

A box highlighting recommendations from the end-user workshop was added.

Information on cyanobacterial taxonomy updates was provided, including a table of the name changes relevant to common New Zealand species.

Section 1.1: What is the purpose of these guidelines?

Information on the Ministry of Health's 'Guidelines for Drinking-Water Quality Management for New Zealand' was updated as the document is no longer a draft document.

The web addresses included were updated.

Section 1.2: What does this document cover?

No changes were made to this section.

Section 1.3: Who should use these guidelines?

No changes were made to this section.

Section 1.4: Status of this guidance

A new paragraph has been added noting that this document is an update from the 2009 interim guidelines, and is intended to be a 'living' document.

6.4. Section 2: Framework

Section 2.1: Why monitor for cyanobacteria in fresh water?

No changes were made to this section.

Section 2.2: What is contact recreation?

No changes were made to this section.

Section 2.3: The overall approach

No changes were made to this section.

Section 2.4: Roles and responsibilities

A box highlighting recommendations from the end-user workshop was added.

A sentence suggesting the annual review of roles and responsibilities was added.

Section 2.5: Cost and resource implications

No changes were made to this section.

Section 2.6: Conditions of using these guidelines: A disclaimer

No changes were made to this section.

6.5. Section 3: The Guidelines

Section 3.1: Planktonic cyanobacteria: An introduction

Information on the areas of interest identified at the end-user workshop were compiled as appendices and referenced at relevant points in this section.

A sentence from the final paragraph was removed as the content is now covered in the following section.

Section 3.2: Alert-level framework: Planktonic cyanobacteria

A box highlighting recommendations from the end-user workshop was added.

The table of toxin-producing cyanobacteria identified in New Zealand was updated and the table of international toxin-producing cyanobacteria was moved to the appendices.

The proposed restructured alert level framework for planktonic cyanobacteria was included but requires more work before it is able to be implemented (see Section 4.1). The original alert level framework was removed.

The text was revised to reflect the proposed restructured alert level framework for planktonic cyanobacteria.

Section 3.3: Details of the framework: Planktonic cyanobacteria

A box highlighting recommendations from the end-user workshop was added.

A brief description of the rationale for using cell biovolumes was included.

The wording was updated to reflect the proposed restructured alert-level framework for planktonic cyanobacteria; however, more work is required before the restructured alert-level framework is suitable for implementation.

A paragraph describing how the biovolume thresholds for the previous Situation 1 and Situation 2 was deleted as it will no longer be relevant under the proposed restructured alert-level framework for planktonic cyanobacteria.

A sentence provided recommendation on toxin testing frequencies was removed.

A sentence that related to the previous threshold values in respect to a situation at Lake Rotoiti was deleted.

Section 3.4: Benthic cyanobacteria: Introduction

A box highlighting recommendations from the end-user workshop was added.

A statement specifying that the section primarily relates to benthic *Microcoleus* was added.

A statement on knowledge surrounding downstream impacts of *Microcoleus* blooms was included.

Updated information on extracellular levels of anatoxins in river water and SPATT sampling was included.

A reference to an appendix on benthic cyanobacteria in lakes was included at the end of this section.

Section 3.5: Alert-level framework: Benthic cyanobacteria

A box highlighting recommendations from the end-user workshop was added.

Section 3.6: Details of the framework: Benthic cyanobacteria

A box highlighting recommendations from the end-user workshop was added.

A sentence related to using flow measurements to make health alert decisions was removed.

Section 3.7: Benthic cyanobacteria and river flows

Much of this section was deleted as the use of flow measurements to make health alert decisions has been shown to not be good practice, and instead a sentence on site-specific models was included.

Section 3.8: Cyanotoxin accumulation in aquatic organisms

Information on the accumulation of nodularin in New Zealand shortfin eels was added.

6.6. Section 4: Sampling

Section 4.1: Health and safety

A box highlighting recommendations from the end-user workshop was added.

Section 4.2: Biosecurity

This section was updated to include more modern examples of aquatic threats and to highlight land-based biosecurity threats such as *Mycoplasma bovis*.

Section 4.3: Planktonic cyanobacteria

No changes were made to this section.

Section 4.4: Benthic cyanobacteria

A sentence implying that site selection would be the biggest time investment was removed.

The information provided on underwater viewers was updated.

Wording that supported the use of single person site surveys was removed as this is no longer acceptable health and safety practice.

Section 4.5: Sample storage and transport

No changes were made to this section.

Section 4.6: Susceptibility of a water body to a cyanobacterial bloom or benthic mat event

This section was removed.

6.7. Appendices

Several new appendices were developed to provide information on specific areas of interest identified at the end-user workshop. Subsequently, appendices were re-numbered. We have reviewed the other sections of the Guidelines to adjust the references to the appendices throughout the main text.

Appendix 1: Effect of climate change on cyanobacterial blooms

This appendix was added upon the recommendation of the end-user workshop.

Appendix 2: Management of freshwater cyanobacteria blooms

This appendix was added by the authors as they felt it would provide useful information for the end-users of the guidelines.

Appendix 3: The impact of toxic freshwater cyanobacteria on marine environments

This appendix was added upon the recommendation of the end-user workshop.

Appendix 4: Cyanotoxins and their distribution in New Zealand (previously Appendix 1)

This appendix has been updated with the new information published since 2009 on cyanotoxins in New Zealand and significant knowledge advances from the international literature.

