Evaluation of the Evidence for the Recreational Water Quality Guidelines by the National Health and Medical Research Council
Section: Cyanobacteria and Algae

Evidence Evaluation Report

Report to the Recreational Water Quality Advisory Committee of the National Health and Medical Research Council

Australis Water Consulting

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Australis Water Consulting Pty Ltd
ABN: 12 621 158 487
T: +61 411 521 570
E: mike.burch@australiswater.com.au

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Contact for this report:	**Mike Burch** **T: +61 411 521 570** **E: mike.burch@australiswater.com.au**	Australis Water Consulting
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Abbreviations

ACT	Australian Capital Territory
ANZECC	Australia and New Zealand Environment and Conservation Council
AWC	Australis Water Consulting
BMAA	β-methylamino-L-alanine
CASP	Critical Appraisal Skills Programme
CDC	Centers for Disease Control and Prevention
CI	confidence interval
Czech	Czech Republic
d	day
EFSA	European Food Safety Authority
ELISA	enzyme-linked immunosorbent assay
fg	femtogram
g	gram
GI	gastrointestinal infection
GIS	geographical information system
GRADE	Grading of Recommendations Assessment, Development and Evaluation
GV	guideline value
h	hour
HABs	harmful algal blooms
HACCP	Hazard Analysis and Critical Control Points
kg	kilogram
L	litre
LC-MS	liquid chromatograph-mass spectrometry
LOAEL	lowest observed adverse effect level
LOD	limit of detection
LPS	Lipopolysaccharide
MeSH	Medical Subject Headings
m	metre
max	maximum
min	minute
minn	minimum
mg	milligram
ng	nanogram

NHMRC	National Health and Medical Research Council
NOAEL	no observed adverse effect level
NRMMC	Natural Resource Management Ministerial Council
NSW	New South Wales
OEHHA	Office of Health Hazard Assessment (California, USA)
OHAT	Office of Health Assessment and Translation
OR	odds ratio
PECO	Population Exposure Comparator Outcome
PCR	polymerase chain reaction
PFT	pulmonary function test
QMRA	quantitative microbial risk assessment
RfD	Reference Dose
RoB	Risk of Bias
RWQAC	Recreational Water Quality Advisory Committee (NHMRC) (termed 'the Committee')
SEQ	South-East Queensland
sp./spp.	species
Tas	Tasmania
tiab	title and abstract
TDI	tolerable daily intake
µg	microgram
UF	uncertainty factors
UK	United Kingdom
UNEP	United Nations Environment Programme
UNESCO	United Nations Educational, Scientific and Cultural Organization
USA	United States of America
USEPA	United States Environmental Protection Agency
Vic	Victoria
WA	Western Australia
WBDOSS	Waterborne Disease and Outbreak Surveillance System (CDC)
WHO	World Health Organization
WSAA	Water Services Association of Australia
y	year

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1 Executive Summary

1.1 Background

This Evidence Evaluation Report together with the associated Technical Report comprise a narrative review for the topic of Cyanobacteria and Algae to inform the update to the NHMRC *Guidelines for Managing Risks in Recreational Water* (2008).

The Evidence Evaluation Report contains:

- Background
- Purpose
- Methodology (summary only)
- Results (summary only)
- Discussion
- Conclusions

The Methodology section in the Evidence Evaluation Report provides a brief outline and summary of the approach only, with the full details being provided in Section 2 of the Technical Report.

The Results section in the Evidence Evaluation Report gives a summary of the findings represented in a PRISMA flow diagram. This outlines the results from the identification and screening of the literature and assessment for study quality to identify and evaluate evidence from the studies. Full details of all results are provided in Section 3 and in the Appendices of the Technical Report.

This review was structured around answering a series of specified research questions in relation to the sub-topic of Cyanobacteria and Algae. The questions comprised one primary question and five secondary questions.

1.2 Research Questions

The review set out to answer one Primary Question and five Secondary Questions.

Primary Question

What is the risk of any adverse health outcome for water users from exposure to cyanobacteria or algae in recreational water?

Secondary Questions

1. What are the indicators/surrogates of this/these hazard/s? What are the advantages and disadvantages of using surrogates versus monitoring specific toxins?

2. What guidelines, guidance and implementation practices are in place in comparable countries to minimise or manage this/these hazards and risks/s?

3. What are the specific exposure scenarios that might increase risk for sub-populations (e.g., infants playing in shallow waters in presence of benthic mats, water skiers/beach goers inhaling aerosolised cells/toxins) and how are these managed by other organisations?

4. What is the extent of evidence of adverse effects due to recreational exposure to marine cyanobacteria or algae (e.g., skin irritation due to *Lyngbya majuscula* or inhalation-related symptoms due to cells/toxins aerosolised by wave action, boats, jet-skis, etc.)? Are there any existing guidelines that address these exposure risks?

5. Much of the evidence for freshwater benthic cyanotoxin production in Australia is anecdotal and often linked to dog deaths following swimming in water bodies (e.g., at least 4 dog deaths in Lake Burley Griffin). It would be useful to try to collate the grey literature evidence to provide a clearer picture of the extent of any risk.

Several additional supplementary searches were carried out to explore evidence related to topics (other cyanobacterial components) identified by the Recreational Water Quality Advisory Committee (the Committee). These were an examination of the potential adverse health effects of the cyanobacterial components, endotoxins/LPS and the amino acid, β-methylamino-L-alanine (BMAA) in a recreational exposure setting. A specific search was also carried out to assess the relevance of cyanobacteria and algae to the public health of Australian indigenous people.

1.3 Methods

The review process to answer the research questions included four components. Each component had a different methodological approach selected to optimise information collection and evidence evaluation to answer the specific question. These components were:

1. A conventional systematic literature search and review of primary studies to address the Primary Question about the risk of adverse health outcomes from exposure to cyanobacteria and algae in recreational water.

2. A review of selected reviews to address Secondary Question 1 related to the indicators/surrogates of hazards posed by cyanobacterial toxins.

3. A review of guidelines, guidance, and implementation practices in place in comparable countries from grey literature obtained from organisational or jurisdictional agency websites to address Secondary Question 2.

4. A systematic review of selected primary studies and other reports derived from the search to answer the Primary Question, and additional supplementary searches and other sources specifically related to Secondary Questions 3, 4 and 5.

The search strategy developed to find and select the evidence for the Primary Question involved a number of steps. The databases PubMed® and Scopus® were searched to capture the conventional peer-reviewed published literature. The searches employed advanced search techniques which involved the development of a structured search that was able to capture literature based upon concepts of cyanobacteria/algae/toxins combined with both water-based recreation and health outcomes for the freshwater and marine environments. The review considered papers and reports published from 2006 onwards and search results were restricted to English language publications only.

A range of other publications were also assessed to source reports and publications that would also provide evidence that may be relevant to answer the questions. This was done by citation searching which involved review of the bibliography of selected publications within the date range for the review (2006-2021).

In addition to the database searches, a grey literature search was conducted using the Google search engine to identify studies not in the published, peer-reviewed literature and to source guideline values used for cyanobacteria in recreational freshwater and marine water in other jurisdictions. These searches were also carried out to gather information specifically required to address Secondary Question 2.

The searches were screened to select studies to include for full-text review. These studies were critically appraised for relevance and suitability for the update of the Guidelines. The aim of full-text review was to identify primary studies that could be included in the assessment for study quality by risk of bias assessment using an adaptation of the OHAT risk of bias tool (OHAT, 2019). This included assessing the certainty of the body of evidence where appropriate. The process for identification, screening and eligibility assessment of literature used for the evidence evaluation and review was summarised in a Prisma Flow Diagram.

1.4 Results

The results of the searches in PubMed® and Scopus® databases and the records identified from other sources were combined to produce 1,693 studies. After removal of duplicates a total of 1,237 records were screened in a two-stage process to select papers for full-text review. Following screening, the number of records assessed by full-text review for eligibility to answer the Primary Question, for both freshwater and marine cyanobacteria and algae, was 143. This was comprised of 89 freshwater studies and 54 marine studies. The full-text review identified 51 studies that were primary studies. However, from these, only the human exposure studies were included in the risk of bias assessment. These consisted of 11 freshwater and 22 marine studies.

1.5 Discussion and Conclusions

1.5.1 Primary Question

What is the risk of any adverse health outcome for water users from exposure to cyanobacteria or algae in recreational water?

The literature search and subsequent screening identified 51 primary studies to further assess for answering the Primary Question. From these studies, however, only the human exposure studies were included for further assessment of study quality by risk of bias assessment. These were comprised of 11 freshwater and 22 marine studies.

The freshwater studies consisted of 5 cohort, 3 observational and 3 case studies. The marine studies consisted of 12 cohort, 4 observational and 6 case studies. There were two Australian investigations in the freshwater primary studies, and both were epidemiological studies related to exposure to cyanobacteria in recreational waters (Pilotto *et al.,* 1997; and Stewart *et al*., 2006). The study by Pilotto *et al*., (1997) was included in the review although it was outside the date range specified (2006-2021). This was because it was a highly relevant Australian epidemiological study designed at the time to gather information to inform exposure to toxic cyanobacteria in recreational water environments. There were also only two Australian-based investigations within the marine primary studies. These were both related to health effects associated with exposure to the marine cyanobacterium *Lyngbya majuscula* in Queensland (Osborne *et al.*, 2007; and Osborne and Shaw, 2008).

The risk of bias assessment is designed principally for the assessment of the validity of studies for the evaluation of clinical outcomes. The type of studies reviewed here were either field-based observational and case studies, or cohort studies associated with environmental contaminants, so not all of the usual bias domains were applicable.

The conclusion from the risk of bias assessment was that there was a clear and consistent pattern in the types of bias in all of the marine and freshwater studies assessed. The majority of the studies suffered from shortcomings in some of the major bias domains including:

- failing to include suitable comparators or control groups

- not considering potential confounders (i.e., factors or causes for adverse outcomes other than cyanobacteria, algae or toxins)
- not adequately accounting for exposure characterisation for these organisms and compounds in an environmental setting
- many studies had a reliance on self-reporting as part of outcome assessment

These limitations in design reflect that none of the studies assessed were designed as randomised controlled trials or similar clinical trials. Only about 50% of both the freshwater and marine and studies were cohort studies, with the remainder being observational and case studies.

Consequently, all of the primary studies assessed for study quality by risk of bias assessment were regarded as having significant weaknesses in study quality across multiple bias domains. The conclusion was that the body of evidence overall was rated as having a "definitely high risk of bias". This led to the conclusion that there was insufficient confidence in the studies. As a consequence, there was insufficient information to determine if there were any further reasons to upgrade the certainty of the overall body of evidence from 'very low certainty' using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) framework.

These shortcomings considered together led to the conclusion that there was insufficient confidence in the findings of the available studies. It is worth noting that methods and approaches for systematic reviews of environmental health evidence is still an area of research and development, and further modification of the available frameworks and tools is beyond the scope of services required for this review.

Despite this, the review clearly identified a wide range of studies where exposure to freshwater cyanobacteria and marine algae and their toxins in recreational waters caused adverse health outcomes ranging from respiratory, gastro-intestinal and irritation effects.

1.5.2 Secondary Questions

Secondary Question 1 - Indicators/Surrogates

The surrogates that are used widely for monitoring cyanobacteria and cyanotoxins are cyanobacterial cell counts, biovolume and the measurement of chlorophyll-a and phycocyanin pigments. The surrogate most-commonly used in guidelines is cell counts followed by chlorophyll-a and biovolume. Phycocyanin is not used in any guideline.

While cell counts are widely used in guidelines, a significant drawback for this measurement is the potentially long delay required for providing results due to the time required for sample collection and processing. Another disadvantage of cell count measurement is associated with the diversity in the range of shapes and sizes of cyanobacterial cells (Wood *et al.*, 2008 in Health Canada, 2020). This can result in very large differences in estimates of cyanobacterial biovolume and hence toxin quantity for equivalent cell count values of different species. In addition, the high variability in toxin cell quotas (toxin content per cell) between individual clones within natural populations is a major source of uncertainty. These factors are all potential limitations for the use of cell counts as a surrogate for cyanotoxin monitoring.

Cyanobacterial biovolume is a more accurate indicator of cyanobacterial biomass than total cell counts. Cyanotoxin concentrations have also been found to relate more directly to cellular biomass than to cell numbers. The World Health Organization (WHO) have discontinued the use of cell numbers in the setting of guidance or Alert Levels for recreational exposure in their most recently issued guidelines and moved to the use of biovolumes. This change reflects experience that the use of

cell number thresholds may lead to undue restrictions of recreational use if the dominant cyanobacteria are species with very small cells. This is because toxin concentrations relate to biomass rather than cell numbers.

Chlorophyll-a has frequently been used as an index for eutrophication. It can be used as part of a cyanobacterial alert system to trigger further investigation and action. The use of monitoring by pigment fluorescence, of either chlorophyll or phycocyanin, can potentially be useful to provide continuous and real time data of cyanobacterial hazards. This is particularly the case when using on-line probes and after calibration for the local population.

Molecular methods for monitoring of microorganisms in environmental samples can be used to generate information on the presence of potential toxins in short time frames. These methods detect specific genes that identify cyanobacterial species as well as the presence of the toxin-producing genes. It is suggested that these molecular methods have a role as a screening tool to determine the presence of cyanobacterial species and to provide an indication of the potential for toxin production, particularly as the use of the technology becomes more widespread.

It must be noted that none of the surrogates will provide an indication of free dissolved toxin in water that has been released or liberated from cells. This can be substantial after a bloom has collapsed and will be unknown unless toxin is measured directly.

Irrespective of which method is used, it is strongly recommended that all surrogate measurements need to be locally calibrated against toxin concentration.

Secondary Question 2 - Guidelines/Guidance and Implementation

Guideline Derivations: The review of the published guidelines found that the majority of cyanotoxin guidelines have been derived following a conventional regulatory model using experimental animal studies rather than human exposure data derived from field studies. This approach uses laboratory animal toxicological studies with pure compounds or characterised cyanobacterial extracts combined with an uncertainty or safety factor approach to determine TDIs or RfDs and subsequent use of allocation factors. The rationale for adopting the animal model approach is related to the overall limitations of interpreting and applying data from the the available human exposure studies. The collation and assessment of all available derivations for cyanotoxin guidelines in different jurisdictions highlighted the wide variation in approach, which resulted in the observed differences in final guideline values. These variations included the choice of animal model, different approaches to calculation of the TDI or RfD, through to the choice of uncertainty factors applied to these studies and the use of local conventions for body weight, water ingestion volumes and duration of exposure.

Guidelines and Guidance: The review found recreational water quality guidelines for freshwater cyanobacteria and cyanobacterial toxins for 42 jurisdictions. These were from 17 jurisdictions that represented international and national agencies and 25 jurisdictions within the USA, which were assessed separately. Across these jurisdictions and by class, the most frequently issued guideline was for microcystin (34), followed by cylindrospermopsin (19), anatoxin-a (16), saxitoxin (10) and nodularin (1). In relation to surrogates or other indicators, chlorophyll-a was used in 7 guidelines and biovolume was used in 8 guidelines. The presence of cyanobacterial scum was used as an Action level within 18 guidelines. The most authoritative recent guidelines with comprehensive assessments and supporting information are those released by WHO (2020), and the USEPA (2019a).

The review found that most Australian states have continued to use the NHMRC (2008) guideline of 10 (µg/L) for microcystin, except for SE Queensland who have adopted 2-tier system at the Action

level for 5 classes of toxins (microcystin, cylindrospermopsin, anatoxin-a, saxitoxin and nodularin) (Veal *et al*., 2018). International guidelines vary over a relatively wide range. The most recent guidelines released by WHO (2020) for four classes of toxin (defined variously as 'guidelines', 'provisional guidelines' and 'health-based reference values') have the following values, microcystin: ≥24 µg/L; cylindrospermopsin: ≥6 µg/L anatoxin-a and analogues: ≥59 µg/L and saxitoxins: ≥30 µg/L. National guidelines in non-US jurisdictions have yet to take a lead from these recently published values and have earlier issued guidelines, usually for microcystin only, in the range of 10 to 25 µg/L.

Guidelines or Action levels in US jurisdictions are highly variable and have a range of definitions based across jurisdictions which make them difficult to compare exactly. The most recent the USEPA (2019a) guidelines published are 'human health recreational ambient water quality criteria' or 'swimming advisories' for 8 µg/L microcystins of 15 µg/L for cylindrospermopsin. Many individual US states and jurisdictions have guidelines (Action levels) for microcystins in the range of 6 to >2,000 µg/L. Many states follow the USEPA advisory for cylindrospermopsin of 15 µg/L as an Action level while the most variation is seen for anatoxin-a which range from 1 to 300 µg/L as an Action level.

New Zealand is currently the only country or jurisdiction that specifically considers guidance for the hazard posed by benthic cyanobacteria.

This review found that Australian states with marine guidelines (NSW and WA) primarily follow the NHMRC (2008) guideline of ≥10,000 cells/L (Tier 2) for the dinoflagellate *Karenia brevis* and advice for the visible presence of 'moderate', or 'high' numbers of the marine cyanobacterium *Lyngby majuscula*. The only other international guideline for comparison to Australia are the Action levels of >100,000 cells/L – 1,000,000 cells/L (Medium) and >1,000,000 cells/L (High) for *Karenia brevis* from Florida (USA) related to medium and high likelihood or risk of respiratory irritation. These are one to two orders of magnitude greater than the current Australian advice.

Secondary Question 3 - Exposure Scenarios and Risk for Sub-populations

The specific exposure scenarios leading to an increased risk for sub-populations that have been identified include infants playing in shallow waters in the presence of cyanobacterial blooms, and exposure of sub-groups such as asthmatics and workers such as lifeguards on beaches. These groups are considered more vulnerable than the general population when exposed to aerosolised marine algal or cyanobacterial toxins.

Organisations manage the increased risk for these sub-populations in multiple ways. Firstly, within the development of regulations, risk is accounted for by the approach of selecting body weight and water ingestion volumes relevant to children and by the use of uncertainty factors in guideline derivation (see Secondary Question 2). Secondly, agencies use a range of strategies to guide and influence the behaviour of recreational water users to avoid the hazard. Options for this range from informing users by creating awareness and enabling individual responses to bloom situations to temporarily banning waterbody use for the duration of the bloom.

Secondary Question 4 - Evidence of Adverse Effects from Marine Cyanobacteria and Algae

The review found 22 primary studies regarding evidence of adverse health effects due to recreational exposure to marine cyanobacteria. Most of these studies (12/22: 55%) related to exposure to brevetoxins, often via aerosols from the marine dinoflagellate *Karenia brevis* associated with red tides in Florida, USA. There were three studies related to dermal effects associated with exposure to the marine cyanobacterium *Lyngbya majuscula*, of which two were Australian studies in Queensland. All of these marine primary studies were assessed for study quality by risk of bias assessment and found

to have a range of sources of bias. They were considered as having significant weaknesses in study quality across multiple bias domains.

In relation to existing guidelines that address these exposure risks, only four recreational water quality guidelines for marine algae and cyanobacteria were found. No guidelines for marine algal or cyanobacterial toxins were found. It is important to note that no national or local jurisdiction has yet developed any guidelines for specific marine toxins for recreational water quality in the marine environment. The four existing guidelines consisted of cell number guidelines for the dinoflagellate *Karenia brevis* from Florida, USA, and cell number guidelines for dinoflagellates and various marine cyanobacteria from three Australian sources.

Secondary Question 5 - Evidence for Risk from Benthic Cyanobacteria and Cyanotoxins

The review found a large body of evidence from primary studies that confirmed the relationship between dog deaths and exposure to both freshwater benthic and planktonic cyanobacteria. Most of the studies reported ingestion as the exposure pathway, with one also reporting dermal exposure. A high proportion of the animal primary studies recorded death as the end point, so it was often possible, by veterinary post-mortem examination, to provide strong evidence for a causal link between the exposure to cyanobacteria and the observed health outcomes for the animals. The evidence suggested that animals are susceptible to poisoning by cyanotoxins and can become very ill, or potentially die, due to exposure in recreational water environments. It is not clear whether dogs are any more sensitive than other animals or that they simply have opportunities for exposure to very high concentrations. Exposure in dogs is unpredictable because they may consume both scum at the shoreline and drying algal mats that wash up on shore. Anecdotal evidence indicates that dogs may be attracted to consume cyanobacterial benthic mat material due to its strong odour. They are also exposed by cleaning cyanotoxin-containing material from their coats after being in the water.

A high-level summary of findings for both the Primary Question and Secondary Questions is given at the end of the Executive Summary.

1.5.3 Additional and Supplementary Searches

Endotoxins/LPS: The supplementary search for Endotoxins/LPS related to the Primary Question indicated that there is limited evidence for the assessment of the potential significance of cyanobacterial lipopolysaccharides to determine their relevance for adverse human health effects in a recreational water exposure setting.

BMAA: The supplementary search for the potentially toxic amino acid BMAA, combined with terms for cyanobacteria to determine the extent of literature on this compound, returned a moderate number of publications (399 results; 2006-2020). These were not screened or considered separately from the assessment undertaken to answer the Primary Question for the review. The significance of the compound for human health is currently controversial.

Assessment of the Significance of the Topic for Indigenous Health: The searches for this review were combined with an indigenous search term string to determine the relevance of this topic to public health of Australian indigenous people/s. The outcome was that no results were found that related to indigenous studies or health outcomes and the Primary Question.

1.5.4 Implementation of Guidelines

A range of resources was identified during the search of grey literature. These are considered to have potential value for organisations that are required to implement recreational guidelines, or for others that may have to deal with the range of impacts on both humans and animals (e.g., physicians,

veterinarians, dog owners, farmers, etc.). The material covers the following topics: local action plans, field identification of cyanobacteria, fact sheets about cyanobacterial blooms, sampling and monitoring advice, and advice for veterinarians, dog owners, physicians, general homeowners, irrigators, and livestock owners.

1.5.5 High-Level Summary of Findings for the Primary and Secondary Questions

Primary Question – High-Level Summary of Findings

Primary Question: ***What is the risk of any adverse health outcome for water users from exposure to cyanobacteria or algae in recreational water?***
Search Results and Study Types
• The literature search identified 51 primary studies to assess for the Primary Question. From these, 11 freshwater and 22 marine studies involving human exposure (33 studies) were further assessed for study quality by risk of bias assessment. The freshwater studies consisted of 5 cohort, 3 observational and 3 case studies and the marine consisted of 12 cohort, 4 observational and 6 case studies. • There were two Australian investigations which were epidemiological studies in the freshwater primary studies (Pilotto *et al.,* 1997; Stewart *et al*., 2006). and two Australian-based investigations within the marine primary studies (Osborne *et al.*, 2007; Osborne and Shaw, 2008).
Quality of Studies
• All of the primary studies assessed for study quality by risk of bias assessment were regarded as having significant weaknesses in study quality across multiple bias domains.
Quality of Body of Evidence
• The risk of bias assessment concluded that the body of evidence overall was rated as having a "definitely high risk of bias". These shortcomings considered together led to the conclusion that there was insufficient confidence in the findings of the available studies. • There was insufficient information to determine if there were any further reasons to upgrade the certainty of the overall body of evidence from 'very low certainty' using the GRADE system.
Evidence of adverse health outcomes from exposure in recreational water
• The review clearly identified a limited range of studies where exposure to freshwater cyanobacteria and marine algae and their toxins in recreational waters caused adverse health outcomes ranging from respiratory, gastro-intestinal and irritation effects. • Selected examples of some of the primary studies that were notable for showing a relationship between exposure to freshwater cyanobacteria and/or cyanotoxins, and marine algae and/or their toxins and adverse health outcomes were: Freshwater Studies: Pilotto *et al*., (1997), Vidal *et al*., (2017), Giannuzzi *et al*., (2011). Marine Studies: Backer *et al*., (2003), Fleming *et al*., (2005), Lin *et al*., (2016), Milian *et al.*, (2007), Backer *et al*., (2005). • Many of these studies, as for most of the primary studies reviewed, suffered from design deficiencies related to a lack of control groups, confounding, inadequate exposure characterisation for either organism types, toxins or associated biomarkers that did not correspond with the exact exposure site and time. There were also limitations with regard to the type and degree of health assessment.

Secondary Questions – High-Level Summary of Findings

Secondary Question 1: Indicators/Surrogates

What are the indicators/surrogates of this/these hazard/s? What are the advantages and disadvantages of using surrogates versus monitoring specific toxins?

- Surrogates that are used widely for monitoring cyanobacteria and cyanotoxins are cyanobacterial cell counts, biovolume and the measurement of chlorophyll-a and phycocyanin pigments
- The surrogate most-commonly used in guidelines is cell counts followed by chlorophyll-a and biovolume. Phycocyanin is not used in any guideline
- Although cell counts are widely used in guidelines, they have disadvantages that are potential limitations as a surrogate for cyanotoxin monitoring. These include:
    - the potentially long delay required for providing results due to the time required for sample collection and processing
    - The diversity in the range of shapes and sizes of cyanobacterial cells can result in large differences in estimates of cyanobacterial biovolume and hence toxin quantity for equivalent cell count values of different species
    - the high variability in toxin cell quotas (toxin content per cell) between individual clones within natural populations is a major source of uncertainty
- Cyanobacterial biovolume is a more accurate indicator of cyanobacterial biomass than total cell counts
- Pigment monitoring by fluorescence (of either chlorophyll or phycocyanin) can be useful to provide continuous and real time data of cyanobacterial hazards.
- Molecular methods for monitoring of microorganisms in environmental samples can be used to generate information on the presence of potential toxins in short time frames.
- None of the surrogates will provide an indication of free dissolved toxin in water that has been released from cells.
- It is recommended that all surrogate measurements need to be locally calibrated against toxin concentration.

Secondary Questions – High-Level Summary of Findings (continued)

Secondary Question 2: Guidelines/Guidance and Implementation

What guidelines, guidance and implementation practices are in place in comparable countries to minimise or manage this/these hazards and risks/s?

Guidelines and Guidance

- The majority of cyanotoxin guidelines have been derived with a conventional regulatory model using experimental animal studies rather than human exposure data from field studies.
- The reason for this relates to the overall limitations of interpreting and applying the data of variable quality from the human exposure studies
- There is wide variation in the approach used in different jurisdictions for derivation of cyanotoxin guidelines which results in significant differences in final values
- The review found recreational water quality guidelines for freshwater cyanobacteria and cyanotoxins for 42 jurisdictions, comprised of 17 jurisdictions from international and national agencies and 25 jurisdictions within the USA
- Across these jurisdictions the most frequently issued guideline was for microcystin (34), followed by cylindrospermopsin (19), anatoxin-a (16), saxitoxin (10) and nodularin (1)
- In relation to surrogates, chlorophyll-a was used in 7 guidelines and biovolume in 8 guidelines
- The most recent guidelines released by WHO (2020) for four classes of toxin (defined variously as 'guidelines', 'provisional guidelines' and 'health-based reference values') have the following values - microcystin: $\geq$24 µg/L; cylindrospermopsin: $\geq$6 µg/L anatoxin-a and analogues: $\geq$59 µg/L and saxitoxins: $\geq$30 µg/L
- The most recent the USEPA (2019a) guidelines published are 'human health recreational ambient water quality criteria' or 'swimming advisories' for 8 µg/L microcystins of 15 µg/L for cylindrospermopsin
- New Zealand is currently the only country or jurisdiction that specifically considers guidance for the hazard posed by benthic cyanobacteria

Implementation

- A range of resources was identified that have potential value for agencies required to implement recreational water guidelines

Secondary Question 3: Exposure Scenarios and Risk for Sub-populations

What are the specific exposure scenarios that might increase risk for sub-populations (e.g., infants playing in shallow waters in presence of benthic mats, water skiers/beach goers inhaling aerosolised cells/toxins) and how are these managed by other organisations?

- The specific exposure scenarios that might lead to an increased risk for sub-populations include infants playing in shallow waters in the presence of cyanobacterial blooms, and exposure of sub-groups such as asthmatics and workers such as lifeguards on beaches
- These groups are considered more vulnerable than the general population when exposed to aerosolised marine algal or cyanobacterial toxins
- Organisations manage the increased risk multiple ways:
    - firstly, risk is accounted for within guidelines by often selecting body weight and water ingestion volumes relevant to children
    - secondly, agencies use a range of strategies to guide recreational water users to avoid the hazard

Secondary Questions – High-Level Summary of Findings (continued)

Secondary Question 4: Evidence of Adverse Effects from Marine Cyanobacteria and Algae

What is the extent of evidence of adverse effects due to recreational exposure to marine cyanobacteria or algae (e.g., skin irritation due to Lyngbya majuscula or inhalation-related symptoms due to cells/toxins aerosolised by wave action, boats, jet-skis, etc.)? Are there any existing guidelines that address these exposure risks?

- The review found 22 primary studies regarding evidence of adverse health effects due to recreational exposure to marine cyanobacteria
- Most of these studies related to exposure to brevetoxins, often via aerosols from the marine dinoflagellate *Karenia brevis* associated with red tides in Florida, USA
- There were three studies related to dermal effects associated with exposure to the marine cyanobacterium *Lyngbya majuscula*, of which two were Australian studies from Queensland
- In relation to existing guidelines that address these exposure risks, only four recreational water quality guidelines for marine algae and cyanobacteria were found
- No national or local jurisdiction has yet developed any guidelines for specific marine toxins for recreational water quality in the marine environment
- The four existing guidelines consisted of cell number guidelines for the dinoflagellate *Karenia brevis* from Florida, USA, and cell number guidelines for dinoflagellates and various marine cyanobacteria from three Australian sources

Secondary Question 5: Evidence for Risk from Benthic Cyanobacteria and Cyanotoxins

Much of the evidence for freshwater benthic cyanotoxin production in Australia is anecdotal and often linked to dog deaths following swimming in water bodies (e.g., at least 4 dog deaths in Lake Burley Griffin). It would be useful to try to collate the grey literature evidence to provide a clearer picture of the extent of any risk.

- The review found a large body of evidence from primary studies that confirmed the relationship between dog deaths and exposure to both freshwater benthic and planktonic cyanobacteria
- Most of the studies reported ingestion as the exposure pathway, with one also reporting dermal exposure
- A high proportion of the animal primary studies of dogs recorded death as the endpoint and it was often possible by veterinary post-mortem examination to provide strong evidence for a causal link between the exposure to cyanobacteria and the observed health outcomes
- It is not clear whether dogs are any more sensitive than other animals or that they simply have opportunities for exposure to very high concentrations

2 Introduction

2.1 Background Information

The National Health and Medical Research Council (NHMRC) through the Recreational Water Quality Advisory Committee (the Committee) will update the *Guidelines for Managing Risks from Recreational Water* (2008) during 2021-22.

As part of this update a series of Narrative Reviews were conducted by contractors to gather evidence to answer research questions on Microbial Risks, Chemical Hazards and Free-living Organisms, as determined by the Committee. Australis Water Consulting (AWC) was engaged to undertake the Narrative Review for the sub-topic of Cyanobacteria and Algae to inform the update to Chapters 6 and 7 of the *Guidelines for Managing Risks in Recreational Water* (2008).

2.2 Purpose of this Review

The update of the *Guidelines for Managing Risks in Recreational Water* (2008) includes a Risk Management Framework (referred to as the Framework). The proposed Framework for the updated Australian Recreational Water Quality Guidelines (the Guidelines) is a new feature developed by the NHMRC that provides a structured process for identifying, planning for, and managing risks related to recreational water quality.

As such, the Framework is intended as an overarching risk assessment and management framework for recreational water quality. To support this Framework, the Guidelines will provide comprehensive elements including guideline values, technical fact sheets and specific technical guidance along with citing of associated evidence.

The Narrative Reviews, comprising of Evidence Evaluation and Technical Reports, as part of this project are designed to gather, assess and contribute to the detailed and up-to-date evidence. They will provide the rigour to support the above comprehensive information components contained within the Framework and the Guidelines.

2.3 Approach

The approach for this review is provided in detail in the Technical Report (Section 1.3). This outlines the context and target audience for the updated Guidelines, the risks to be included and excluded from the framework, and the definitions applied for recreational water, recreational water use and recreational water users.

3 Methodology

A summary overview of the Methodology for this review is provided here with further details given in the Technical Report (Section 2).

The detailed description of methods in the Technical Report covers: the literature review protocol; the process for critically appraising the evidence; the search strategy and selection of evidence; the search protocol development and structure; the screening methods; the methods for additional and supplementary searches and grey literature searches; the assessment of the study quality (risk of bias) of individual studies and; the assessment of the certainty in the body of evidence. In addition, the Technical Report includes a compilation of the full search structure, the terms used, and results for all search iterations in the databases as they progressively evolved and were refined. It includes an

assessment of a selected range of international and national recreational water guidelines in relation to a suite of administrative and technical criteria for comparison to NHMRC procedures and requirements.

3.1 Literature Review Protocol

This review was comprised of answering a series of questions to inform the update of the NHMRC *Guidelines for Managing Risks in Recreational Water* in relation to the sub-topic of Cyanobacteria and Algae. The research questions to be addressed consisted of one primary question and five secondary questions (Table 1).

Table 1: Research Questions for the Narrative Review: Cyanobacteria and Algae (provided by the Committee)

Research Questions
Primary Question: What is the risk of any adverse health outcome for water users from exposure to cyanobacteria or algae in recreational water? **Secondary Questions:** 1. What are the indicators/surrogates of this/these hazard/s? What are the advantages and disadvantages of using surrogates versus monitoring specific toxins? 2. What guidelines, guidance and implementation practices are in place in comparable countries to minimise or manage this/these hazards and risks/s? 3. What are the specific exposure scenarios that might increase risk for sub-populations (e.g. infants playing in shallow waters in presence of benthic mats, water skiers/beach goers inhaling aerosolised cells/toxins) and how are these managed by other organisations? 4. What is the extent of evidence of adverse effects due to recreational exposure to marine cyanobacteria or algae (e.g. skin irritation due to *Lyngbya majuscula* or inhalation-related symptoms due to cells/toxins aerosolised by wave action, boats, jet-skis, etc.)? Are there any existing guidelines that address these exposure risks? 5. Much of the evidence for freshwater benthic cyanotoxin production in Australia is anecdotal and often linked to dog deaths following swimming in water bodies (e.g. at least 4 dog deaths in Lake Burley Griffin). It would be useful to try to collate the grey literature evidence to provide a clearer picture of the extent of any risk.

The review process to answer the research questions included four components. Each component had a different methodological approach selected to optimise information collection and evidence evaluation to answer the specificquestion. These components were:

1. A conventional systematic literature search and review of primary studies to address the Primary Question about the risk of adverse health outcomes from exposure to cyanobacteria and algae in recreational water.
2. A review of selected reviews to address Secondary Question 1 related to the indicators/surrogates of hazards posed by cyanobacterial toxins.
3. A review of guidelines, guidance, and implementation practices in place in comparable countries from grey literature obtained from organisational or jurisdictional agency websites to address Secondary Question 2.

4. A systematic review of selected primary studies and other reports derived from the search to answer the Primary Question, and additional supplementary searches and other sources specifically related to Secondary Questions 3, 4 and 5.

The justification and details of this differential approach related to the different questions is provided in the Technical Report (Section 2.1.1).

3.1.1 Population, Exposure, Comparator, Outcome (PECO) Table

The context for the review was set by the 'PECO' (Population, Exposure, Comparator, Outcome) assessment developed by the Committee. This was used to scope and guide the evidence collection and analysis. The PECO table is given in Table 2.

Table 2: PECO for the Narrative Review: Cyanobacteria and Algae (provided by the Committee).