A revised table of international toxin-producing cyanobacteria was included.

A section on inter-strain variability was added upon the recommendation of the end-user workshop.

Revised toxin structures were included to better represent the presently known structural diversity of cyanotoxin congeners.

Information on the accumulation of relevant cyanotoxins in foodstuffs was included, as suggested by participants at the end-user workshop.
Up-to-date information on analytical methods for cyanotoxins was provided.

Appendix 5: Derivation of guideline values (previously Appendix 2)
The calculations in Box A5.1 (previously Box A2.1) were revised to be in line with modern practices and using a more robust microcystin quota value. The revision resulted in a reduction in the Action Level threshold for *Microcystis* from 19,000 cells mL^{-1} to 12,100 cells mL^{-1}.

To align with an alert-level framework for planktonic cyanobacteria based on cell concentrations of toxin-producing species identified in New Zealand, the conversion of the *Microcystis* cell concentration to biovolume and subsequent assessment of relevance to anatoxin-producing *Cuspidothrix issatschenkoi* were deleted.

Information on other toxin-producing planktonic cyanobacteria frequently observed in New Zealand lakes and the necessary steps to develop threshold values for them was included as a reminder that further work is required before the proposed restructure alert-level framework is suitable for implementation.

Appendix 6: Photographs of planktonic blooms (previously Appendix 3)
No changes were made to the content of this appendix, but a box indicating a recommendation from the end-user workshop was added.

Appendix 7: Biovolumes explained (previously Appendix 4)
A box indicating a recommendation from the end-user workshop was added.

The taxonomy of relevant cyanobacteria species was updated and following that, the order of species in the table was revised to alphabetical order.

Appendix 8: Media release – Planktonic cyanobacteria (previously Appendix 5)
No changes were made to the content of this appendix, but participants at the end-user workshop highlighted the importance of promoting a communication strategy beyond traditional media releases.

Appendix 9: Health warning sign for planktonic cyanobacteria (previously Appendix 6)
No changes were made to the content of this appendix, but a box indicating a recommendation from the end-user workshop was added.

Appendix 10: Benthic cyanobacterial and other benthic algae photos (previously Appendix 7)

A box indicating a recommendation from the end-user workshop was added.

The images of blooms of *Nostoc* sp. from Lake Taupo were removed from this appendix as these photos are now included in Appendix 11 (Benthic cyanobacteria mats in lakes). Note: this deletion was not marked with a strikethrough so that the page formatting could be adjusting properly.

Appendix 11: Benthic cyanobacterial mats in lakes

This appendix was added upon the recommendation of the end-user workshop.

Appendix 12: Cyanobacteria and cyanotoxin capabilities in New Zealand (previously Appendix 8)

The service providers listed in the two tables were updated.

Service providers who are no longer active were removed (Stephen Moore from Landcare Research, Paul Broady from the University of Canterbury and David Hamilton from the University of Waikato).

Phil Novis from Manaaki Whenua/Landcare Research was added to the list of freshwater micro-algae/cyanobacterial analysis providers.

A box indicating a recommendation from the end-user workshop was added.

Appendix 13: Health warning signs for benthic cyanobacteria (previously Appendix 9)

No changes were made to the content of this appendix, but a box indicating a recommendation from the end-user workshop was added.

Appendix 14: Media release – Benthic cyanobacteria (previously Appendix 10)

No changes were made to the content of this appendix, but participants at the end-user workshop highlighted the importance of promoting communication strategy beyond traditional media releases.

Appendix 15: Example pamphlet – Nelson City Council / Tasman District Council (previously Appendix 11)

The pamphlet example was changed to a pamphlet on toxic algae developed by Nelson City Council and Tasman District Council as it was more up-to-date than the previous example.

Appendix 16: Example field sampling sheet for planktonic cyanobacteria (previously Appendix 12)

No changes were made to the content of this appendix, but participants at the end-user workshop commented that a simplified sampling sheet would be useful.

Appendix 17: Example field sampling sheet for benthic cyanobacteria (previously Appendix 13)

No changes were made to the content of this appendix, but participants at the end-user workshop commented that a simplified sampling sheet would be useful.

Lugol's solution appendix

This appendix was deleted.

7. RECOMMENDATIONS

This 2018 review of the 'Interim New Zealand Guidelines for Cyanobacteria in Recreational Fresh Waters' has highlighted several areas that require further work to improve their robustness and usability.

Recommendation 1: Conduct additional work required for the new combined cell-count/biovolume based planktonic cyanobacteria alert-level framework to be implemented (see Section 4.1).

The research and monitoring work conducted over the past nine years has established that at least three planktonic toxin-producing cyanobacteria are present in New Zealand lakes (microcystin-producing *Microcystis* sp., nodularin-producing *N. spumigena* and anatoxin-a-producing *C. issatschenkoi*). With the oral toxicity work that has recently been conducted on anatoxin-a, it may now be possible to devise cell concentration thresholds for *C. issatschenkoi*. As the present alert-level framework for planktonic cyanobacteria is based solely on the toxicity calculations for microcystins and cell quota values from *Microcystis* sp., developing thresholds for each of these toxin-producing cyanobacteria will form a more robust framework. Feedback from the end-user workshop suggested that the present alert-level framework for planktonic cyanobacteria is difficult to manage because of variability in biovolumes and uncertainty around how to handle potentially toxic cyanobacterial taxa.