Population	Exposure	Comparator	Outcomes
The general population *May also need to consider:* Do specific subpopulations need additional attention • Elderly • Infants and children • Pregnant women • Indigenous Australians (Aboriginal and Torres Strait Islander peoples) • Any groups that might be exposed more frequently as a result of inequity (e.g. geographic location, socioeconomic status) or lifestyle/occupation.	**Freshwater pelagic cyanobacteria** and toxins of interest: • *Cylindrospermopsis raciborskii, Microcystis spp., Dolichospermum circinale, Nodularia spumigena, Lyngbya wollei,* Total cyanobacteria. • Microcystins, cylindrospermopsins, saxitoxins, anatoxin-a, nodularin, LPS endotoxins	Control group of people with no exposure; where available/included and reported	• Gastrointestinal illness • Pneumonia-like symptoms • Hepatotoxicity • Neurotoxicity • Dermal irritation or allergic reaction • Inhalation-related symptoms (e.g. induction of asthma, shortness of breath)
As above.	**Freshwater benthic cyanobacteria** and toxins of interest: • *Phormidium, Geitlerinema, Nostoc, Oscillaroria, Schizothrix*, Total cyanobacteria. • Microcystins, cylindrospermopsins, saxitoxins, anatoxin-a, nodularin, LPS endotoxins	Control group of people with no exposure; where available/included and reported	• Gastrointestinal illness • Pneumonia-like symptoms • Hepatotoxicity • Neurotoxicity • Dermal irritation or allergic reaction
As above.	**Marine algae and cyanobacteria** and toxins of interest: • *Lyngbya majuscula, Oscillaroria, Trichodesmium, Karenia brevis, K. spp., Pfiesteria, Alexandrium, Gymnodinium, Dinophysis.* • lyngbyatoxin, applisiatoxin, pectenotoxin, saxitoxins, other marine toxins (e.g. brevetoxins, domoic acid).	Control group of people with no exposure; where available/included and reported	• Inhalation-related symptoms (e.g. induction of asthma, shortness of breath) • Dermal irritation or allergic reaction
Domestic, farm or wild animals exhibiting adverse health effects or death as evidence for the presence of toxin-producers in recreational waters.	**Algae or cyanobacteria** and toxins of interest: • Algae or cyanobacteria in general. • Any toxin type listed above or unidentified toxins.	Control group of animals with no exposure; where available/included and reported	• Gastrointestinal illness • Pneumonia-like symptoms • Hepatotoxicity • Neurotoxicity • Dermal irritation or allergic reaction • Inhalation-related symptoms (e.g. induction of asthma, shortness of breath)

Further detailed description of the Methodology for the review in the Technical Report includes:

Components of the **Literature Review Protocol** (Section 2.1):

- Retrieval of Publications (Section 2.1.3)
- Process for Extracting and Presenting Data (Section 2.1.4)
- Process for Critically Appraising the Evidence (Section 2.1.5)

The **Search Strategy and the Selection of Evidence** is described in Section 2.2 and includes:

- Databases searched (Section 2.2.1)
- Publication Dates and Language criteria applied (Section 2.2.2)

The **Search Protocol Development and Structure** is described in Section 2.3

Accessing Evidence from Other Sources is described in Section 2.4, and includes:

- Screening Methods (Section 2.4.1)

The review included some **Additional and Supplementary Searches** (Section 2.5) identified by the Committee that were required to complement the searches for the primary question. The additional topic searches were:

- Endotoxins/LPS (Section 2.5.1)
- BMAA (Section 2.5.2)
- Assessment of the Significance of the Topic for Indigenous Health (Section 2.5.3)

The review also required extensive grey literature searches to identify studies not in the published, peer-reviewed literature and to source guideline values used for cyanobacteria in recreational fresh- and marine water in other jurisdictions. These searches were carried out specifically to gather information required to address Secondary Question 2: "What guidelines, guidance and implementation practices are in place in comparable countries to minimise or manage this/these hazards and risks/s?" The search required the coverage of an extensive list of key international agencies which have potentially developed guidelines and the full list of these and the approach applied for the **Grey Literature** searching is given in Section 2.6 of the Technical Report.

3.2 Assessment of the Study Quality (Risk of Bias) of Individual Primary Studies

A central component of this review was the assessment for study quality to evaluate the evidence from the primary studies reviewed. This involved assessment of risk of bias and the approach used for this was an adaptation of the OHAT risk of bias tool (Appendix 1) (OHAT, 2019). The full detail of how studies were evaluated on applicable risk of bias questions based on study design is provided in Section 2.7 of the Technical Report.

The process used to assess the certainty in the body of evidence was based on the OHAT (2019) approach to using the GRADE system and is provided in Section 2.8 of the Technical Report.

4 Results

4.1 Primary Question Search

As described in the methodology (Technical Report: Section 2.2), searches to answer the primary question were developed using logic grids for three individual concepts: Cyanobacteria/Algae/Toxins; Recreation/Recreational; Health Outcomes. The concepts were then combined into single comprehensive searches. The results for both the individual concept searches and multiple combined searches performed in different databases (PubMed® and Scopus®) are given in the Technical Report (Section 3.1).

4.2 Inclusion/Exclusion of Literature and PRISMA Flow Diagram

The Prisma Flow Diagram (Figure 1) summarises the process for identification, screening and eligibility assessment of literature used for the evidence evaluation and the narrative review.

The first stage for the identification of studies involved combining the results of the database searches and studies from other sources to produce 1,693 records. After removal of duplicates (n=456) the number of records identified to proceed to screening was 1,237. Following screening (see Technical Report: Section 2.4) the number of papers that proceeded to full text review was 143, comprised of 89 freshwater and 54 marine studies.

The aim of the full-text review was to identify primary studies that contained suitable data that could be included in the assessment for risk of bias and further exclude other studies that did not meet this criterion.

The definition of primary studies applied here was those studies that contain original primary data which report measurements of effects or observations of health outcomes from exposure to cyanobacteria, algae or their toxins. This is opposed to secondary reporting and publication of data taken from primary studies.

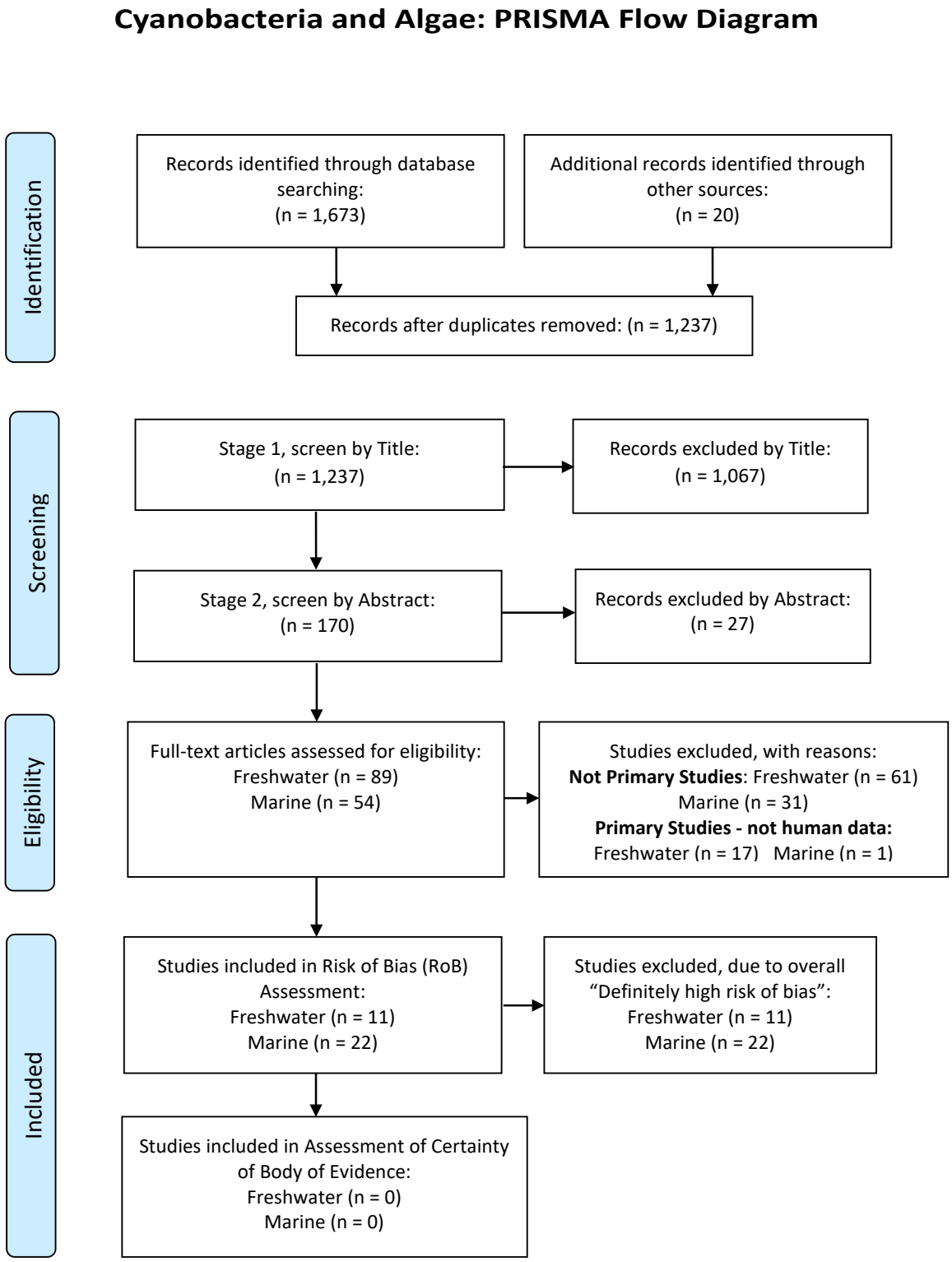
A list of freshwater and marine studies that were excluded from further assessment after full-text review with reasons for exclusion is given in Appendix 3 of the Technical Report.

The output from the full-text review identified 51 studies that were regarded as primary studies that contained suitable data that could potentially be included in the assessment for risk of bias. However, only the human exposure studies were included in the risk of bias assessment, and this excluded a further 18 studies (11 freshwater; 1 marine). The numbers of primary studies therefore that proceeded through the full risk of bias assessment were 11 freshwater and 22 marine studies. The other primary studies which were not related to human exposure, provided data that was useful for answering the Secondary Questions in some cases. A list of the primary freshwater and marine studies excluded from the risk of bias assessment is given in Appendix 4 of the Technical Report with explanations for their exclusion.

All studies assessed for risk of bias assessment were determined to have overall “definitely high risk of bias”. A subsequent assessment of certainty in the body of evidence was done and an overall certainty rating was assigned to each evidence stream as ‘very low confidence’ across all study types. This was based on downgrading any evidence streams with an initial ‘low’ or ‘very low’ confidence rating to ‘very low’ across the board for serious risk of bias.

These shortcomings considered together led to the conclusion that there was insufficient confidence in the findings of the available studies. This is explained in further detail in Sections 5.1.2 and 5.1.3.

Figure 1: PRISMA flow diagram outlining the identification and screening of literature and assessment for study quality to identify and evaluate evidence from the studies.



4.3 Additional and Supplementary Searches

4.3.1 Endotoxins/LPS

A supplementary search for Endotoxins/LPS was used with the Recreation/al and Health outcomes concepts previously developed for the full combined searches to determine the potential significance of these compounds to health outcomes in recreational water situations. The full details and results of this search are given in Section 3.3.1 of the Technical Report. The results returned from this combined search (Endotoxins/LPS; Recreation; Health) were low – only 170 studies/papers and these were of very limited or no relevance to environmental exposure to Endotoxins/LPS in recreational water situations.

4.3.2 BMAA

A supplementary search for the potentially toxic amino acid BMAA was combined with a limited range of terms for cyanobacteria to determine the extent of literature on this compound in association with cyanobacteria. The full details and results of this search are given in Section 3.3.2 of the Technical Report. The specific individual search for BMAA terms (5 terms only) returned 399 results (from 2006-2020). The combined cyanobacteria and BMAA search returned 234 results for (2006-2020). This combined result of 234 suggested that the association of BMAA with cyanobacteria is a recent popular research topic and approximately 60% of the publications from 2006 that mentioned BMAA also mentioned cyanobacteria (234 from 399).

It must be noted this search return was for the terms “cyanobacteria” and “BMAA” found in titles and abstracts only, and the relevance of this for the public health hazard of BMAA can only be confirmed by a detailed assessment of these publications. This search was regarded as satisfactory to assess the extent of literature on this topic for information of the Committee.

4.3.3 Assessment of the Significance of the Topic for Indigenous Health

A supplementary search was developed and carried out to assess relevance of the topic of cyanobacteria and recreational water environments to public health of Australian indigenous people/s. The full details and results of this search are given in Section 3.3.3 of the Technical Report. This search was tested only within one database (PubMed®) and returned no results related to indigenous studies or health outcomes and the Primary Question. This was regarded as a sufficient indication that there is limited or no published literature on this topic in conventional databases.

4.4 Assessment of Primary Studies and Grey Literature

4.4.1 Assessment of Primary Studies with regard to the Primary Question

A detailed assessment of the primary freshwater and marine studies selected for full-text review was made by extracting and analysing information on a range of data and experimental design elements from each study. This assessment included a breakdown of key parameters for each study such as the type of water recreational environment (e.g. lake, river, pond); the cyanobacterial type (e.g. planktonic, benthic); the peer review status of the study; whether toxins or their surrogates were determined or analysed for both within the exposure environment and/or within the subject of the exposure; and the type and degree of health assessment undertaken and health outcomes reported from human primary and animal exposure studies. A detailed analysis of this data is given in Section 3.4.1 of the Technical Report.

4.4.2 Assessment of Grey Literature with regard to the Secondary Questions

Detailed assessment and analysis of the results from the grey literature searches that were used to answer the five Secondary Questions is given in Section 3.4.2 of the Technical Report.

5 Discussion

5.1 Assessment of Key Questions

5.1.1 Primary Question

What is the risk of any adverse health outcome for water users from exposure to cyanobacteria or algae in recreational water?

5.1.2 Assessment of the Study Quality (Risk of Bias) of the Primary Studies

The results of the literature search and subsequent screening to identify studies to answer the Primary Question identified 51 studies that could potentially be included in the assessment for study quality by risk of bias assessment. However only the human exposure studies were included in the risk of bias assessment, and this excluded a further 18 studies (11 freshwater; 1 marine). The numbers of primary studies that proceeded through the full risk of bias assessment were 11 freshwater and 22 marine studies. Details of these studies listed by study type and including a summary of the key findings and comments are given in Tables 3 and 4, respectively. The freshwater studies consisted of 5 cohort, 3 observational and 3 case studies. The marine studies consisted of 12 cohort, 4 observational and 6 case studies. There were only two Australian investigations in the freshwater primary studies, and both were epidemiological studies related to exposure to cyanobacteria in recreational waters (Pilotto *et al.,* 1997; and Stewart *et al*., 2006). The study by Pilotto *et al*., (1997) was included in the review although it was outside the date range specified (2006-2021). This was because it was a highly relevant Australian epidemiological study designed at the time to gather information to inform exposure to toxic cyanobacteria in recreational water environments. There were also two Australian-based investigations within the marine primary studies. These were both related to health effects associated with exposure to the marine cyanobacterium *Lyngbya majuscula* in Queensland (Osborne *et al.*, 2007; and Osborne and Shaw, 2008).

As described in Section 3.2 and in full detail in Section 2.7 of the Technical Report, the methodological quality of included studies was assessed using an adaptation of the OHAT risk of bias tool (OHAT, 2019). Studies were evaluated using risk of bias questions that were applicable based upon the type of study design. The areas of bias covered by the OHAT tool are selection bias, confounding bias, attrition/exclusion bias, detection bias, selective reporting bias and other sources of bias.

It must be noted that risk of bias assessment has principally been designed and used for assessment of the validity of studies either for the evaluation of clinical outcomes or other public health interventions or diseases. Thetypes of studies assessed here were either field-based observational and case studies, or cohort studies associated with environmental contaminants, so not all of the usual bias domains were applicable.

Each of these specific areas of bias are discussed overall below are based upon the key observations from the individual studies given in Tables 3 and 4, respectively. The risk of bias assessments for individual studies, with detailed comments about each bias criteria and number coding for the individual studies are given in Appendix 5 of the Technical Report. Some of the comments and observations included below were also identified as risk of bias issues by the authors for their own or other studies. This discussion of the bias domains is followed by a summary of the risk of bias assessments for all the primary freshwater and marine studies (Tables 5 and 6, respectively).

SELECTION BIAS

Comparison groups

From the primary studies approximately one-quarter of both the marine and freshwater studies were case reports (marine: 6/23; 26% and freshwater: 3/11; 27%). These studies had no comparator group, as would be expected. An example of an extensive report in this category is the comprehensive report by Hilborn *et al*. (2014) presenting the CDC's Waterborne Disease and Outbreak Surveillance System in the USA in 2009-2010. This identified a number of reports which contain substantial evidence of the exposure to and uptake of cyanobacteria and a likely connection to the symptoms observed. In this study, 11 outbreaks were associated with cyanobacteria, and in all cases because of the nature of the data from outbreak incidents no comparator group was identified or presented.

The remainder of the studies reviewed had comparators in some form, however the majority had limitations and weaknesses in the selection and numbers allocated as controls.

In the studies of recreational exposure reviewed here a large number were biased by targeting specific sub-groups of the general population, particularly in the marine studies. These sub-groups were lifeguards (Backer *et al*., 2005) or asthmatics (Bean *et al*., 2011; Cheng *et al*., 2010; Fleming *et al*., 2005, 2007, 2009; Kirkpatrick *et al*., 2011; Milian *et al*., 2007; Pierce *et al*., 2005). These population-biased studies accounted for 53% of the marine primary studies (9/17), excluding the 6 case studies.

CONFOUNDING BIAS

While some studies (Backer *et al*., 2008, 2010; Honner, 2010; O'Halloran *et al*., 2017) attempted to account for any confounding factors that may have impacted the health outcomes reported, generally this was not widely considered. For example, Levesque *et al*. (2014) discussed confounders, specifically other cyanotoxins and *Aeromonas* strains associated with cyanobacteria, but no measures of these parameters were included.

Several studies (Levesque *et al*., 2014; Stewart *et al*., 2006) considered only faecal coliforms as a confounding variable but in some studies, this was not comprehensive. For example, in the study by Stewart *et al*. (2006) samples for faecal coliforms were taken only when an exposure day was followed by a routine working day (39% of exposure events).

There is likely to be a large range of possible causes which may be confounding factors for the health outcomes (irritation, respiratory, gastrointestinal and fever or headache) considered in these recreational exposure studies. The potential confounding factors could include for example bacterial and viral pathogens, airborne irritants from local wild-fires or aerial pesticide spraying, and airborne pollen. However, it is recognised that it is largely not practical or economically feasible for these types of field studies of recreational exposure to consider all potential confounding factors.

ATTRITION/EXCLUSION BIAS

In most cases the studies reviewed did not exclude data or observations and there were no cases of significant attrition reported for prospective or other studies in both the freshwater to marine literature. An example of an exception was the case series of adverse health outcomes reported from exposure to marine dinoflagellates in the Mediterranean by Tichadou *et al*. (2010). These authors reported that a limitation was that the data reported was from presentations to a Poisons Control Centre and clinical manifestations were sometimes non-specific. In these circumstances only cases where the dinoflagellate was considered a plausible case were included. It is possible that this

occurred in similar studies, as it is a realistic judgement by authors to exclude cases in field case where observations are not clear cut or definitive and may not have been reported by authors.

DETECTION BIAS

Exposure Characterisation

Exposure characterisation for epidemiological studies related to recreational exposure to cyanobacteria has been identified as a major issue in the adequacy of these studies in the recent WHO-supported review by Chorus and Testai (2021). These authors examined many of the studies also reviewed here (Pilotto *et al*., 1997; Stewart *et al*., 2006; Backer *et al.*, 2008, 2010; Lévesque *et al*., 2014) and also earlier work. They make a general statement related to all of the epidemiological studies conducted between 1990 and 2011, that "levels of exposure were usually poorly characterized and hence these studies are inadequate for risk assessment purposes".

General & specific comments on the risk of bias associated of exposure characterisation for studies reviewed here in relation to exposure time and environmental exposure data (sampling, etc.) are as follows.

Exposure time

Exposure time between individuals varied in many of the beach studies in an unknown manner since participants were allowed to leave the beach at any time if they felt symptomatic (Bean *et al*., 2010; Fleming *et al*., 2005, 2007 and 2009). Fleming *et al*. (2005) also noted study participants were residents of the region which had a history of red tide exposure. Consequently, these participants may have experienced intermittent aerosolised brevetoxin exposure which was unmeasured during the study period. Schaefer *et al*. (2020) also observed in a nasal swab study that microcystins were detected in nasal passages among persons who denied having direct contact with impacted water. Indirect exposure in the absence of direct contact with the impacted waterways is possible. The aerosolisation of cyanotoxins makes it nearly impossible for subjects to be unexposed unless participants are sourced at a significant distance away from the impacted waterbody.

In one study (Backer *et al*., 2003) the two cohorts (exposed and unexposed) were exposed at widely different times (separated by months) and at different locations. The non-exposed group participated in the study in February 1999 at Sarasota (Florida) while the exposed group participated in the study in October 1999 at Jacksonville (Florida), which are over 400km apart. In addition to this variation in exposure scenario between the two cohorts, individual exposure times varied widely during the study, ranging from 10 min to 8 h. Variations in exposure time are often an uncontrollable factor in volunteer-recruited studies.

Environmental exposure data

Many of the observational and/or case studies provided either very limited or no environmental data to allow exposure to be assessed. These included seven marine studies (Gallitelli *et al*., 2005; Osborne *et al*., 2008; Lee *et al*., 2009; Namendys-Silva *et al*., 2018; Reddy *et al*., 2019; Steensma *et al*., 2007; Werner *et al*., 2012) and two freshwater studies (Slavin *et al*., 2008; Trevino-Garrisson *et al*., 2015).

Another major issue with the field studies is that often the location of water sampling for exposure characterisation and the location of exposure did not coincide. In a few cases water sampling occurred at the exact location of exposure and in a timely manner (Gianuzzi *et al*., 2011) but this was not always possible. Water sampling was more often carried out as part of a routine sampling program not related to the study and could not be linked directly to the exact time and location of each reported exposure

(e.g. Morris Jr. *et al*., 2006; Schaefer *et al*., 2020; Vidal *et al*., 2017). Morris Jr. *et al*. (2006), in a study of an occupational cohort, noted that the zones of work area grids of study participants (fisher people) and water monitoring grids did not provide certainty regarding the temporal overlap of work exposure and *Pfiesteria* detection.

In some studies, environmental data were missing, and interpolation was required. For example, in the large cohort study by Levesque *et al*. (2014) where the participants lived adjacent to a lake in Canada the authors used interpolation to assign data to exposure periods which were missing information for *E. coli*, cyanobacterial counts and microcystin data from other sampling days which were closest in time to the exposure days where no sampling was carried out.

The well-known spatial and temporal variability in the distribution of an algal and cyanobacterial blooms also poses an issue for exposure characterisation. Schaefer *et al*. (2020) noted variability in concentrations of microcystins in nasal swabs and attributed it to the patchy distribution of cyanobacteria across the sampling regions, as well as variation in the extent of exposure.

In marine studies of aerosolised brevetoxins, several authors noted that exposure assessment is complicated by weather factors including wind direction and wind speed. Funari *et al.* (2015) noted that in 2010 and 2012 blooms of *Ostreopspis* cf. *ovata* that cell numbers reached very high densities (> 5 million cells/mL), but no adverse health effects were reported. However, in 80 cases of inhalation effects reported in 2007, the cell count was recorded as 36,400 cells/mL. They noted that weather conditions (e.g. presence of onshore winds, favouring aerosol formation, and turbulent conditions trigger the release of algal cells from the substrate into the water column) have a major impact on whether or not adverse health effects are observed.

A further complication with studies of aerosolised brevetoxins is that *K. brevis* produces a natural inhibitor of brevetoxin, brevenol, that has been shown to block bronchoconstriction in the allergic sheep model (Abraham *et al.,* 2005 in Fleming *et al.,* 2007). Some studies measured brevenol in environmental samplers during the exposure period (Cheng *et al*., 2005; Pierce *et al.,* 2005) and this would be expected to complicate the exposure assessment. In an indeterminate number of cases this may be a potentially unknown and unaccounted co-factor associated with exposure to brevetoxin that potentially moderates its toxicity.

Outcome Assessment

Two studies involved assessment of exposure of recreational users to aerosolised toxins originating from either marine algae (Pierce *et al*., 2005) or freshwater cyanobacteria (Schaefer *et al*., 2020) but both failed to provide information about health outcomes.

Pierce *et al*., (2005) undertook an investigation as part of other studies to establish types and amounts of brevetoxins and *K. brevis* cells that marine beachgoers were exposed to during a 3-day cohort study reported by Fleming *et al.* (2004, 2005) and Backer *et al* (2005).

Similarly, the freshwater study by Schaefer *et al*., (2020) set out to investigate potential exposure to microcystins by measuring concentrations in nasal swabs from 125 participants in Florida, USA. The study reported on the significance of this exposure route only and provided no health outcome data.

Many of the study designs relied upon participants self-reporting health outcomes following exposure to cyanobacteria or algae in recreational situations. This was the case for both freshwater (5/11: 45%) and marine (6/23: 26%) studies. There are numerous issues associated with self-reported health symptom data. For example, symptoms such as throat and skin irritation, that are common with a wide range of causes, may be under-reported since the subjects may not associate these symptoms

with recreational exposure to cyanobacteria (Backer *et al.*, 2010). Backer *et al*. (2010) found self-reported symptom data had limited value in assessing acute exposures to low environmental concentrations of microcystins. Similarly, Tichadou *et al*. (2010) noted the non-specific nature of clinical manifestations probably resulted in under-diagnosis and thus under-reporting associated with self-reporting.

Furthermore, even when symptoms are medically diagnosed it has been suggested that a healthcare provider may find it difficult to confirm cyanobacterial toxins are the cause of the illness based upon symptoms alone, and hence under-reporting may occur (Hilborn *et al*., 2014; Trevino-Garrison, *et al*., 2015).

Some of the methods used to assess health outcomes were also questioned by authors in their studies. Fleming *et al*. (2009) commented that a major limitation in the interpretation of all asthma literature is the inconsistency in the definition of response to pulmonary function testing (PFTs). The study by Fleming *et al*. (2009) also noted that in their study the PFTs may have been delayed by hours or even days for some subjects and thus where no response was detected it may reflect the time delay rather than a response that would be detected by an immediate PFT.

Backer *et al*. (2005) also noted spirometry tests have limitations since it is almost impossible to reproduce three spirograms within the guidelines without maximal effort. Kirkpatrick *et al*. (2011) reported the handheld peak flow meters used to assess respiratory function are relatively inaccurate.

These methodological and instrumental issues compound problems with the assessment of outcomes.

SELECTIVE REPORTING BIAS

For this body of studies there were no cases of non-reporting of outcomes.

OTHER SOURCES OF BIAS

An issue noted with this body of studies that falls under responder-bias is the potential for participants judgement and experience to influence self-reporting of their exposure. The response of subjects regarding symptoms may be influenced by their awareness of the environmental conditions at the time of exposure and non-exposure (e.g. visual observation of a “red tide” or scum). Backer *et al*. (2005) noted this concern but claimed that since the participants did not know the exposure status (environmental analyses) at the time of collection of symptom data, it was less likely that study participants could influence results. The absence of environmental data however does not remove the effect of any visual influences upon participants’ responses.

Other authors noted that responder-bias may be associated with the nature of the cohort in the study. For example, Fleming *et al*. (2005) noted this for study participants that were residents of the region which had a history of red tide exposure. These residents may have adapted to chronic red tide aerosol exposure, and this may have influenced their self-reported health outcome responses.

Backer *et al*. (2010) raised the issue of responder bias if the participants perceived that reporting adverse health impacts following recreational exposure to cyanobacteria may negatively impact upon the community in either a regulatory or economic manner. For example, this may be a particular issue in areas where the communities rely upon local water bodies for tourism or if there are concerns that community access to recreation areas may be impacted.

Responder-bias may also occur when the study subjects are not individually interviewed. For example, in Lin *et al*. (2016) one household member responded on behalf of all members who were exposed. Stewart *et al*. (2006) tried to minimise this aspect of bias, but exceptions were made in the case of

children, where a parent or guardian was asked to decide whether or not their child would participate in the follow-up interview directly.

Levesque *et al*. (2014) noted in a study of residents around three lakes that people in better health may have had more frequent contact with the lakes, thereby resulting in an underestimation of relative risks of recreational exposure. However, if people are not intending to participate in recreational activities in waterbodies, then they will not be exposed to the hazard.

An important comment on significant responder bias related to self-reporting was given for the Australian study related to health effects associated with exposure to the marine cyanobacterium *Lyngbya majuscula* in Queensland (Osborne *et al.*, 2007). Osborne *et al*., (2007) noted that the possibility of non-respondent bias in their study was high since only 27% of individuals replied. However, they accounted for this by claiming that the demographics of the respondents generally resembled the Australian Bureau of Statistics population data for study area of Bribie Island.

Summary of the Assessment of Study Quality

There was a clear and consistent pattern in the types of bias in all of the marine and freshwater studies reviewed here that led to weaknesses overall in study quality and in the resulting body of data. The majority of the studies suffered from shortcomings in some of the major bias domains including:

- failing to include suitable comparators or control groups
- not considering potential confounders (i.e. factors or causes for adverse outcomes other than cyanobacteria, algae or toxins)
- not adequately accounting for exposure characterisation for these organisms and compounds in an environmental setting
- many studies had a reliance on self-reporting as part of outcome assessment.

These limitations in design reflect that none of the studies reviewed were designed as randomised control trials or similar clinical trials. Only about 50% of both the freshwater and marine and studies were cohort studies, with the remainder being observational and case studies. As a consequence, all of the studies reviewed by the risk of bias assessment were determined to have an overall “definitely high risk of bias”.

Across the entire body of studies and data it was not possible to extract a subset of data that was not conflicted by design weaknesses that led to the bias limitations described above. Almost all studies exhibited a high risk of bias in one or another of the domains which would preclude the data being extracted and considered for being upgraded. The most significant limitations related to lack of comparators, presence of confounders, exposure characterisation and very high reliance on self-reporting.

These shortcomings considered together led to the conclusion that there was insufficient confidence in the studies. As a consequence, there was insufficient information to determine if there were any further reasons to upgrade the certainty of the overall body of evidence from ‘very low certainty’ using the GRADE system. See further discussion of this below in Section 5.1.3.

Table 3: Freshwater primary studies included in the risk of bias assessment grouped by study type with a summary and comments for each study. The study number aligns with the summary of RoB assessments in Table 5 and with assessment of individual studies in Appendix 5 of the Technical Report1.

Study No.	Authors	Summary	Comments
Cohort Studies			
1	Backer *et al*., 2008	*Recreational exposure to low concentrations of microcystins during an algal bloom in a small lake.* This cohort study followed 96 subjects exposed to an algal bloom during recreational activities on a lake and 7 who used a nearby lake with no bloom (unexposed). The small lake name and location was not provided, but was in either Michigan, New York or Ohio, USA. The recreational activities included swimming, water skiing, jet skiing, or boating during an algal bloom, and it was expected that people involved in these activities may ingest water or inhaling aerosols and should receive enough exposure to allow detection of microcystins in their blood. Recreational activities and symptoms were self-reported by interviews. Symptom data was collected 7 days before the study, immediately before and after the recreational activities and 7-10 days after the recreational activity. Blood samples were collected from all subjects and analysed for microcystins. Only one blood sample had detectable (>1 µg/L) microcystin concentrations but was thought to be a false positive since LC/MS showed absence of microcystin-LR, -RR and -YR. Water samples were collected for algal identification, cell counts, chlorophyll and microcystin analyses. Air samples were collected from personal samplers or from samplers on boats owned by subjects for measuring microcystin concentrations. Low levels of microcystins were found in the water (2-5 µg/L) and aerosol (< 0.1 ng/m^3) samples. The range of phytoplankton concentrations was 175,000 to 688,000 cells/mL and > 95% of the cells were cyanobacteria. The dominant genera of potentially toxic cyanobacteria reported in water samples were *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, and *Microcystis*. The two documented microcystin-producing genera present were *Anabaena* and *Microcystis*. Given that toxin levels measured were very low, it was not possible to determine any potential relationship between the number of microcystin-producing cyanobacteria and concentrations of microcystins. Study participants reported no symptom increases following recreational exposure to microcystins.	This was a very comprehensive study with reasonable numbers of well-characterised participants, and it is one of few studies to attempt to determine microcystin exposure by the analysis of blood to use as a biomarker. The study was designed with a small unexposed group (comparator or control) who undertook recreation in a nearby bloom-free lake. Confounding variables were considered by analysing presence of adenoviruses and enteroviruses in the lake water. Health outcome assessment was self-reported. The study found no increases in symptoms reported post-exposure for the levels of microcystins seen in the lake at the time of the study. Environmental data (sampling, etc.) was not provided for the unexposed site. It was not stated whether it was collected. The absence of this data decreases the confidence in the exposure assessment for the study, otherwise exposure was systematically well-designed and performed. In addition, 6 individuals in the comparator group reported that they had participated in activities at the exposed site in 7 days prior to the study.

Table 3: (continued)

Study No.	Authors	Summary	Comments
2	Backer *et al.*, 2010	*Recreational exposure to microcystins during algal blooms in two California lakes.* This cohort study followed participants exposed to an algal bloom during recreational activities over 3-days in two lakes with an algal bloom ("Bloom lakes"; exposed, n=81) and one lake with no bloom ("Control lake"; unexposed, n=7). Participants provided pre- and post-water activity nasal swabs and questionnaire responses and a single post-water blood sample. A follow up questionnaire was completed 7-10 days after lake exposure. Water samples were collected for algal taxonomy and measuring microcystin concentrations. Air samples were collected by ambient samplers and personal samplers for measuring microcystin concentrations. Phytoplankton cell concentrations were in the range 100, 000–2,000,000 cells/ml. The predominant phytoplankton present were *Microcystis* spp. followed by *Aphanizomenon flos-aquae.* The study found highly variable microcystin concentrations across sites in the two Bloom Lakes (<10 µg/L to >500 µg/L); microcystin was not detected in the Control Lake. Low microcystin concentrations were found in personal air samples (<0.1 ng/m^3 [limit of detection]–2.89 ng/m^3) and nasal swabs (<0.1 ng [limit of detection]–5 ng). In addition, microcystins were detected in air samples on only 1 of the 3 days of the study. Microcystin concentrations in the water-soluble fraction of all plasma samples were below the limit of detection (1.0 µg/L). They did not detect adenoviruses or enteroviruses in any of the lakes. The study concluded that toxin-producing cyanobacterial blooms can generate aerosolised cyanotoxins, making inhalation a potential route of exposure. Participants reported more symptoms during the 7 days before the study than either during the study or during the 7-10 days after the study period.	This is a very comprehensive study which found with no increases in symptoms reported post-exposure. Confounding variables were considered by analysing presence of adenoviruses and enteroviruses in the lake water. The authors hypothesised that inhaled cyanotoxins may subsequently be absorbed into the body through either upper or lower airway mucosal surfaces. However, they did not demonstrate a detectable internal MC dose as measured by plasma toxin analysis or a significant increase in addition to the main finding of no increases in self-reported acute symptoms after exposure. Health outcome assessment was self-reported, and the authors note that self-reported symptom data have limited value in assessing acute exposures to low environmental concentrations since the respiratory or dermal irritation symptoms are commonly associated with exposure to other environmental contaminants and infections.

Table 3: (continued)

Study No.	Authors	Summary	Comments
3	Levesque *et al.*, 2014	*Prospective study of acute health effects in relation to exposure to cyanobacteria.* This was a cohort study of participants living around three lakes in Canada who were asked to keep daily journals of symptoms and contact (full or limited) with the water body. The study involved contacting a large number of families and the eventual number in the study were 466 subjects from 267 families. Study participants had to reside in the targeted residence for > 2 weeks during the study period (11 weeks). Water samples were collected for measuring cyanobacterial cell counts and microcystins. Water samples were collected daily from multiple locations and depths, which were then pooled into a range of composite types for analysis. Cyanobacterial types were not reported, and counts are given as cell totals only. The range was highly variable, and results were presented as medians and maximum concentration, and it is not clear which data or concentrations were used for multivariate analysis with symptoms. The range of symptoms examined that were regarded potentially associated with exposure to cyanobacteria were: gastrointestinal: 2 indices (GI1: diarrhea or abdominal pain or nausea or vomiting; GI2: diarrhea or vomiting or [nausea and fever] or [abdominal cramps and fever]); upper and lower respiratory tract; eye; ear; skin; muscle pain; headaches; mouth ulcers). The results showed that only GI symptoms only were associated with contact with the lakes. The authors indicate that for exposure by full or limited contact to cyanobacterial concentrations higher than 100,000 cells/mL may expose the population to substantial risk of the gastrointestinal effects (i.e. RR of 3.28 for the GI2).	The study found a large variation in exposure time for participants. A potential complication related to exposure was that some of the residents also received treated drinking water which originated from one of the lakes, while others had alternative sources (e.g. wells). Confounding factors were considered by measuring *E. coli* in water. *E. coli* contamination was low and not associated with GI symptoms in residents that had contact with the water bodies. Authors note a potential uncorrected selection bias. They suggest that it was possible that people in better health had more frequent contact with the lake thereby resulting in an underestimation of relative risks of exposure. Confounders discussed but not measured include other cyanotoxins and *Aeromonas* strains associated with cyanobacteria.

Table 3: (continued)

Study No.	Authors	Summary	Comments
4	Stewart *et al.*, 2006	*Epidemiology of recreational exposure to freshwater cyanobacteria – an international prospective cohort study.* This is primarily an Australian prospective cohort study of health impacts in individuals exposed to cyanobacteria through recreational activities. A total Participants were recruited over a 3-year period (1999-2002) at lakes and river sites in Florida, USA and in two Australian states (Qld, NSW). A total of 3,595 participants across all sites completed a questionnaire before departure from the study site and were interviewed as soon as practicable after three days from the exposure. Water samples for phytoplankton and cyanotoxin analysis were collected twice daily from 1-4 locations on the exposure day. Cyanotoxins in the study waters were rarely found and when present they were at low concentrations. Cyanobacteria were identified and counted at 3 separate laboratories associated with the location of the study sites. Types and cell number data were not provided, and the information was converted to cyanobacterial cell surface area as the exposure variable of interest and classified in classes as low (total cyanobacterial cell surface area <2.4 mm^2/mL), intermediate (2.4–12.0 mm^2/mL) and high (>12.0 mm^2/mL) based upon guidelines from the Queensland Department of Natural Resources and Mines. Individuals exposed to recreational waters from which total cyanobacterial cell surface area >12 mm^2/mL (high level) were more likely to report symptoms. The authors' analysis was that "when grouping all reported symptoms, individuals exposed to high levels of cyanobacteria were 1.7 (95%CI: 1.0–2.8) times more likely to report symptoms than their low-level cyanobacteria-exposed counterparts.	Confounding variables were limited to faecal coliform analysis, but these samples were taken only when an exposure day was followed by a routine working day (39% of exposure events). The use of cyanobacterial cell surface area was as the principal exposure variable resulted in limited ability for exposure assessment to different cyanobacterial types, genera or cyanotoxins.