Recommendation 2: Conduct additional research on benthic cyanobacteria in lakes to identify if there is a health risk and whether alert level thresholds can be developed.

Recently, there have been several incidences of benthic cyanobacteria blooms in lake environments. Feedback from the end-user workshop highlighted that benthic cyanobacteria in lakes are not well covered by the present guidelines and there is a lack of guidance on how to monitor, sample and when to issue warnings for this group. Research is required to identify: which species are toxin producers; whether benthic mats (detached or attached) pose a health risk; methods for monitoring; and to consider whether thresholds (perhaps based on coverage) can be developed to assist with risk management.

Recommendation 3: Consider developing the new guidelines sections suggested by participants at the end-user workshop (see Section 7 of Cawthron Report 3181 (Wood et al. 2018)).

The end-user workshop raised many ideas and opportunities that if actioned will improve cyanobacterial monitoring and management in New Zealand. However, many of the suggestions are outside the scope of this guidelines review project. We therefore recommend that MfE and MoH, in concert with stakeholders, prioritise these

suggestions and commission work to incorporate end-user-identified issues into the revised guidelines.

Recommendation 4: Amend the next version of the guidelines to improve document functionality.

During the 2018 review it has become apparent that certain sections of the guidelines require more frequent updating to remain current (e.g., tables of toxin producers, background information on cyanobacteria and cyanotoxins). Whilst it is important to maintain the integrity of the advice provided in the guidelines and the alert-level frameworks through strict document control, it might be possible to place some of this background information online and to commission more frequent updates. It was also apparent during the end-user workshop that much of the information desired by participants was already present in the guidelines but was difficult to find. Improving the layout or ability to search the guidelines might improve the functionality and use of the guidelines by end-users. This step could begin immediately with suitable sections and information from the current interim guidelines been placed on a suitable webpage.

Recommendation 5: Address the knowledge gaps required to refine the Quantitative Microbial Risk Assessment (using a second-order Monte Carlo approach) to determine the risk posed by exposure to anatoxin in river water. Other approaches may also need to be explored. The five key knowledge gaps identified are given below along with a recommendation on how to progress these areas:

1. There is a lack of toxicological data for the congeners dihydrohomoanatoxin-a and homoanatoxin-a. We recommend further investment is provided to complete the purification and determine the toxicity of dhHTX and to explore the feasibility of sourcing HTX.

2. There is only acute toxicological data for anatoxin-a and dihydroanatoxin-a. We recommend a toxicologist is commissioned to explore the feasibility of acquiring data on chronic exposure, defining the endpoint for risk assessments (i.e., death or illness) and determining the TDI based on the current data.

3. The risk of *Microcoleus* mat consumption needs to be incorporated into risk models. We recommend an expert in soil ingestion (or similar) risk modelling should be consulted.

4. There is insufficient information on concentrations of anatoxins released into river water. We recommend a study to collect data from 30-50 rivers.

5. There is uncertainty as to how anatoxins thresholds (if they can be calculated) will be translated into a metric that can be measured by water managers (i.e., percent cover of *Microcoleus* mats). We recommend a workshop is convened which brings

together ecologists/water quality scientists, chemists, risk modellers, toxicologists and policy makers.

8. ACKNOWLEDGEMENTS

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10. APPENDICES

Appendix 1. New literature on toxic cyanobacteria in New Zealand identified during 2009-2017. Each section is organised by date of publication.

New cylindrospermopsin literature identified

Wood SA, Pochon X, Luttringer-Plu L, Vant BN, Hamilton DP 2014. Recent invader or indicator of environmental change? A phylogenetic and ecological study of *Cylindrospermopsis raciborskii* in New Zealand. Harmful Algae 39: 64-74.

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Smith FMJ, Wood SA, van Ginkel R, Broady PA, Gaw S 2011. First report of saxitoxin production by a species of the freshwater benthic cyanobacterium, *Scytonema* Agardh. Toxicon 57: 566-573.

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Wood SA, Dietrich DR 2011. Quantitative assessment of aerosolized cyanobacterial toxins at two New Zealand lakes. Journal of Environmental Monitoring 13: 1617-1624.

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Somdee T 2010. Biodegradation of cyanobacterial hepatotoxins [Dha^{7}] MC-LR and MC-LR by natural aquatic bacteria. PhD thesis: Massey University, Wellington, NZ.

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Appendix 2. Updated microcystin toxicity calculations to derive the Action Level Threshold for planktonic cyanobacteria.

Below is an updated version of Appendix 2 in the 2009 interim guidelines (Ministry for the Environment and Ministry of Health 2009); major changes have been highlighted.

Derivation of planktonic cyanobacteria guideline values

Based on animal toxicological studies, guidelines for exposure to microcystins via ingestion have been developed for the action level (red mode) – Situation 1. The guideline values are extrapolated from animal experiments and make various assumptions about exposure, including uncertainty factors. Uncertainty factors are used to account for safety margins, errors in extrapolation from animal experiments to human risk, and other limitations associated with experiments or limited data.

In this document, tolerable daily intakes (TDIs) for microcystins are calculated based on data from two separate animal toxicological studies; a 13-week mouse study (Fawell et al. 1999) conducted with purified microcystin-LR via gavage, and a 44-day pig study (Falconer et al. 1994) (see Box A2.1) carried out with cyanobacterial bloom material in the drinking water (containing at least nine microcystin congeners but not microcystin-LR). The toxin content of this bloom material was estimated via an LD50 assay over 24 h by intraperitoneal injection in mice, high-performance liquid chromatography analysis, and the protein phosphatase inhibition assay, all three yielding different toxin concentrations.