Table 3: (continued)

Study No.	Authors	Summary	Comments
5	Pilotto *et al.*, 1997	*Health effects of exposure to cyanobacteria (blue-green algae) during recreational water-related activities.* This prospective cohort study is included as it is the early comprehensive Australian epidemiological study examining specific exposure to cyanobacteria in recreational situations. Over two months participants were interviewed on selected Sundays at several water recreation sites in southern Australia (South Australia, New South Wales and Victoria). Subjects either had recreational exposure to water (exposed =777) or did not (unexposed =75). On the day of exposure participants were interviewed about their health status and recreational water activities for the day of the interview and for the previous five days. Follow up interviews were conducted 2 and 7 days later about a range of symptoms. On the interview day water samples were collected twice daily at evenly spaced distances and in a regular pattern across the exposure site and then pooled. The sampling involved 10 samples across the exposure zone being pooled to form a composite sample. Cyanobacterial cell counts of the dominant types were determined at one laboratory using a technique to achieve a specified level of precision. Dominant types across all sites included *Microcystis aeruginosa*, *Microcystis* sp., *Anabaena* sp., *Aphanizomenon* sp., and *Nodularia spumigena.* Potential cyanobacterial toxicity was measured on specific concentrated sample using mouse bioassay. Hepatotoxicity was identified in the concentrated samples at one site on two separate interview days, and also at three other sites on one day only. No toxin identification or quantification was done by a chemical analytical technique. Total cell counts were used for the analysis to correlate to symptom occurrence rates. Symptoms assessed and recorded included vomiting or diarrhoea, cold and flu-like symptoms, mouth ulcers, eye irritation, ear irritation, skin rash and fever. Symptom rates were pooled for the analysis. In the two days after exposure there was no significant differences in the occurrence of symptoms between the exposed and unexposed subjects. In addition, there was no significant trend in increasing symptom rates with increasing duration of water contact or cyanobacterial cell counts. Seven days after exposure there was a significant trend of increasing symptom rates with increasing duration of exposure, after exclusion of previously ill or exposed subjects. Participants exposed to > 5,000 cells/mL for >1 h had a significantly higher symptom occurrence rate than the unexposed. The authors concluded that symptom occurrence was associated with duration of contact with water containing cyanobacteria, and with cyanobacterial cell density.	Stewart *et al*. (2006) commented on this study and queried whether non-bathers as control subjects might differ from those subjects that chose to go into the water. It was suggested they may under-report illnesses. They suggest that a control group of bathers is preferred as it also accounts for possible effects of water immersion that may be unrelated to water quality. Authors noted that although hepatotoxicity was identified at one site on 2 separate days and at 3 sites one day only there was no significant association between hepatotoxicity and symptom occurrence. This was not unexpected as the symptoms reported were not specific to liver injury and rather to allergic reactions to cyanobacterial cells. They noted however that they could not exclude hepatotoxins from being responsible for symptom development in some participants. No other cofounders were considered. The authors suggest that the Australian safety threshold of 20,000 cells/mL may be too high. Note: This study by Pilotto *et al.,* (1997) was included in the review although it was outside the date range specified (2006-2021). This was because it was a highly relevant Australian epidemiological study designed at the time to gather information to inform exposure to toxic cyanobacteria in recreational water environments.

Table 3: (continued)

Study No.	Authors	Summary	Comments
Observational Studies			
6	Hilborn *et al.*, 2014	*Algal bloom-associated disease outbreaks among users of freshwater lakes – United States, 2009-2010.* This report represents a compilation summary of human health data and water sampling results voluntarily reported to CDC's Waterborne Disease and Outbreak Surveillance System (WBDOSS) and the Harmful Algal Bloom-Related Illness Surveillance System (HABISS)* for the years 2009–2010 in the USA. The report found that for 2009–2010, 11 waterborne disease outbreaks associated with algal blooms were reported and these HABs all occurred in freshwater lakes. The outbreaks occurred in three states and affected at least 61 persons. Health effects included dermatologic, gastrointestinal, respiratory, and neurologic signs and symptoms. The report provides water quality indicator data where it was available including the presence of cyanobacteria, *E. coli* and a range of toxin types and concentrations. The data was limited and varied in the time period after exposure associated with the disease reports.	This study had limited environmental data. There were no details of the water sampling protocol. Only maximum cyanotoxin concentrations were reported. No comparator groups were identified. Confounding variables were limited to *E. coli* measured in outbreak. The authors note the limitations of this data compilation in that reporting is voluntary, so outbreaks are likely to be under-reported. Also outbreak detection varies among and localities.
7	Schaefer *et al.*, 2020	*Exposure to microcystin among coastal residents during a cyanobacteria bloom in Florida.* This study investigated potential exposure to microcystins by measuring concentrations in nasal swabs from 125 participants in Florida, USA. Participants were recruited during a *Microcystis* bloom and completed a questionnaire about recreational and occupation exposure with impacted waterways over 10 d. Nasal swabs were taken from participants to measure microcystin concentrations. Bi-weekly water samples were collected for measuring microcystin concentrations. The study found that 95.0 %, i.e. 115 of the 121 participants who provided nasal swabs had concentrations of MC above the limit of detection. There were significant differences ($p < 0.01$) in mean MC concentration between individuals with direct contact with impacted waters compared to those with no recent contact. Higher concentrations were observed among occupationally exposed individuals. In addition, nasal concentrations of MC varied significantly over time and location of exposure to the bloom and was related to concentrations in water samples. The authors suggest that inhalation of aerosols may be an important pathway for exposure to MC. Nasal MC concentrations were generally highest during periods when concentrations in the surrounding waters peaked.	There was an issue in this study with participants recollection of exposure which was requested for the last 10 days. The study reported on the significance of this exposure route only and provided no health outcome data. The duration of exposure was not measured.

Table 3: (continued)

Study No.	Authors	Summary	Comments
8	Vidal *et al.*, 2017	*Recreational exposure during algal bloom in Carrasco Beach, Uruguay: A liver failure case report.* This paper reports on a family (3 adults and a 20-month-old child) who were exposed to an algal bloom while bathing at beaches in Uruguay. A few hours after the last exposure all family members developed diarrhea. While the adults soon recovered the child's symptoms continued for 5 d until she was admitted to a hospital intensive care unit. A liver transplant was performed on the child 20 d after the hospital admission. The extensive hospital serology tests for hepatitis A, B, and C, Epstein-Barr virus, and cytomegalovirus were negative. Histological studies and microcystin determination were performed on the explanted liver. The analysis of MCs revealed the presence of two microcystin toxins: Microsytin-LR (MC-LR) and [D-Leu^{1}]MC-LR, which was considered to confirm the role of microcystins in the development of hepatitis in this this child. Water sampling occurred once a week as part of a monitoring program by the Montevideo authorities. During the exposure period blooms of mainly *Microcystis* with the presence of "foam" (scum) being observed. Faecal coliforms < 1,000 cfu/dL and very high microcystin levels (mean 2.9 mg/L and max 8.2 mg/L).	This study provides extensive details about outcome assessment for cases of severe exposure. Despite the water sampling potentially not being at the exact location as exposure, the detection of microcystins in the explanted liver provided good evidence of exposure. There is no comparator group for this observational case study.

Table 3: (continued)

Study No.	Authors	Summary	Comments
Case Studies			
9	Giannuzzi *et al.*, 2011	*An acute case of intoxication with cyanobacteria and cyanotoxins in recreational water in Salto Grande Dam, Argentina.* This is case report of a 19-year-old man who was accidentally immersed in an intense *Microcystis* sp. bloom for 2 h after falling off his jet ski in lake in Argentina. He swam back to shore and a few hours later began to experience GI symptoms, malaise, nausea, vomiting and muscle weakness. His condition worsened and he was hospitalized and diagnosed with a liver disorder. He was discharged from intensive care after 8 d. Water samples were collected for a quantitative phytoplankton and toxin analysis on the same day and at the same place where the patient was immersed within 4 h of the incident. Total phytoplankton ranged between 33,680 and 35,740 cells/mL. The most abundant species was *Microcystis wesenbergii*, with cell numbers between 30,600 and 31,600 cells/mL. *Microcystis aeruginosa* was also detected in the range of 3,080–4,100 cells/mL. High levels of Microcystin-LR were detected in water samples (48.6 ± 15 µg/L).	The authors indicate that this is the first report an acute case of cyanobacterial poisoning in Argentina due to an accidental exposure of a man to a cyanobacterial bloom with confirmation of the presence of cyanotoxins. No confounders were considered.
10	Slavin, 2008	*The tale of the allergist's life: A series of interesting case reports.* This report is a short paragraph about 2 case reports. The first is a 33-year-old man who experiences severe rhinoconjunctivitis after he fished on inland lakes. The second was a 7-year-old girl who experienced urticaria (hives) and respiratory symptoms while swimming in a lake. The author makes association between a range of possible environmental causes including algae infestation in the lakes	This report provides no significant environmental data to confirm any sort of significant exposure and limited details of outcome assessment.
11	Trevino-Garrison *et al.*, 2015	*Human illnesses and animal deaths associated with freshwater harmful algal blooms – Kansas.* The study summarises a series of case studies from the Kansas Dept of Health and Environment, USA. They received 25 reports of human illnesses potentially associated with freshwater harmful algal blooms in Kansas, USA, in 2011 and this paper reports on 7 of the confirmed human illnesses. Environmental data is provided for only two cases – in one case water analyses on the same day as exposure confirmed cyanobacterial cell concentrations and microcystin toxin levels at a Public Health Warning Level; in the second case the subject fell in the lake that was under a public health Warning also due to the presence of high cyanobacterial cell concentrations and microcystin levels. The predominant cyanobacterial type in the lakes was *Microcystis* spp. Both cases were assessed were severe illness and were medically after admission to hospital emergency departments with one diagnosed with pneumonia and the second with cyanobacteria toxicosis.	The study provided limited environmental data to accompany the reports and determine exposure characterisation. The authors note a healthcare provider may find it difficult to confirm that cyanobacterial toxins are the cause of the illness based upon symptoms alone. Hence under-reporting may have occurred.

Table 4: Marine primary studies included in the risk of bias assessment grouped by study type together with a summary and comments for each study. The study number aligns with the summary of risk of bias assessments in Table 6 and with risk of bias assessments of individual studies given in Appendix 5 of the Technical Report.

Study No.	Authors	Summary	Comments
Cohort Studies			
1	Backer *et al.*, 2003	*Recreational exposure to aerosolized brevetoxins during Florida red tide events.* This cohort study reports personal interviews and pulmonary function tests performed on one group of people that were unlikely to be exposed to aerosolised toxins of *Karenia brevis* (Location: Sarasota, USA) (non-exposure) and a second group that were exposed to aerosolised toxins due to strong onshore winds (Location: Jacksonville, USA). At both locations, the study was conducted over 2-days. One hundred and twenty-nine people participated in the study. Exposure was categorised into three levels: low/no exposure, moderate-exposure, and high-exposure. Nasal-pharyngeal (nose and throat) swabs for cytologic evaluation of epithelial and inflammatory cells and brevetoxin analyses were taken from participants before and after going to the beach in the Jacksonville "onshore" event only, i.e., those who experienced moderate or high exposure. Pulmonary function tests were also performed on participants before and after beach exposure. Seawater samples (11) were collected twice daily determining *K. brevis* cells and brevetoxins. Six air samplers were placed 65m apart in the study area to capture airborne particles for brevetoxin analyses in a grid sample matrix. In Sarasota "offshore" (non-exposure), few people reported symptoms after spending time on the beach. In Jacksonville, on the high-exposure day people reported an increase in lower respiratory symptoms and on the moderate exposure day there was a significant increase in reports of upper respiratory symptoms. Lower respiratory symptoms (e.g., wheezing) were reported by 8% of unexposed people, 11% of the moderately exposed people, and 28% of the highly exposed people. The authors found an inflammatory response in over 33% of these participants and did not find any clinically significant changes in pulmonary function test results; however, they indicate that the study population was small.	The two groups were exposed at different times and different locations – the "Offshore" event at Sarasota in February, 1999 (non-exposure, i.e. "control"); and the "Onshore" red tide event (exposure) in October, 1999 at Jacksonville. The events were therefore separated both in location and in time by 8-months. Individual exposures varied widely during the study, ranging from 10 min to 8h. An issue was raised about whether the symptoms reported at Jacksonville were the result of acute exposure on the day of study or the result of previous periodic exposures since a red tide had been offshore for a week before the study commenced.

Table 4: (continued)

Study No.	Authors	Summary	Comments
2	Bean *et al.*, 2011	*Florida red tide toxins (brevetoxins) and longitudinal respiratory effects in asthmatics.* This cohort study is a collation of 11 studies over 7 years of the longer-term health effects in asthmatics from intermittent (> 1 h) environmental exposure to brevotoxins in Florida (USA). Each asthmatic participated in at least one evaluation during an active *K. brevis* bloom (exposure) and during a period without a bloom (non-exposure). *K. brevis* cell counts were measured in water and brevetoxins were measured in air and water. Thirty-eight participants were involved with only 1 exposure study and 36 participated in $\geq$ 4 studies. The 36 asthmatics participating in ≥4 exposure studies demonstrated no significant change in their standardized percent predicted pre-exposure pulmonary function over the 7 years of the study. These results indicate that stable asthmatics living in areas with intermittent Florida red tides do not exhibit chronic respiratory effects from intermittent environmental exposure to aerosolized brevetoxins over a 7-year period.	Participants self-reported that their asthmatic status had been diagnosed by a physician. Participants had different exposure time periods since they could leave the beach at any time if they felt symptomatic.
3	Cheng *et al.*, 2010	*Personal exposure to aerosolized red tide toxins (brevetoxins).* This cohort study is a report on the suitability of using personal air samplers to monitor exposure of study participants to aerosolised brevetoxins and the correlation in concentrations measured with the personal air samplers and those measured by high-volume samplers. Aerosolised brevetoxins from the personal sampler were in modest agreement with the concentrations measured from the high-volume sampler. Results from the analysis of nasal swab samples for brevetoxins demonstrated 68% positive samples in one sampling event when air concentrations of brevetoxins were between 50 to 120 ng/m as measured with the high-volume sampler. However, they found that there were no statistical correlations between the amounts of brevetoxins detected in the swab samples with either the environmental or personal concentration. Results suggested that the personal sample might provide an estimate of individual exposure level. Nasal swab samples also showed that brevetoxins were inhaled and deposited in the nasal passage during one of the red tide events.	Participants self-reported that their asthmatic status had been diagnosed by a physician. Health effects were reported in Fleming *et al*. (2005; 2007).

Table 4: (continued)

Study No.	Authors	Summary	Comments
4	Fleming *et al.*, 2005	*Initial evaluation of the effects of aerosolized Florida red tide toxins (brevetoxins) in persons with asthma. (Brevetoxins: Mini-Monograph).* The cohort study followed the same 59 asthmatics before and after going to the beach (>1 h) on 3 days with exposure ("exposure") and 3 days without exposure ("non-exposure") to *Karenia brevis* red tide events in the Gulf of Mexico, USA. Data for the exposure and non-exposure days were pooled. To achieve exposure and non-exposure conditions the evaluation was carried out for two separate events separated in time by 2 months. (Non-exposure - Jan 2003; Exposure event - March 2003). Cell counts were made in water samples and brevetoxins were measured in water and air samples. Participants were significantly more likely to report symptoms and have measurable respiratory impairment symptoms after the red-tide exposure event. There was considerable variation in respiratory function during the non-exposure event. Results showed that the participants demonstrated small but statistically significant decreases in forced expiratory volume in 1 sec, forced expiratory flow between 25 and 75%, and peak expiratory flow after exposure, particularly those regularly using asthma medications. Similar evaluation during non-exposure periods did not significantly differ. The study claims to be the first to show objectively measurable adverse health effects from exposure to aerosolized red tide toxins in persons with asthma.	This study involved the same cohort being studied during a non-exposure and an exposure period. Participants self-reported that their asthmatic status had been diagnosed by a physician. *K. brevis* cells were found in the waters at the beach study site even during the "non-exposure" period. Participants were residents of the region, and many had a history of red tide exposure. These participants may have experienced intermittent aerosolised brevetoxin exposure which was unmeasured during the study periods. Furthermore, these residents may have adapted to chronic red tide aerosol exposure. For the exposure days the brevetoxin in the air ranged from <LOD to 36.57 ng/m^3 and in the seawater from 3.31 – 14.01 µg/L. See Backer *et al.* (2005) for more detail about spirometers.

Table 4: (continued)

Study No.	Authors	Summary	Comments
5	Fleming *et al.*, 2007	*Aerosolized red-tide toxins (brevetoxins) and asthma.* This cohort study was part on the on-going evaluation of aerosolised *K. brevis* brevetoxin exposure in Florida, USA, also detailed in Fleming *et al* (2005). The study followed 97 asthmatics before and after going to the beach (>1 h) with exposure ("exposure") and without exposure ("non-exposure") to *Karenia brevis* red tide events. Ninety-seven subjects participated in at least one evaluation during an exposure event (March 2003 or March 2005) and a non-exposure event (January 2003, May 2004 or October 2004). The participants were evaluated by questionnaire and spirometry. The study also involved concomitant environmental monitoring, water and air sampling, and personal monitoring for brevetoxins. After 1h beach exposure to brevetoxins increased respiratory symptoms and decreased respiratory function were observed. The study results reported that participants demonstrated small, but statistically significant, decreases in FEV_1, midexpiratory phase of forced expiratory flow and peak expiratory flow after exposure, particularly among those participants regularly using asthma medications. There were no significant changes in symptoms or respiratory function following 1 h beach exposure in an area without an active *K. brevis* bloom. (i.e. during non-exposure periods).	See comments for Backer *et al.* (2005); Fleming *et al.* (2005). The study includes environmental data from Jan 2003 (unexposed) and Mar 2003 (exposed) which is reported in Fleming *et al.* (2005). It is considered that this study may not be a "new" group of 97 but include data for the 59 asthmatics previously reported in Fleming *et al.* (2005).

Table 4: (continued)

Study No.	Authors	Summary	Comments
6	Fleming *et al.*, 2009	*Exposure and effect assessment of aerosolized red tide toxins (brevetoxins) and asthma.* This cohort study was part on the on-going evaluation of aerosolised *K. brevis* brevetoxin exposure in Florida, USA, detailed in Fleming *et al* (2005; 2007). The study followed of 87 asthmatics before and after going to the beach (>1 h) with exposure ("exposure") and without exposure ("non-exposure") to *Karenia brevis* red tide events. This study examined the possible dose-response relationship between health effects (i.e., reported symptoms and pulmonary function testing (PFT) results) and exposure to brevetoxins measured using personal air samplers, and hourly ambient measurements by ELISA and LC-MS. Strong associations were found between the brevetoxin concentrations measured by the personal air sampler and the hourly ambient measurements. A positive relationship between reported asthma symptoms with both ambient measures. The results showed that after only 1 h of exposure to aerosols containing brevetoxin concentrations at > 57 ng/m^3, asthmatics had statistically significant increases in self-reported respiratory symptoms and total symptom scores. However, they did not find any expected corresponding changes in PFT results, i.e., there was no association between pulmonary function changes and the three brevetoxin measures. There were also significant increases in self-reported symptoms observed for those not using asthma medication and those living ≥ 1 mile from the coast.	See comments for Backer *et al.* (2005); Fleming *et al.* (2005). This paper includes environmental data from March 2005 (exposed) which is reported in Fleming *et al* (2007). The authors note that a major limitation in the interpretation of all asthma literature is the inconsistency in the definition of the response to pulmonary function testing (PFTs). Stemple and Fuhlbrigge (2008) concluded response must be defined as a combination of self-report of symptoms and objective measures. Also, PFTs may have been delayed by hours or even days for some subjects and thus any reported changes in PFT measurements were not associated by immediate testing after exposure.

Table 4: (continued)

Study No.	Authors	Summary	Comments
7	Kirkpatrick *et al.*, 2011	*Aerosolized red tide toxins (brevetoxins) and asthma: Continued health effects after 1 h beach exposure.* This cohort study is another paper associated with the series of related studies from work on "red tides" done in Florida, USA, over several years by the same combination of authors. This study investigated if there were latent and/or sustained effects in asthmatics in the days following the initial beach exposure during periods with without an active Florida red tide. Symptom data and spirometry data were collected before and after 1-h of beach exposure. Subjects kept daily symptom diaries and measured their peak flow each morning for 5-days following beach exposure. Results showed that during non-exposure periods, there were no significant changes in symptoms or pulmonary function either acutely or over 5 days of follow-up. However, after exposure during an active red tide, the subjects had elevated mean symptoms which did not return to the pre-exposure baseline for at least 4 days. In addition, the peak flow measurements decreased after the initial beach exposure, and decreased further within 24-h, and continued to be suppressed even after 5 days. The conclusion therefore was that the greatest mean number of reported symptoms occurred after 1-h exposure to the red tide, and these symptoms lasted for at least 5 days after exposure.	The same cohort was studied during a non-exposure and an exposure period. Participants had different exposure time periods since they could return at any time from the beach if they felt symptomatic. The authors report the handheld peak flow meters used to assess respiratory function are relatively inaccurate. These meters were only used to measure peak flow post 1 h exposure and not prior to exposure. Brevetoxins had been measured inland so it is possible that the subjects were exposed after the 1 h beach exposure.

Table 4: (continued)

Study No.	Authors	Summary	Comments
8	Lin *et al.,* 2016	*A prospective study of marine phytoplankton and reported illness among recreational beachgoers in Puerto Rico, 2009.* This study is a large prospective cohort (n=15,726) study of the relationship between phytoplankton cell counts and self-reported illnesses following recreational exposure at beach over 26 days at Boqueron Beach, Puerto Rico. The study involved using interviews at three time points (Enrolment, Beach exit, Follow-up (10-12d later)) to assess baseline health, water activities, and subsequent illness. Associated water samples were collected daily and quantitatively analysed for phytoplankton cell counts. The interview results were analysed using logistic regression models, adjusted for age and sex, to assess the association between exposure to three categories of phytoplankton concentration and subsequent illness. A summary of the results is as follows: Daily total phytoplankton cell counts ranged from 346 to 2,012 cells/mL (median, 712 cells/mL). The category with the highest (≥ 75th percentile) total phytoplankton cell count was associated with eye irritation [adjusted odds ratio (OR) = 1.30; 95% confidence interval (CI): 1.01, 1.66], rash (OR = 1.27; 95% CI: 1.02, 1.57), and earache (OR = 1.25; 95% CI: 0.88, 1.77). In phytoplankton group-specific analyses, the category with the highest Cyanobacteria counts was associated with respiratory illness (OR = 1.37; 95% CI: 1.12, 1.67), rash (OR = 1.32; 95% CI: 1.05, 1.66), eye irritation (OR = 1.25; 95% CI: 0.97, 1.62), and earache (OR = 1.35; 95% CI: 0.95, 1.93). The conclusion was that an association was found between recreational exposure to total marine phytoplankton cell counts and eye irritation, respiratory illness, earache, and rash at a tropical beach in the absence of an algal bloom.	There was potential for risk of bias associated with exposure assessment. Water sampling was systematic at multiple sites at the beach. Phytoplankton cell counts were performed on a daily composite sample and were quantitatively assayed for both totals and major phytoplankton group counts resulting in a low level of discrimination of potentially toxic or problematic organisms in the analysis. The high-level taxonomic groups used were Cyanobacteria; Dinophyta (dinoflagellates); Bacillariophyta (diatoms); and miscellaneous other groups. The counting protocol involved comprehensive identification of all genera and types, however this data was not used in the logistic regression models. The data was however used to determine associations between major groups and major symptom classes. This showed an association (non-significant) between earache and cyanobacteria. Also, although water samples were analysed for two different cyanotoxins (Debromoaplysiatoxin and lyngbyatoxin-a), there were no detections and concentrations were reported as all <LOD. The authors identified a possibility for responder bias since one adult was allowed to answer questions for all household members.

Table 4: (continued)

Study No.	Authors	Summary	Comments
9	Milian *et al.*, 2007	*Reported respiratory symptom intensity in asthmatics during exposure to aerosolized Florida red tide toxins.* This cohort study represents a further paper in the series of work done on "red tides' in Florida, USA. It was comprised of a study of 97 asthmatics before and after going to the beach (>1 h) with (exposure) and without (non-exposure) to *Karenia brevis* red tide events. *Karenia brevis* cell counts were measured in seawater and brevetoxins were measured in seawater and air. Participants were evaluated utilizing questionnaires and pulmonary function testing before and after a 1-h beach walk. Respiratory symptom intensity scores were determined using a modified Likert scale. Asthmatics reported increased respiratory symptom intensity after 1-h exposure, while no change in respiratory symptom intensity was reported during non-exposure.	This study was different to earlier investigations by this group in that they attempted to examine the intensity of these self-reported symptoms in asthmatics. Previous studies only examined the report of a respiratory symptom if the participant reported no symptoms prior to exposure to red tide. The study showed that in asthmatics, respiratory symptom intensity increased during a 1-hour exposure to Florida red tide, while respiratory symptom intensity did not change significantly after a 1-hour beach walk when unexposed to Florida red tide. An issue in this study that relates to definitions of exposure in the study design. The study reported that both *K brevis* cells and brevetoxins were also present during what was defined as the non-exposure study periods: "the *K. brevis* cell counts in this area of the Gulf of Mexico were between < 1,000 and 6,000 cells/L, and the concentrations of brevetoxins in the water ranged from < 0.01 to 0.20 μ m/L. The concentrations of brevetoxins in the aerosol did not exceed 0.2 ng/m^3 but were often much lower. During exposure study periods, there were *K. brevis* cell counts between 14,000 and 200,000 cells/L in the water; the concentrations of brevetoxins in the water ranged from 0.50 to 29.20 μ m/L; and the concentrations of brevetoxins in the aerosol from 0.02 to 76.6 ng/m^3 (with higher levels during direct onshore winds)". There was approximately an order of magnitude difference in the exposure agent between exposed and non-exposed periods, which may suggest a threshold, however the importance of this is unknown.

Table 4: (continued)

Study No.	Authors	Summary	Comments
10	Morris Jr *et al.*, 2006	*Occupational exposure to Pfiesteria species in estuarine waters is not a risk factor for illness.* This cohort study reports a study of 107 persons ("Watermen") who had regular, occupational exposure to the Chesapeake Bay, over 4 summer "seasons". Participants self-reported exposure to any type of known chemical toxicants and selected symptoms provided to them based on "possible estuary-associated syndrome". A neuropsychological screening was performed on participants pre- and postseason for 4 y. *Pfiesteria* and other harmful algal blooms were measured in water samples as part of an ongoing monitoring program. There were no significant differences in performance for several neuropsychological tests when exposed and unexposed watermen were compared. The Conclusions reached were that "although high-level or outbreak-associated exposure to *Pfiesteria* species (or specific strains within a species) may have an effect on health, routine occupational exposure to estuarine environments in which these organisms are present does not appear to pose a significant health risk."	The exposure data for *Pfiesteria* in this study was not quantitative and was only recorded as positive or negative based upon number of samples positive for *P. piscicida* and *P. shumwayae* based upon a PCR-test. In addition, the exposure assessment was based around a routine ongoing monitoring program by the Maryland Department of Natural Resources during 1999 – 2002 where samples were obtained from the tributaries where the enrolled watermen worked. The overlapping study participant work area grids and water monitoring grids did not provide certainty regarding the temporal overlap of work exposure and *Pfiesteria* detection. *Pfiesteria* was monitored using a PCR test that detected strains that had both toxic and nontoxic phenotypes. Absence of human health effects may have been due to lack of toxic *Pfiesteria* strains during the study period. The sampling protocol was modified part-way through the study to improve the assessment of specific exposure at the workplace. The revised protocol involved potentially exposed cohort members from three general areas taking water samples before departing their work area at the end of the day. In 2001, watermen collected samples on a biweekly basis (*n*= 426), and in 2002, on a weekly basis (*n*= 1,677).

Table 4: (continued)

Study No.	Authors	Summary	Comments
11	O'Halloran *et al.*, 2017	*Respiratory problems associated with surfing in coastal waters.* This cohort study reports from a pilot project to examine the health status and possible adverse health effects associated with seawater exposure (microbial water-quality indicators and phytoplankton abundance and their toxins) of surfers in California, USA. Forty-eight surfers enrolled in the study conducted over 8 months and completed an initial health background survey and weekly health surveys online. Symptoms were self-reported via the surveys. Their most common health problems reported by the respondents were allergies and asthma. During the study, 10% of the surfers reported gastrointestinal symptoms and 29% reported upper respiratory symptoms. This study suggests surfers were significantly more likely to report upper respiratory symptoms when they had a history of allergies, housemates with upper respiratory symptoms, and/or a history of previous adverse health symptoms while surfing during a "red tide" (i.e. an event often associated with the presence of phytoplankton toxins).	The authors note the retrospective report of adverse health effects after exposure was a weakness. They also note that confounding factors that may have been responsible for the adverse health outcomes, such as local wildfires and aerial pesticide spraying that were not considered. Exposure assessment was based around a sampling program from weekly samples from the end of a wharf over the 8-months of the study to determine chlorophyll *a,* phytoplankton cell concentrations of *Pseudo-nitzschia australis* and *Alexandrium catenella* and domoic acid toxin (Domoic Acid produced by *P. australis*). While these samples were in the Monterey Bay area, they were not necessarily representative of the surfers' exposure zone.

Table 4: (continued)

Study No.	Authors	Summary	Comments
12	Backer *et al.*, 2005	*Occupational exposure to aerosolized Brevetoxins during Florida red tide events: Effects on a healthy worker population.* Study of 28 lifeguards who performed spirometry tests and reported symptoms before and after an 8-hour shift when there was no red tide (unexposed period) and again when there was a red tide (exposed period). *Karenia brevis* cell counts were measured in seawater and brevetoxins were measured in seawater and air. The group of lifeguards reported more upper respiratory symptoms during the exposed periods. Compared with non-exposure periods the lifeguards reported more upper airway but not lower airway discomfort during the red tide exposure periods.	The same cohort was studied during a non-exposure and an exposure period. The comparison was therefore the same group at different times. Symptoms were self-reported. However, exposure status (environmental analyses) was not known at time of collection of symptom data, making it less likely that study participants could influence results. Spirometry tests have limitations since it is almost impossible to reproduce 3 spirograms within the guidelines without maximal effort. A limitation was associated with characterising aerosol exposure measurement. This covered in authors statement that: "the traditional approach to individual occupational exposure assessment would be to have the lifeguards wear the personal samplers. However, there was concern that the personal samplers would interfere with emergency response activities or be destroyed by immersion in seawater. Instead, personal exposure was measured by placing personal samplers……. on the lifeguard towers near the lifeguards' breathing zones".

Table 4: (continued)

Study No.	Authors	Summary	Comments
Observational Studies			
13	Gallitelli *et al.*, 2005	*Respiratory illness as a reaction to tropical algal blooms occurring in a temperate climate.* This paper is a short 3-page research letter. Over two summers, 28 people reported a range of symptoms (respiratory, irritation and fever) during recreational or working activities on a beach where a 'mild macroalgal mucilage was floating on the water'. Complaints occurred concurrent with the algal blooms and disappeared when the *Ostreopsis* population decreased.	Exposure characterisation was limited as phytoplankton presence/abundance was measured at three days after the onset of symptoms during both summers. Results are reported only as: "an unusual proliferation of the tropical microalga *Ostreopsis* genus (more than 1 million cells/L) during both episodes."
14	Osborne *et al.*, 2007	*Health effects of recreational exposure to Moreton Bay, Australia waters during a Lyngbya majuscula bloom.* This study is a report of a postal survey of residents in Queensland who live in an area subject to annual toxic cyanobacterial (*Lyngbya majuscula*) blooms. The authors summary of the study findings was: "Of those having marine recreational water activity, 34% reported at least one symptom after exposure to marine waters, with skin itching the most reported (23%). Younger participants had greater water exposure and symptoms than older participants. Participants with greater exposures were more likely to have skin and eye symptoms than less exposed groups, suggesting agents in the marine environment may have contributed to these symptoms. Of those entering Moreton Bay waters 29 (2.7%) reported severe skin symptoms, 12 of whom attended a health professional. Six (0.6%) reported the classic symptoms of recreational water exposure to *L. majuscula*, severe skin symptoms in the inguinal region. Participants with knowledge of *L. majuscula* were less likely to report less skin, gastrointestinal and fever and headache symptoms. In conclusion, high numbers of participants reported symptoms after exposure to waters subject to *L. majuscula* blooms but only a small number appeared to be serious in nature suggesting limited exposure to toxins".	Limitations for this study were: 1. The outcomes given were self-reported symptoms. 2. There was no concurrent or reported exposure characterisation associated with the survey period. This was even though the survey covered 7-months (January to July) since previously this was when blooms of *L. majuscula* had occurred. 3. Authors note the possibility of non-respondent bias was potentially high. This is because postal survey was mailed to 5,000 residents with a response rate of 27%. High numbers of people (78%) responding to the survey reported recreational water activity in Moreton Bay. However, the demographics of the respondents generally resembled the Australian Bureau of Statistics population data for Bribie Island, Queensland.

Table 4: (continued)

Study No.	Authors	Summary	Comments
15	Osborne and Shaw, 2008	*Dermatitis associated with exposure to a marine cyanobacterium during recreational water exposure.* This study represents an investigation of data from the collation of 176 presentations to first aid stations on Fraser Island, Queensland for the summers of 1998-2001. These years were selected as there were anecdotal cases reported in the summer of 1998. The majority (81%) of *Lyngbya*-like symptoms occurred over a 7-week period in Jan – Feb 1998. The authors conclusions are principally by association that "during a bloom of *L. majuscula* there were numerous reports of symptoms that could be attributed to dermotoxins found in *L. majuscula*. The other four years examined had no *L. majuscula* blooms and the number of *L. majuscula* symptoms was much reduced."	Exposure characterisation and assessment was based solely upon National Parks staff reporting *Lyngbya* being present in early 1998 and not afterwards. Signs had been erected warning of 'harmful algae' at a location where *Lyngbya*-like symptoms were reported.
16	Tichadou *et al.*, 2010	*Health impact of unicellular algae of the Ostreopsis genus blooms in the Mediterranean Sea: experience of the French Mediterranean coast surveillance network from 2006 to 2009.* This paper is a collation of clinical and medical data collected by the French Mediterranean Coast *Ostreopsis* Surveillance Network from 2006 to 2009. The network operates June 15 to Sept 15 each year, which is the most favourable time for *Ostreopsis* blooms. Results given were that a total of 47 patients presented symptoms of involving benign or mild skin, mucosal, and/or respiratory irritation that regressed spontaneously without treatment within 12–72 h (4–12 h with nonsteroidal anti-inflammatory drugs). Clinical findings observed after direct exposure to *O. ovata* were variable. Skin irritation was the most common manifestation. Outcome assessment is detailed since it is medically diagnosed but authors note there was likely under diagnosis, particularly when there are low concentrations of *O. ovata* in the water and it remains mainly attached to macrophytes.	The authors note that the nonspecific nature of clinical manifestations probably resulted in under-diagnosis and thus under-reporting. Only cases in which *Ostreopsis* was considered a plausible cause were included based on the identification of compatible clinical features in at least 2 persons in a location where a bloom was demonstrated. Timely exposure characterisation is limited/poor as seawater and/or macrophyte analyses could only be done the day after symptoms are reported and several hours may elapse between occurrence of symptoms and reporting to the poison control centre. *Ostreopsis* blooms can last only a few hours so the delay in sampling may miss a bloom occurrence.

Table 4: (continued)

Study No.	Authors	Summary	Comments
Case Studies			
17	Honner *et al.*, 2010	*Bilateral mastoiditis from red tide exposure.* This paper is a short 4-page clinical communication. Case report of a 53-year-old woman presenting with bilateral mastoiditis four days after scuba diving during red tide algal bloom in California, USA. Authors indicated that levels of coliform bacteria recorded at the time and location of her dive exceeded health regulatory limits and correlate with her atypical culture results. They conclude that the elevated bacterial counts that result from harmful algal blooms may account for this rare infection.	The study has detailed information about the health assessment. The only environmental data to accompany the exposure period and location is from weekly monitoring of ocean levels of total bacteria, faecal bacteria and enterococci. Two days prior to the woman scuba diving the faecal bacteria and enterococci levels exceeded regulatory limits.
18	Lee *et al.*, 2009	*Surfer's asthma.* This paper is a short 3-page clinical communication. The case report is of a 42-year-old man with a 2 year history of respiratory symptoms that were associated with surfing in California, USA. He had no difficulties while he surfed but symptoms were noted 2-3 h later. The symptoms would last 1-2 days and then self-resolve. He reported the association with symptoms on days when he saw the red tide glow and less so during the seasons that were not associated with red tides.	The study has no environmental data to accompany the exposure period, and the only observations made by the subject were reported. It therefore represents a potential association with red tide only with no sampling-based exposure characterisation.
19	Namendys-Silva *et al.*, 2018	*Acute respiratory distress syndrome potentially caused by respiratory syncytial virus and a diatom.* This study is a short 1-page case report of a 56-year-old man reporting with a 7 d-history of fever and dyspnea and hypoxemic respiratory failure, Mexico. A microorganism (compatible with a marine diatom) was found in the bronchoalveolar lavage sample	The report has no environmental exposure data given and no identification of the diatom.
20	Reddy *et al.*, 2019	*A rare case of hypersensitivity pneumonitis due to Florida red tide.* This study is a short 3-page case report of a 50-year-old man presenting with a 4-week history of progressively worsening breathlessness in Florida, USA. The symptoms began after he swam into a large area of red tide.	The report has limited environmental data for any suitable exposure characterisation. The study presents state records of *Karenia brevis* cell concentration data integrated for a 1-month period from the Florida Fish and Wildlife Commission monitoring program at the same time as the incident.