Recent studies (e.g., Fischer et al. (2010)) suggest that other microcystin congeners such as microcystin-LW, -LF, and -LA may be more toxic than microcystin-LR due to more rapid uptake kinetics. To account for this, an additional safety factor of 2 is included in the toxicology calculations (see Box A2.1).

A TDI is defined as an estimate of the intake of a substance over a lifetime that is considered acceptable without appreciable health risk. The TDIs were used to calculate maximum allowable values (MAVs) of microcystins in recreational water under the following assumptions: the proportion of microcystin intake from recreational water is 100 per cent (i.e., there are no other microcystin sources or exposures), and there is an average consumption of 100 mL of water per day (see Box A2.1).

Usually only a small proportion (less than 30 per cent) of the total microcystin load in a water body is extracellular (outside the cells), when cells are healthy (Wood et al. 2011b; 2012b). Therefore, cells can only provide an approximate measure of microcystin concentrations present in a sample. The microcystin MAVs can be translated to an equivalent worst-case cell density of *Microcystis* sp. based on toxin quota data (Table 1 and Figure 1 of this document; Box A2.1).

We have selected the highest microcystin quota measured for the calculations (5.949 g cell-1 or 5.949 × 10-6 µg cell^{-1}; excluding the data from Puddick et al. (2016a). The rationale is that this value represents the highest known risk from microcystins in New Zealand water bodies and thus will provide for a level of redundancy in the assessment of safety (i.e., use of the worst-case scenario).

The rationale for using cell counts, rather than toxin concentrations to prompt management actions is that for most practical purposes cell counting is still primarily used by water managers to detect algae/cyanobacterial-related water-quality problems. Cell counts (or biovolumes) must, however, be regarded as an indicator or 'surrogate' for a potential toxin hazard. These should be used to prompt actions, such as toxin monitoring, that are outlined in the alert levels framework (Section XXXX). The calculations given in Box A2.1 also include a temporal allocation factor (TAF). We have set this to 0.083 (8.3%). This value corresponds to an adult/child typically undertaking recreational activities through 1 month of the year. We have set the allocation factor (AF) to 100%, which assumes that that the only source of microcystins is from recreational activities (i.e., no additional toxin is consumed from drinking water or food sources).

Box A2.1: Derivation of a guideline for microcystin and cyanobacterial exposure during recreational activities over a lifetime

Tolerable daily intakes for recreational exposure to microcystins were calculated (Table A2.1) using data from a 13-week mouse study (Fawell et al. 1999) and a 44-day pig study (Falconer et al. 1994) and the following equation:

$$TDI = \frac{\text{NOAEL or LOAEL} \times AF}{\text{uncertainty factors} \times TAF} \quad (1)$$

AF: allocation factor, in this case the exposure to the toxins is assumed to come 100% from recreational activities, therefore AF = 1.0.
TAF: Temporal allocation factor, in this case exposure is assumed to occur 8.3% of the time during a year, as lakes would be used only during 1 month per year, therefore TAF = 0.083.

Table A2.1: Summary of data and uncertainty factors used to calculate TDIs for microcystins.

Study	Falconer et al. (1994)	Fawell et al. (1999)
Test animal	Pigs	Mouse
Duration	44 days	13 weeks
Material/toxin	Cyanobacterial bloom material, containing at least nine microcystin congeners but no microcystin-LR, via drinking water	Purified microcystin-LR via gavage
LOAEL (µg kg^{-1} bw)[a]	88[c]	
NOAEL (µg kg^{-1} bw)[b]		40
Uncertainty factors		
MC-Intercongener differences in toxicity	2	2
Intraspecies variability	10	10
Interspecies variability	10	10
LOAEL to NOAEL	2	–
Lifetime exposure	5	5
Sum of uncertainty factors	2000[d]	1000[d]
TDI excluding TAF (µg kg^{-1} bw day^{-1})	0.044	0.04
TDI including TAF (µg kg^{-1} bw day^{-1})	0.53	0.48

[a] LOAEL = lowest observed adverse effect level – the lowest dose at which adverse health effects are observed.
[b] NOAEL = no observed adverse effect level – the highest dose at which no adverse health effects are observed.
[c] As measured by PP2A assay (worst-case scenario).
[d] The risk scenario of a swimmer, kayaker, sailor, etc. (whether adult or child) being repeatedly but discontinuously exposed during short visits, should primarily present with liver damage as the main endpoint (point of departure for Risk Assessment). These safety factors do not incorporate an additional safety factor for tumour promotion. Incorporation of tumour promotion, and thus an additional safety factor, would be required where there is continuous exposure (e.g., via drinking water or food).

The TDIs are used to calculate maximum allowable values (MAVs) for microcystins during recreational exposure.

MAVs based on TDIs (including TAF), derived from Falconer et al. (1994):

Child = 0.53 µg kg^{-1} day^{-1} × 15 kg × 0.1 = 79 µg L^{-1} total microcystins (2)

Adult = 0.53 µg kg^{-1} day^{-1} × 70 kg × 0.1 = 370 µg L^{-1} total microcystins (3)

where:

- 0.53 µg kg^{-1} body weight per day is the TDI (including the TAF).
- 15 is the average weight of a child in kg (Equation 2) and 70 is the average weight of an adult in kg (Equation 3).
- 0.1 L is the amount of water accidentally swallowed per day.

MAVs based on TDIs (including TAF) derived from Fawell et al. (1999)

Child = 0.48 µg kg^{-1} day^{-1} × 15 kg × 0.1 = 72 µg L^{-1} total microcystins (4)

Adult = 0.48 µg kg^{-1} day^{-1} × 70 kg × 0.1 = 336 µg L^{-1} total microcystins (5)

where:

- 0.48 µg kg^{-1} body weight per day is the TDI (including the TAF).
- 15 kg is the average weight of a child (Equation 4) and 70 kg is the average weight of an adult (Equation 5).
- 0.1 L is the amount of water accidentally swallowed per day.

The child exposure guideline derived for microcystins from the Fawell et al. (1999) study (measured as total microcystins and noting that microcystin-LR is no longer considered the most toxic congener; thus a safety factor of 2 is included), provided the lowest MAV (72 µg L^{-1}) and is used to determine the *Microcystis* cell concentration for the action level (red mode) – Situation 1 guideline.

To derive a cell number that is equivalent to this toxin hazard, a microcystin cell quota of 5.949×10^{-6} µg total microcystins $cell^{-1}$ is assumed. This data is given in Section 2 of this document. Therefore, the equivalent concentrations of toxic cells of *Microcystis* sp. that are tolerable for a small child and an adult during recreational activities are:

$$\textbf{Child} = \frac{72\ \mu g\ L^{-1} \times 10^{-3}\ L\ mL^{-1}}{5.949 \times 10^{-6}\ \mu g\ cell^{-1}} = 12{,}103 \text{ cells } mL^{-1} \text{ (rounded to 12,100)} \quad (6)$$

$$\textbf{Adult} = \frac{336\ \mu g\ L^{-1} \times 10^{-3}\ L\ mL^{-1}}{5.949 \times 10^{-6}\ \mu g\ cell^{-1}} = 56{,}480 \text{ cells } mL^{-1} \quad (7)$$

where:

- 72 µg L^{-1} is the MAV guideline (Equation 4) for cyanobacterial exposure in children (Equation 6), and 336 µg L^{-1} is the MAV guideline (Equation 5) for cyanobacterial exposure in adults (Equation 7).
- 5.949×10^{-6} µg $cell^{-1}$ is the toxin cell quota for total microcystins.

Based on these calculations we recommend that 12,100 cells mL^{-1} is used as the Action Level (red mode) for *Microcystis* sp.

Appendix 3. A quantitative health risk assessment framework for human exposure to anatoxins produced by *Microcoleus*.

This contribution arises from suggestions made in a report advising on the possible development of a benthic cyanobacteria attribute for the National Policy Statement for Freshwater Management (Wood et al. 2015). That report raised the possibility of using 'Monte Carlo' models to better inform health risk assessment. The aim of this section was to further develop this concept and to highlight knowledge gaps (see Section 5). Introductory material to this Appendix is provided in Section 5.

A 3.1 Dose-response functions for discrete variables

For reasons that will become obvious, we first present dose-response functions for discrete quantities (viruses), such as used in QMRA (Quantitative Microbial Risk Assessment - Haas et al. 1999, McBride 2005). Figure A3.1 displays the generic form of typical dose-response functions, relating dose to the probability of infection. Both single-parameter and double-parameter curves are shown.

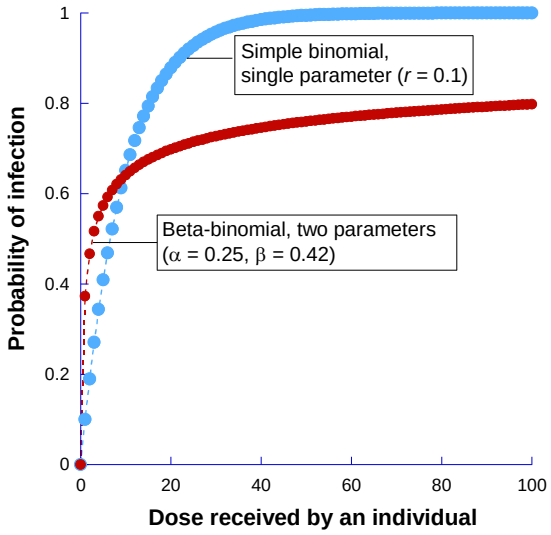

Figure A3.1. Single-parameter and double-parameter dose-response functions.[3,4,5]

[3] The single-parameter (simple-binomial) function displayed in Figure A3.1 is $Pr_{inf} = 1 - (1 - r)^i$ where r is the probability that a pathogen survives the host's defences and reaches an infection site and i is the dose received by an *individual*. In risk modelling, this equation is applied to multiple individuals on each exposure occasion. If only the mean dose is known for each exposure occasion (as is common in clinical trials) these are assumed to be randomly distributed and so follow a Poisson distribution. In that case we obtain the 'simple exponential function) $Pr_{inf} = 1 - e^{-rd}$, where d is the *mean* dose on each exposure occasion.