Table 4: (continued)

Study No.	Authors	Summary	Comments
21	Steensma, 2007	*Exacerbation of asthma by Florida red tide during an ocean sailing trip.* This study is a short 2-page case report of a 36-year-old man reporting respiratory symptoms that began during a coastal ocean sailing excursion, in Florida, USA. Before the sailing excursion the patient's symptoms were well controlled. During the week of the sailing trip government and county departments reported very high cell counts of *K. brevis* (> 1 million cells/mL). During the day sailing trip, the boat criss-crossed the thick bloom of red tide. The patient's symptoms began about 20 m from the edge of the bloom and dissipated a few minutes after crossing the bloom area.	This study had limited environmental data for exposure characterisation. Cell concentrations of *Karenia brevis* in the area of the sailing trip during the week of the incident and exposure came from data from the Florida Fish and Wildlife Commission monitoring program.
22	Werner *et al.*, 2011	*Lyngbya dermatitis (toxic seaweed dermatitis).* This study is a short 3-page case report of a 13-year-old girl presenting with dermal irritation 1 d after swimming in rough surf conditions in Hawaii, USA. The case was reported as having the typical histopathological findings of *Lyngbya* dermatitis.	This report has no environmental monitoring data to allow for exposure characterisation.

Table 5: Overall risk of bias assessment (body of evidence by study type) for the freshwater studies (protocol adapted from OHAT Handbook, OHAT, 2019). Study numbers correspond to studies listed in Table 3.

Bias Domains & their associated risk of bias Questions	**Cohort Studies**					**Observational Studies**			**Case Studies**		
Freshwater Study Number[1.]	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Selection bias											
1. Randomization											
2. Allocation concealment											
3. Appropriate comparison groups	--	--	--	--	++	--	++	--			
Confounding bias											
4. Confounding (design/analysis)	-	--	--	--	--	--	--	--	--	--	--
Performance bias											
5. Identical experimental conditions											
6. Blinding of researchers during study											
Attrition/Exclusion bias											
7. Missing outcome data	+	++	--	--	--	--	--	++			
Detection bias											
8. Exposure characterisation	--	--	--	--	-	--	--	--	+	--	--
9. Outcome assessment	--	--	--	--	--	--	--	++	++	--	--
Selective Reporting bias											
10. Outcome reporting	+	+	-	--	++	N/A	N/A	N/A	++	N/A	N/A
Other sources of bias											
11. Other threats	--		-						++		

Definitely low risk of bias	++	Probably low risk of bias	+	Probably high risk of bias	-	Definitely high risk of bias	--

[1] Refer to Appendix 5 of the Technical Report for study details and full risk of bias assessment of individual studies.

Table 6: Overall risk of bias assessment (body of evidence by study type) for the marine studies (protocol adapted from OHAT Handbook, OHAT, 2019). Study numbers correspond to studies listed in Table 4.

Bias Domains & their associated risk of bias Questions	Cohort or Prospective Studies												Observational Studies				Case Studies					
Marine Study Number[1.]	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
Selection bias																						
1. Randomization																						
2. Allocation concealment																						
3. Appropriate comparison groups	-	--	--	--	--	--	--	--	--	++	--	-	--	++	--	--						
Confounding bias																						
4. Confounding (design/analysis)	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Performance bias																						
5. Identical experimental conditions																						
6. Blinding of researchers during study																						
Attrition/Exclusion bias																						
7. Missing outcome data	++	++	N/A	++	++	++	++	-	++	++	++	++	++	--	--	--						
Detection bias																						
8. Exposure characterisation	--	--	-	--	--	--	--	--	-	--	--	--	--	--	--	--	--	--	--	--	--	--
9. Outcome assessment	--	--	N/A	--	--	--	--	--	--	--	--	--	-	--	--	--	++	-	-	-	-	-
Selective Reporting bias																						
10. Outcome reporting	++	-	++	--	-	-	++	-	++	++	++	++	++	++	-	--	++	++	++	++	++	++
Other sources of bias																						
11. Other threats	--	-	--	--	--	--	--	++	--			--		--			N/A	N/A				

Definitely low risk of bias	++	Probably low risk of bias	+	Probably high risk of bias	-	Definitely high risk of bias	--

[1] Refer to Appendix 5 of the Technical Report for study details and full risk of bias assessment of individual studies.

5.1.3 Assessment of Certainty in the Body of Evidence for the Primary Studies

As outlined in the Methodology (Section 2.8 of the Technical Report) a process based on the OHAT (2019) approach to using the GRADE system was used to assess the certainty of the body of evidence.

In the Research Protocol (also see Section 2.8 of the Technical Report) it was anticipated that the evidence streams for the following four topics would be listed together in a summary table for GRADE assessment: freshwater pelagic cyanobacteria and toxins (human exposure); freshwater benthic cyanobacteria and toxins (human exposure); marine algae and cyanobacteria and toxins (human exposure); algae or cyanobacteria and toxins (animal exposure). However, this approach was changed based upon the lack of reported information regarding benthic vs pelagic cyanobacteria in freshwater. Instead, the evidence streams for human health effects from recreational water exposure to freshwater and marine cyanobacteria and algae were grouped together respectively (Table 7).

It was also decided that separating out human health outcomes from the available studies would be very difficult given the nature and low quality of the available evidence and was not attempted. Further analysis and evaluation of the primary studies by the Committee can be undertaken if required.

The review had specified that animal studies for recreational water exposure to cyanobacteria and algae would be included in the certainty assessment. However, upon further discussion with NHMRC, it was clarified that animal studies were excluded from the primary research question, which related to human health exposure and outcomes only. Instead, the included animal studies (specifically for dogs) were collated and summarised for Secondary Question 5 (see Section 5.1.3.5) and not evaluated further. Similarly, the remaining secondary questions did not undergo quality or certainty assessment and were collated and summarised for the Committee to consider as supporting information for the Guidelines. Any further appraisal or analysis of this information by the Committee can be undertaken if required.

Initial confidence ratings

Each evidence stream was assigned an initial certainty rating similar to that described in the OHAT Handbook (OHAT, 2019). Cohort studies are categorised in the OHAT Handbook as ‘low to moderate certainty’; however, based on the types of studies found for this research topic, they were downgraded to an initial rating of ‘low certainty’ due to a lack of appropriate comparison groups. Observational studies were also initially graded as low certainty. Case studies (case reports) were categorised as ‘very low certainty’ due to the lack of control/comparison groups and lack of exposure characterisation.

Risk of bias

There was a clear and consistent pattern in the types of bias in all of the marine and freshwater studies reviewed here that led to weaknesses overall in study quality and in the resulting body of data (see Table 7). As discussed in Section 5.1.2, the majority of the studies suffered from shortcomings in some of the major bias domains including:

- failing to include suitable comparators or control groups
- not considering potential confounders (i.e., factors or causes for adverse outcomes other than cyanobacteria, algae or toxins)
- not adequately accounting for exposure characterisation for these organisms and compounds in an environmental setting
- many studies had a reliance on self-reporting as part of outcome assessment.

These limitations in design reflect that none of the studies reviewed were designed as randomised control trials or similar clinical trials. Only about 50% of both the freshwater and marine and studies were cohort studies, with the remainder being observational and case studies. As a consequence, all of the studies reviewed by the risk of bias assessment were determined to have an overall "definitely high risk of bias". This resulted in a rating of 'very serious' across all study types and outcomes.

Unexplained inconsistency

A large amount of heterogeneity was observed across the body of evidence for each outcome; however, this can be explained by the inconsistent nature of the exposure scenarios for recreational water exposure (different recreational water exposures, durations, locations and types) and study designs (if available). This resulted in a rating of 'not serious' across all study types and outcomes.

Indirectness

Most of the included studies were relevant to the primary research question and the populations and recreational exposure types could be assessed for Australian settings. However, the included studies (all types) did not fully characterise recreational water exposure at the time or location of the exposure event in a way that would directly link recreational water exposure to any self-reported or clinically diagnosed health outcomes. This resulted in a rating of 'serious' across all study types and outcomes. However, the certainty of the body of evidence was not downgraded further as this issue had already been considered as part of risk of bias assessment.

Imprecision

Due to the low quality of the available evidence and the types of outcomes reported it was difficult to know how to assess the statistical significance of the findings across the body of evidence and was not attempted. This resulted in a rating of 'unknown' across all study types and outcomes.

Publication bias

Publication bias was not detected.

Reasons for upgrading

There was insufficient information to determine if there were any further reasons to upgrade the certainty of the overall body of evidence from 'very low certainty' using the GRADE system.

Overall certainty rating

An overall certainty rating was assigned to each evidence stream as 'very low confidence' across all study types. This was based on downgrading any evidence streams with an initial 'low' or 'very low' confidence rating to 'very low' across the board for serious risk of bias.

These shortcomings considered together led to the conclusion that there was insufficient confidence in the findings of the available studies. It is worth noting that methods and approaches for systematic reviews of environmental health evidence is still an area of research and development, and further modification of the available frameworks and tools is beyond the scope of services required for this review.

Table 7: Summary of findings – Body of Evidence (adapted from OHAT, 2019)

Body of evidence	**Risk of bias**	**Unexplained inconsistency**	**Indirectness**	**Imprecision**	**Publication bias**	**Magnitude of effect**	**Dose Response**	**Residual confounding**	**Consistency across species/ model**	**Other reason to increase confidence?**	**Final certainty rating**
Evidence stream or study type (# studies) *Initial certainty rating (OHAT,2019)*	*Serious, not serious, unknown* Describe trends, key questions, issues	*Serious, not serious, not applicable (NA)* Describe results in terms of consistency, explain apparent inconsistency	*Serious or not serious, NA* Discuss use of upstream indicators or populations with less relevance, any time-related exposure considerations	*Serious, not serious, unknown, NA* Discuss ability to distinguish treatment from control, describe confidence intervals (if available)	*Detected, undetected* Discuss factors that might indicate publication bias (e.g., funding, lag)	*Large, not large, unknown, NA* Describe magnitude of response or strength of association	*Yes, no, unknown* Outline evidence for or against dose response	*Yes, no, unknown* Address whether there is evidence that confounding would bias toward null	*Yes, no, NA* Describe cross-species, model, or population consistency	*Yes or no* Describe any other factors that increase confidence in the results	***High, moderate, low or very low*** List reasons for down-grading or upgrading
Primary research question: What is the risk of adverse health outcomes from exposure to cyanobacteria and algae in recreational water?											
Body of Evidence for Primary Research Question: *Any human health effects from recreational exposure to cyanobacteria and algae in* ***fresh water***											
Cohort studies (5) **Low certainty** (decreased certainty as may or may not have appropriate comparison groups) **Observational studies (3)** **Low certainty** **Case studies (3)** **Very low certainty**	Very serious **Downgrade** Definitely high risk of bias across all evidence streams due to: • lack of suitable comparators or controls • confounders • inadequate exposure characterisation • self-reported outcomes or recollection of exposure	Unknown Observational studies with different study designs, population groups and exposures explain inconsistency across body of evidence.	Serious Most studies did not fully characterise recreational water exposure at time or location of event. Not downgraded further as this is already considered as part of risk of bias assessment	Unknown	Undetected	Not large or unknown Cohort studies found minimal to null effects only.	Unknown Unable to determine dose response without full exposure datasets (clinical and environmental)	Unknown	NA Animal studies and models not included in review	No	**Very low certainty** **Downgraded once for very serious risk of bias concerns**

Table 7: (continued)

Body of evidence for Primary Research Question: *Any human health effects from recreational exposure to cyanobacteria and algae in* ***marine water***											
Cohort or prospective studies (12) **Low certainty** (initial certainty decreased as may or may not have appropriate comparison groups) **Observational studies (4)** **Low certainty** **Case studies (6)** **Very low certainty**	Very serious **Downgrade** Definitely high risk of bias across all evidence streams due to: • lack of suitable comparators or controls • confounders • inadequate exposure characterisation • self-reported outcomes or recollection of exposure	Unknown Observational studies with different study designs, population groups and exposures explain inconsistency across body of evidence.	Serious Most studies did not fully characterise recreational water exposure at time or location of event. Not downgraded further as this is already considered as part of risk of bias assessment	Unknown	Undetected	Unknown	Unknown Unable to determine dose response without full exposure datasets (clinical and environmental)	Unknown	NA Animal studies and models not included in review	No	**Very low certainty** **Downgraded once for very serious risk of bias concerns**

5.1.4 Secondary Questions

5.1.4.1 Secondary Question 1

What are the indicators/surrogates of this/these hazard/s? What are the advantages and disadvantages of using surrogates versus monitoring specific toxins?

Secondary Question 1 was addressed by a review of selected reviews. These publications were selected by the reviewer based upon his specialist subject knowledge in the topic of monitoring and management of cyanobacteria. Seven publications were included in the assessment. These were Chorus and Testai (2021); Fastner and Humpage (2021); Ibelings *et al.* (2021); Health Canada, (2020); Lu *et al.*, (2019); Srivastava *et al.*, (2013); Zamyadi *et al.*, (2016). The publications chosen were by authoritative experts, were mostly recent and up-to-date and contained comprehensive information on specific components of the question in the context of cyanobacterial toxin monitoring and the use of surrogates. The papers by Chorus and Testai (2021); Fastner and Humpage (2021); and Ibelings *et al.* (2021) were from the recent WHO sponsored publication which is intended to be a manual on all aspects of management of toxic cyanobacteria (Chorus and Welker, 2021). These three publications were selected specifically as they contained extensive compilations of the toxin content of cyanobacteria and in particular all currently published ranges of cell toxin quotas in terms of cell numbers and biovolumes as they relate to monitoring and guidelines. Health Canada, (2020) also represents a recent and thorough technical assessment of cyanobacterial monitoring for recreational water management. Lu *et al.*, (2019) covers aspects of molecular techniques for monitoring toxic cyanobacteria in the context of implementing management frameworks. Srivastava *et al.*, (2013) is a slightly older publication but is a comprehensive review of monitoring approaches for toxic cyanobacterial blooms which discusses all available surrogates. Zamyadi *et al.* (2016) is a more recent review of monitoring technologies for real-time management of cyanobacteria, which specifically focusses on the use of fluorescence techniques to measure pigments as surrogates for cyanobacteria.

In addition, as part of the grey literature search, a broad range of information was found in relation to indicators or measures that were used as surrogates for toxin hazards in a range of published guideline values. This information is given in Table 20 in Section 3.4.2 of the Technical Report and provides a comprehensive overview of current usage and application across jurisdictions. The three surrogates that were used in published guidelines were cell counts, chlorophyll-a concentration and biovolume measurement.

The review of the selected publications and grey literature indicated that the surrogates that are employed widely for monitoring cyanobacteria and cyanotoxins (not just in guidelines) are cyanobacterial cell counts, biovolume and the measurement of chlorophyll-a and phycocyanin pigments. The surrogate most-commonly used in guidelines is cell counts. Cell counts is the only measurement used in any marine recreational guidelines and are used in freshwater guidelines by 13 US and 12 non-US jurisdictions (see Table 20 in the Technical Report). Recently however, WHO removed cell counts from their guidelines (Chorus and Testai, 2021). Three jurisdictions use cell counts only in their guidelines, namely Czech Republic and the US states of Connecticut and Idaho. Chlorophyll-a is used in the guidelines for two US and five non-US jurisdictions while biovolumes are only used by non-US (8) jurisdictions. Phycocyanin is not used in any guideline.

The advantages and disadvantages of these surrogates for monitoring of cyanobacteria and cyanotoxins are summarised in Table 8 and are discussed below.

While cell counts are widely used in guidelines and in the water industry (Lu *et al.*, 2019), a significant drawback for this measurement is the potentially long delay required for providing results due to time requirements for sample collection, transportation, laboratory analysis and reporting and this can further lead to delays in informing management response and actions (Lu *et al.*, 2019). Another disadvantage of cell count measurement is associated with the diversity in the range of shapes and sizes of cyanobacterial cells (Wood et al., 2008 in Health Canada, 2020). This can result in very large differences in estimates of cyanobacterial biovolume and hence toxin quantity for equivalent cell count values of different species.

Depending upon the types of cyanobacteria present, cyanobacterial cell concentrations could exceed the guideline value with no visual evidence of a planktonic bloom. Therefore, when using total cyanobacterial cell counts, it is important to also consider the types of cyanobacteria that are being identified and where possible, their potential for toxin production (Health Canada, 2020). In addition, when total cell counts are decreasing during the dissipation of a bloom, there may still be high levels of cyanotoxins present as the intracellular toxins are released from the dying cells into the surrounding waters. This is important for toxins that are usually contained within intact cells, such as microcystins, but is less of a concern for other toxins, such as cylindrospermopsin, that are released naturally from healthy cells irrespective of cell lysis (Health Canada, 2020).

Recently, the WHO discontinued the use of cell numbers in the setting of guidance or Alert Levels for recreational exposure and moved to the use of biovolumes. This change "reflects experience with cell numbers leading to undue restrictions of recreational use if the dominant cyanobacteria are species with very small cells: as toxin concentrations relate to biomass rather than numbers, even at high cell numbers of very small cells water is clear and toxin concentrations are negligible" (Chorus and Testai, 2021). Others also note that there was no relationship between cell counts and cyanotoxin concentrations for *Planktothrix rubescens* (Manganelli *et al.*, 2010), *Cylindrospermopsis raciborskii* (Veal *et al.*, 2018) and *Microcystis* spp. (Backer *et al*., 2010). Backer *et al*. (2010) found cell counts and toxin concentrations in the water were not well correlated and in open water they found large spatial variability in cyanobacterial cell and toxin concentrations. They concluded that this information individually and in combination was not likely to provide good estimates of human exposure.