[4] The two-parameter (beta-binomial) function treats r as a variable, not a constant, and assumes that it follows a beta distribution (with shape and location parameters α and β). For an individual's dose, the resulting equation is $Pr_{inf} = 1 - B(\alpha, \beta+i)/B(\alpha, \beta)$, where B is the standard beta function. It can be evaluated in Excel. For a mean dose (d) the equation is $Pr_{inf} = 1 - {}_1F_1(\alpha, \alpha + \beta, -d)$, where ${}_1F_1$ is 'Kummer's confluent hypergeometric function'. This function is difficult to evaluate (it cannot be done in Excel) and so is often approximated as the 'beta-Poisson' function, $Pr_{inf} = 1 - (1 + d/\beta)^{-\alpha}$, but with the (sometimes overlooked) requirement that $\beta >> 1$ and $\alpha << \beta$.

[5] An extra shift-parameter would be required to generate a left-tail on these curves.

Note the key features of these curves: the simple binomial curve is of decreasing-slope exponential shape, reaching close to 100% infection probability once a dose of about 40 particles is received. In contrast, the beta-binomial curve starts off steeper but then flattens out such that it doesn't get close to 100% infection probability until much larger does are received. It therefore more adequately applies to situations where there is differential immunity.

It is important to note that dose response curves carry (at times substantial) uncertainty, as depicted on Figure A3.2., showing the uncertainty interval for a dose of 20 particles.[6]

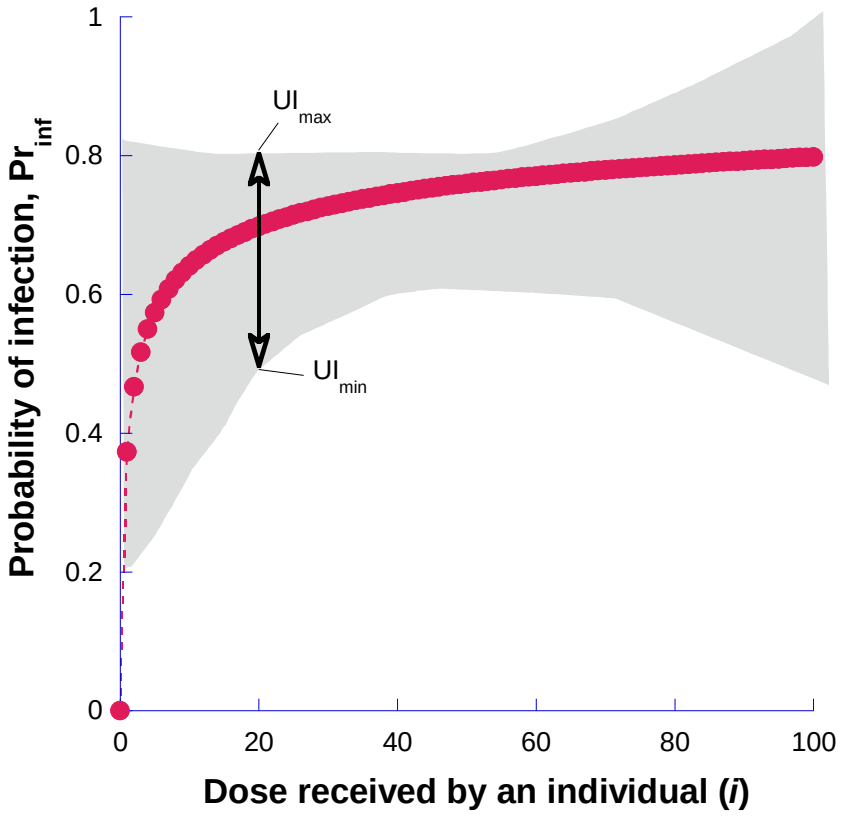

Figure A3.2. Uncertainty intervals around the beta-binomial dose-response curve for a dose of 20: 'UI' denotes Uncertainty Interval.

[6] Strictly, these are Bayesian 'credibility intervals' (with an impartial prior distribution), because probability statements are being made about a *particular* dose-response curve whereas the standard 'frequentist' probability paradigm makes statements about the *average* of many intervals.

A3.2 Calculating risk profiles using Monte Carlo models

Calculating risk profiles, rather than a single risk number, calls for some form of iteration, producing multiple risk levels and their probability of occurrence. In that way informed decisions can be made about risk thresholds, particularly concerning selection of the appropriate burden-of-proof.

A3.2.1 First-order Monte-Carlo models

The calculation procedure for a first-order risk model is shown on Figure A3.3, in which *ranges* of values of exposure duration, ingestion rate and virus concentrations have been given as input to the model. These account for variability (not uncertainty). Statistical distributions are also defined over those ranges from which repeated random samples are drawn: that is, we have an iterative algorithm.[7] Also, a dose-response function has been used to calculate risk for each iteration.

[7] Each iteration is equivalent to the spinning of a roulette wheel at the Monte Carlo casino.

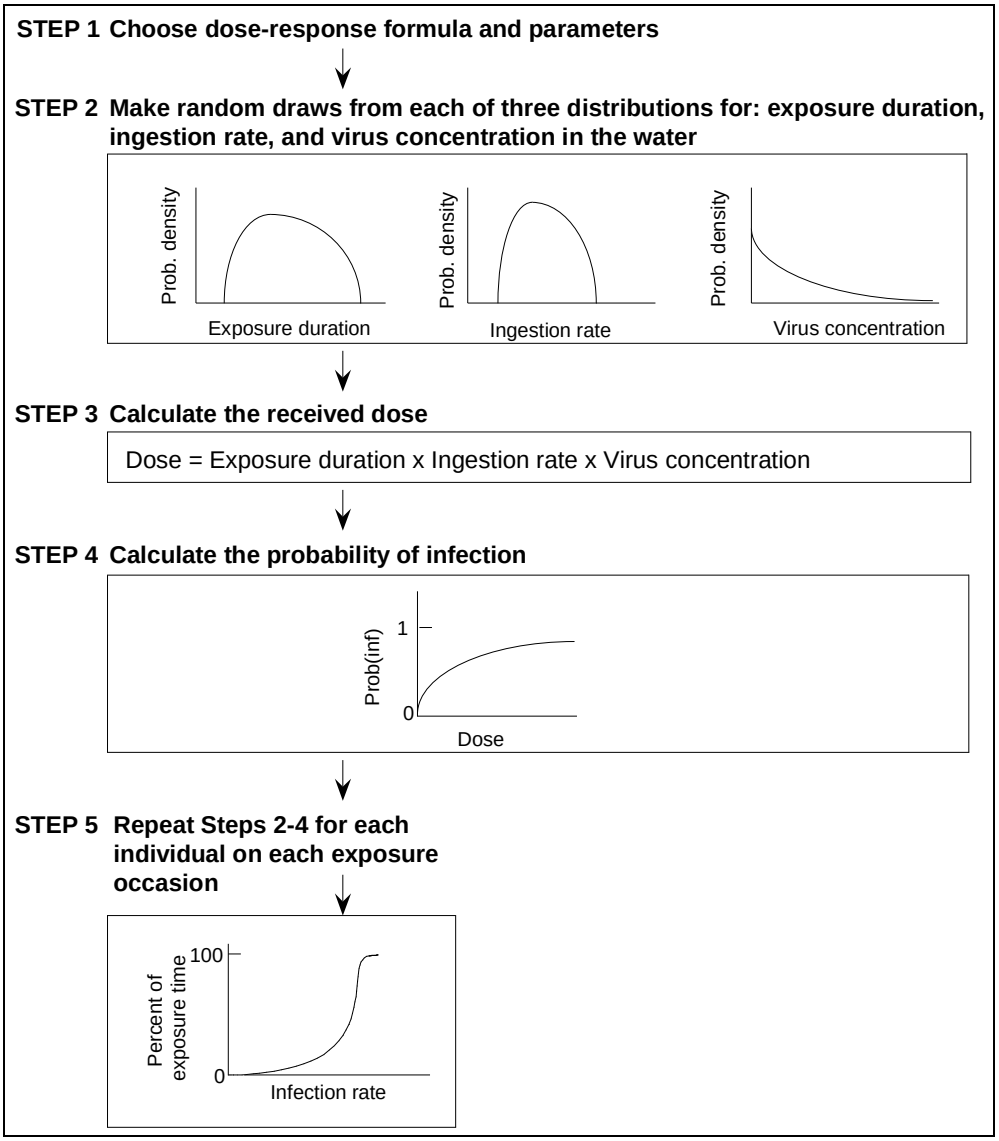

Figure A3.3. First-order Monte Carlo calculation procedure.

A3.2.1 Second-order Monte Carlo Models

Note however, the calculated risk profile in Step 5 is uncertain, because our knowledge of dose-response parameters is limited. To some extent they will be wrong, perhaps substantially so.[8] So, whilst we have accounted for variability in model inputs (via ranges of exposure duration, ingestion rate, virus concentration) we have not accounted for uncertainty. That's where the second-order approach comes into

[8] 'All models are wrong, some are useful'—generally attributed to the statistician George Box.

play, as depicted on Figure A3.4.[9] Note the extra panel its south-east corner (Step 6).[10]

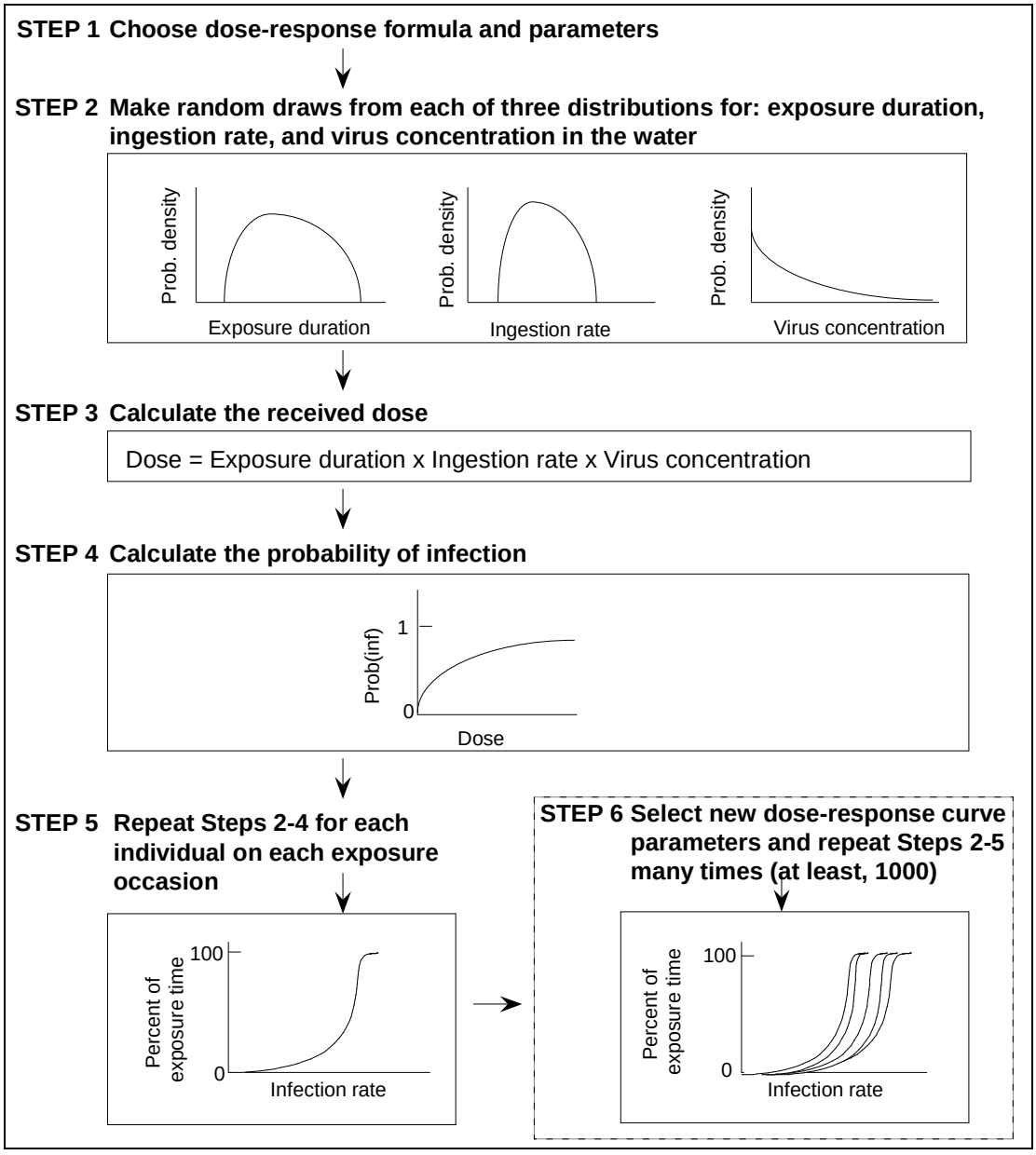

Figure A3.4. Second-order Monte Carlo calculation procedure.[11]

[9] Second-order Monte Carlo has not been adopted often. An exception is Corsi et al. (2015) on swimmers' health risk: 'The computational approach included a two-dimensional Monte Carlo simulation with each simulation consisting of 10 000 iterations in the variability dimension (e.g., swimming ingestion rate) and 1000 iterations in the uncertainty dimension (e.g., dose−response parameters).'

[10] This distinction between variability (in inputs) and uncertainty (in parameters) is somewhat simplistic (but helpful). Uncertainty can also include: absence of specific information; representativeness of experimental data, measurement uncertainty, and selection of statistical distributions (WHO 2016).

[11] A quasi second-order approach has sometimes been used. Its procedure is as given on Figure A3.4, except that on each iteration a random sample is taken from the uncertainty interval, rather than from the dose-response curve.

Comparing Figures A3.3 and A3.4 we see that latter carries a penalty: the computation demand has increased considerably—each first-order iteration is embedded in a higher-level iteration.[12]

So, which profile in the dashed box (in Step 6) should we use? That choice is guided by considerations of burden-of-proof, in three categories (McBride 2005):

- The **face-value** (or **even-handed**) stance would choose a profile in the middle of the range shown in Step 6.
- The **precautionary** stance would take a profile at or near the right-most profile, recognising that the true risk could, by 'bad luck' be higher than the face-value result.
- The **liberal** stance would take a profile at or near the left-most profile, recognising that the true risk could be lower than the face-value result ('good luck').

A3.3 Who decides?

The choice of stance to the burden-of-proof is not a matter for science alone—even though the precautionary approach generally holds sway in matters of public health protection. The choice is typically made by a policy development team (which should include at least some of the risk assessors), but this development should be informed by science, not instructed by science.

A3.4 Dose-response functions for continuous variables—a toxin

A promising approach is to cast toxin data into a number of 'bins', as in the width of bars on a histogram. In that way all the preceding calculation procedures apply, merely by replacing "virus" by "toxin". Note that one piece of extra information is required: the width of each bin.[13] The bin number is then completely identical to the doses on the X-axis of Figure A3.2.

A considerable advantage of such an approach is that the theoretical underpinning of the dose-response curve provides confidence in extrapolating beyond the range of doses used to calibrate it.

This appears to be a novel approach in that the statistical models for environmental toxicology are dominated by empirical relationships, e.g., polynomials, cumulative

[12] These second-order calculations are not possible in Excel and should instead be carried out using specialist R code (NIWA has such code).

[13] This 'floor and ceiling' method has been used in at least one published risk assessment (McBride et al. 2005, pp. 82–85).

distribution functions, logistic curves (Piegorsch & Bailer 1997). But it seems more appealing.

A3.5 Conclusion

The QMRA (Monte Carlo) approach allows us to answer ‘what if?’ questions. It explicitly accounts for variability (exposure duration, ingestion rate, toxin concentration) and uncertainty (regarding dose-response) and we can change any or all of these and thereby assess the sensitivity of the model to such changes. Using a second-order Monte Carlo approach will facilitate the selection of an appropriate burden-of-proof (once this study has been completed) and its results are discussed with a policy development team.