The high variability in toxin cell quotas (toxin content per cell) between individual clones within natural populations is one of the major considerations and a potential limitation for the use of cell counts as a surrogate for cyanotoxin monitoring. Fastner and Humpage (2021) reviewed the available data related to the variability in cellular microcystin content and state that "Microcystin contents in isolates (cultures) of *Microcystis* and *Planktothrix* range over more than two orders of magnitude, from below 100 µg up to more than 10 mg/g dry weight, from traces up to 20 µg/mm^3 biovolume and from a few to around 1,000 fg/cell" (Fastner and Humpage, 2021). Furthermore, they note that environmental factors such as temperature, light, pH, macronutrients, trace elements and salinity can affect the microcystin content or cell quota (Fastner and Humpage, 2021). Ibelings *et al.* (2021) reinforced the variability of cell toxin quotas and concluded that in natural waterbodies individual clones in the cyanobacterial biomass show diverging dynamics. Consequently, there is large variation in average toxin content and the toxin concentration is partly uncoupled from the total cell number. They concluded that accurate predictions of cyanotoxin concentrations from cyanobacterial biomass are limited, even in intensively studied waterbodies.

In this context it must be noted that the selection of published cell quotas for use in guideline derivations for cell counts (Table A6-3; Appendix 6 in the Technical Report) can lead to potentially arbitrary estimates of risk if not related preferably to local data which is strongly recommended for calibration of the toxin cell quota estimates (Chorus and Testai, 2021). Examples of the difference in

cell quotas used in the development of some national guidelines which would result if different estimates of risk based upon cell counts if applied arbitrarily are the Australian, Canadian and New Zealand values. The Australian and Canadian guidelines used a toxin cell quota of 2×10^{-7} µg total microcystins/cell while New Zealand uses quite a different value of 6.3×10^{-7} µg total microcystins/cell. The Australian cell quota was based upon data from a toxic Australian bloom, and this was adopted by the Canadian document, whereas the New Zealand value was based upon their own local data. These examples of the variation in these published values for the development cell number surrogates for toxin risk in guidelines may result in overly conservative estimates or alternatively may underestimate the risk if not calibrated with local data.

Cyanobacterial biovolume is a measure of the planktonic cyanobacterial biomass in a water sample. Biovolume is a more accurate indicator of the cyanobacterial biomass than total cyanobacterial cell counts since this measurement accounts for the surface area of the cell, as well as the mass of all cellular material, or cellular biomass (Saccà, 2016). The use of biovolume measurement, as opposed to total cyanobacterial cell counts, accounts for variable sizes of cells of different types and means that cyanobacteria with small cells do not have a large impact on the calculated measure of biomass. Cyanotoxin concentrations have been found to relate more directly to cellular biomass than to cell numbers (Ibelings *et al.*, 2014; Dong *et al.*, 2016). However, similarly to total cyanobacterial cell counts and depending on the cyanotoxins present, the cyanotoxin concentrations may be high during and immediately following the dissipation of a bloom when the biovolume measurements are likely to be low (Health Canada, 2020). Furthermore, it must be recognised that the first step in determining biovolume is the measurement of cell counts, so the issues of delays in the provision of results to inform management response and actions when there is a bloom still applies equally for biovolume measurements and cell counts. Ibelings *et al.* (2021) reviewed available data related to biovolumes and recommended 3 µg microcystins/mm^3 biovolume as a conservative estimate for setting guidelines and state that this value is not likely to be exceeded in field samples.

Chlorophyll-a has historically and frequently been used as an index for eutrophication. It can be used as part of a cyanobacterial alert system to trigger further investigation and actions (Chorus and Bartram, 1999). Chlorophyll-a is particularly useful if it can be combined with brief qualitative microscopy to assess whether or not the majority of the phytoplankton is comprised of cyanobacteria (Ibelings *et al.,* 2021). Chlorophyll-a measurement has an advantage over other biomass indicators in that the method for detection is simpler and in-situ methods are available allowing for greater temporal and spatial coverage with less expense and effort (Health Canada, 2020). However, chlorophyll-a content of phytoplankton may vary in response to light and nutrient availability by up to a factor of 10 (Ibelings *et al.,* 2021). Ibelings *et al.* (2021) recommended that a maximum ratio of 1 µg microcystins/ µg chlorophyll-a would be a conservative approach, and in most cases the measured microcystin concentrations would be considerably lower than estimations based upon this value.

Phycocyanin, is a photosynthetic accessory pigment found only in cyanobacteria in addition to chlorophyll-a and has also been investigated as a possible specific parameter for cyanobacterial monitoring. Concentrations of these two pigments are highly correlated and, similar to chlorophyll-a, positive correlations have been observed between phycocyanin content and cyanobacterial biomass (Health Canada, 2020). The presence of known microcystin producers has been shown to correlate strongly with phycocyanin concentrations (Oh *et al.*, 2001); however, it does not directly relate to cellular microcystin content as all cyanobacteria possess this pigment (Health Canada, 2020).

Fluorescence probes for chlorophyll-a and/or phycocyanin have been developed and are now widely used for monitoring and have the advantage over traditional enumeration methods of being easily applicable in the field, allowing for continuous and on-line monitoring of blooms to allow for the

provision of instantaneous information (Srivastava *et al.*, 2013). It is important to note that probes provide an estimate of cyanobacterial and/or algal biomass overall and phycocyanin sensors cannot distinguish between different cyanobacterial types or species (Zamyadi *et al.*, 2016). In addition, the disadvantages of these measurements are that both chlorophyll-a and phycocyanin content may vary with species and metabolic state of cells, and the presence of other accessory pigments or suspended particles may interfere with field measurements and probes may be prone to fouling during long-term deployment (Srivastava *et al.*, 2013; Zamyadi *et al.*, 2016). A recent review of NZ Guidelines for Cyanobacteria in Recreational Freshwater noted 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 cyanobacterial biomass" (Wood *et al*., 2018).

Molecular methods for monitoring of microorganisms in environmental samples is becoming increasingly widespread and can result in efficiencies to generate information on the presence of potential toxins in short time frames to inform management actions where the technology is available (Lu *et. al.,* 2019). Molecular techniques are available to detect specific genes that identify cyanobacterial species as well as the presence of the toxin-producing genes. However, the relationship between the results from molecular methods and detection using more traditional methods (i.e., microscopy, enzyme-linked immunosorbent assay [ELISA], physicochemical analysis) is not always clear. Molecular methods are rapid and sensitive, allow the differentiation of toxic and nontoxic strains, allow for high throughput of samples, and provide quantitative analysis of cyanobacterial strains to follow variations in community dynamics (Srivastava *et al.*, 2013). At this stage however, these techniques are expensive, require skilled experts and laboratory facilities that may not be available in regional areas. This means that the techniques potentially suffer the same issues noted for cell counts and biovolume measurements of delays in the provision of results to inform response actions when there is a bloom due to the need for samples to be collected and transported to a specialist laboratory before they can be processed (Srivastava *et al.*, 2013; Zamyadi *et al.*, 2016). Health Canada (2020) have recently suggested using molecular methods as a screening tool to determine the presence of cyanobacterial species and to provide an indication of the potential for toxin production.

Irrespective of which method is used it is strongly recommended that all surrogate measurements need to be locally calibrated against toxin concentration (Chorus and Testai, 2021). To capture the conclusions to this question regarding the advantages and disadvantages of using surrogates versus monitoring specific toxins the statement by Ibelings *et al.* (2021) is a useful summary: "estimates of maximum cyanotoxin concentrations based on surrogate measurements will not be accurate; they merely serve as indicators to support decisions on where to focus efforts for monitoring and for further analyses e.g. of cyanotoxins. Due to their variability over time and between waterbodies, using any of them as an estimate for cyanotoxin concentration implies that follow-up by toxin analysis is most likely to result in considerably lower rather than a higher human health risk."

Table 8: Summary of the advantages and disadvantages of different surrogates for monitoring to estimate cyanobacteria and cyanotoxins.

Surrogate	Advantages	Disadvantages
Cell counts	Used widely in many countries over a long period of time. Allows direct assessment of types and potentially of strains[3.]	High cell numbers of very small cells have negligible toxin concentrations.[1.] Need to be locally calibrated against toxin concentrations.[1.] Microcystin content is widely variable between isolates.[2.] Laborious and time consuming[3.] Skilled expert needed[3.] Cells may be incompletely dispersed in suspension, leading to errors in counting[3.] Dispersal methods may damage cells resulting in an underestimation of cell numbers.[3.] Time delays in the provision of results due to practical requirements for sample collection, transportation, laboratory analysis and reporting[4.] Potentially high and free dissolved and cell-fraction of cylindrospermopsin in the water cannot be accounted for by cell counts[5.] Reliable values for taxon and toxin specific cell quotas are not extensive[5.]
Biovolume	The measurement takes into account the taxonomic composition[5.]	Needs to be locally calibrated against toxin concentrations.[1.] Time delays in the provision of results due to practical requirements for sample collection, transportation, laboratory analysis and reporting[4.] The potentially high dissolved and cell-free fraction of cylindrospermopsin in the water cannot be accounted by cell biovolume measurements[5.]
Chlorophyll	Widely used[3.] Submersible probes are suitable for monitoring variable population compositions[3.]	Needs to be locally calibrated against toxin concentrations.[1.] Interference by other accessory pigments or suspended particles[3.] Conventional laboratory methods are time consuming[3.] Probes are potentially expensive Chlorophyll content may vary with species and metabolic state of cells[3.] Probes may be prone to fouling during long-term deployment.[4.] Chlorophyll containing organisms other than cyanobacteria are included in the measurement so microscopic examination is needed to determine the relative dominance of cyanobacteria in the water body[5.]
Phycocyanin (PC)	Rapid assessment tool[3.] Probes are easily applicable in the field, can monitor blooms daily, and provide instantaneous information[3.] Probes can be suitable for long-term continuous monitoring[4]	Needs to be locally calibrated against toxin concentrations.[1.] PC content may vary with species and metabolic state of cells[3.] Interference by other accessory pigments or suspended particles[3.] Probes may be prone to fouling during long-term deployment.[4.] Probes cannot distinguish between cyanobacterial species.[4.] Probes are potentially expensive

Table 8: (continued)

Molecular approaches	Rapid and sensitive[3.] Differentiation of toxic/nontoxic strains[3.] Potential for high-throughput analysis[3.] Quantitative analysis of cyanobacterial strains and potential for information on variations in community dynamics[3.] Amplification of genes via sensitivity of the techniques allows for early detection of potentially toxic organisms[3.]	Potentially expensive[3.] Not widely available and generally skilled expertise is required Needs to be locally calibrated against toxin concentrations[1.] Mutations in the gene cluster may overestimate potential toxin producers within the bloom[3.] Time delays in the provision of results due to practical requirements for sample collection, transportation, laboratory analysis and reporting[4.]

[1.] Chorus and Testai, 2021;

[2.] Fastner and Humpage, 2021;

[3.] Srivastava *et al.*, 2013;

[4.] Zamyadi *et al.*, 2016;

[5] Lu *et al.*, 2019;

5.1.4.2 Secondary Question 2

What guidelines, guidance and implementation practices are in place in comparable countries to minimise or manage this/these hazards and risks/s?

Derivation of Guidelines

The derivations of recreational water guidelines for freshwater cyanobacteria and cyanobacterial toxins were collated from Australian and international sources and are given in Table A6-1 in Appendix 6 of the Technical Report. It is important to note that none of these guidelines have been derived using human exposure data derived from field studies. The majority of cyanobacterial toxin guidelines have been derived following a conventional regulatory model using laboratory animal toxicological studies with pure compounds or characterised cyanobacterial extracts combined with an uncertainty or safety factor approach to determine TDIs or RfDs and subsequent allocation factors. The exception is the guideline for saxitoxin for some jurisdictions (Oregon, 2019; Washington, 2011; WHO, 2020) which have used human poisoning data (EFSA, 2009).

The rationale for adopting the animal model approach for guideline development is related to the overall limitations of interpreting and applying human exposure data from available studies. These limitations are summarised concisely by Chorus and Welker (2021) in the recent guide related to all aspects of toxic cyanobacteria in water published on behalf of WHO:

"A caveat to keep in mind when assessing reports concerning human exposure to toxic cyanobacteria is that their estimates of exposure are almost always retrospective (it would not be ethically possible to conduct a prospective human study of a toxin at concentrations expected to show effects). That is, they provide information on human symptoms occurring at or just before the time of the study and try to explain these by looking into the past to make an "educated guess" as to what may have caused the observed symptoms. Even cyanotoxins detected in the tissues of people or animals do not solve this problem: while they provide absolute evidence of exposure, they do not necessarily demonstrate cyanotoxins to have been the sole cause of symptoms or elevated serum enzyme levels. Many of the reported symptoms in historical reports are quite general and cannot be considered in isolation as diagnostic of cyanotoxin poisoning. It is also not possible to know whether all potential causes and their interactions have been considered, nor whether the estimates of exposures are accurate. Thus, this type of study cannot prove that a cause–effect relationship exists, nor can it provide a quantitative dose–response estimate. This is why the guideline values (GVs) for all cyanotoxins except saxitoxins (STX) are based on animal studies, despite these also having many limitations. Saxitoxins are an exception due to the rapid onset of highly specific diagnostic symptoms following the consumption of contaminated seafood."

The conventional animal model derivation approach follows a two-stage process. The first stage is the calculation of the TDI or RfD from animal studies. For microcystin, three individual animal studies with different types of animals have been used and the number of jurisdictions that have used these studies are: Falconer *et al.* (1994) – Pig, 2; Heinze (1999) – Rat, 6; Fawell *et al.* (1999a) – Mouse, 2. The TDIs derived for microcystin ranged from 0.02 µg/kg/day (California, 2016) to 0.4 µg/kg/day (WHO, 2020).

For saxitoxin, all three jurisdiction or studies that have derived a TDI for saxitoxin have used the EFSA (2009) human poisoning study, and the final TDI derived for saxitoxin ranged from 0.05 µg/kg/day (Oregon, 2019) to 0.5 µg/kg/day (Washington, 2011; WHO, 2020) based upon the assumptions and conventions of each respective jurisdiction.

For anatoxin-a, six jurisdictions used the Fawell *et al.* (1999b) 28-day mouse study and one jurisdiction used the Astrachan and Archer (1981) 7-week rat study for the derivation of the TDI. The TDIs derived for anatoxin-a ranged from 0.01 µg/kg/day (Oregon, 2019) to 3 µg/kg/day (Washington, 2008).

For cylindrospermopsin, the Falconer and Humpage (2003) 11-week mouse study was used by all four jurisdictions and the TDIs derived for cylindrospermopsin ranged from 0.033 µg/kg/day (Washington, 2011) to 0.1 µg/kg/day (Oregon, 2019; WHO, 2020) (Table A6-1; Appendix 6 of the Technical Report). These variations in the final TDI for the same cyanotoxin arise from differences in LOAEL or NOAEL and uncertainty factors applied in the derivation.

The second stage in the derivation of the guideline is the conversion of the TDI or RfD to the guideline value. This is outlined in Table A6-2 in Appendix 6 of the Technical Report. The final guideline value derived is determined by the variation in the values used for weight, water ingestion, and exposure duration. For example, the weight of the child used in the derivation varied from 15 kg (NZ, 2009; NHMRC, 2008; WHO, 2020) to 35 kg (Massachusetts, 2021) across the derivations for all four cyanotoxins. Even within the same jurisdiction the mass of child used in the derivation was found to vary. For example, in the guideline derivation for anatoxin-a for California the weight of the child is listed as 20 kg for Action Tier 1 but is 30.25 kg for Action Tier 2 (California, 2016). The ingestion volume of surface water is more consistent across all the sources and was 0.05 L/h, however Massachusetts (2021) used a rate of 0.1 L/h for a child. The recreational exposure duration applied ranged from 1 – 5 h/day but again the California (2016) derivation for microcystin used an exposure of 5 h/day for the Alert level but an exposure of 2 h/day for the Action Tier 1 level. These examples highlight the complexity and variation across jurisdictions in the derivation of the guideline values.

Guidelines and Guidance

The grey literature search found recreational water quality guidelines for freshwater cyanobacteria and cyanobacterial toxins for 42 jurisdictions (See Section 3.4.2 in the Technical Report) These can be divided into a cross section of 17 jurisdictions which represented international and national agencies and 25 jurisdictions within the USA (2 Federal and 23 states). The US information was collated and presented separately for the individual states as in some cases it represented a diversity of approaches and eventual guideline values which were useful and instructive to capture individually.

The most authoritative recent guidelines with comprehensive assessments and supporting information are those released by WHO (2020), the USEPA (2019a) and Health Canada (2020). WHO have released background documents for four classes of toxin: microcystins, saxitoxins, anatoxin-a and analogues, and cylindrospermopsins (WHO 2020). Based upon these documents, WHO have issued what are referred to as *Provisional guideline values* for Microcystin-LR and Cylindrospermopsin, a *Health-based reference value* for Anatoxin-a and a *Guideline value* for Saxitoxin (WHO, 2020).

The USEPA have published human health recreational ambient water quality criteria or swimming advisories for microcystins and cylindrospermopsin (USEPA (2019a). The Health Canada (2020) document is a technical document for public consultation for revision of the *Guidelines for Canadian recreational water quality: Cyanobacteria and their toxins*. This Canadian consultation document contains a proposed guideline for total microcystins only.

As introduced with the results in Section 3.4.2 in the Technical Report, the concept of 'Guidance' or 'Alert' levels related to recreational exposure guidelines was first developed and widely promoted by Chorus and Bartram (1999). Following this approach many countries have used this guidance approach as a basis for implementing guidelines or action levels for assessing health risks from cyanobacteria through recreational usage of waterbodies. In general, the jurisdictions have often employed three

alert levels associated with advice, warnings and action related to site usage and/or closure. There are however often considerable differences in the toxin concentrations or cell count levels triggering them and in their assessments of the health risk arising from exposure. For the purposes of this review the 'Alert' level was defined as the stage and threshold where some form of initial advisory or advice was issued, and the 'Action' level was generally the point of declaring the requirement for site or waterbody closure. It was not always easy to find a precise fit to these levels, however the comparison was instructive to achieve a view on the application of guidelines in different jurisdictions.

The full compilation of recreational water guideline values expressed as Action and Alert levels for specific freshwater cyanotoxins, cell counts and other surrogates from Australian and international sources (excluding USA) is given in Table A7-1 in Appendix 7 in the Technical Report). A separate table of the equivalent information for the US federal and state jurisdictions is provided in Table A7-2 in Appendix 7 in the Technical Report). An administrative and technical assessment of existing guidelines from selected jurisdictions (New Zealand, Canada, U.S. EPA, WHO, California, Massachusetts, Oregon, and Washington) is given in Appendix 8 in the Technical Report. This assessment protocol was developed by NHMRC based upon assessment criteria outlined in the AGREE Reporting Checklist (citation: https://www.bmj.com/content/352/bmj.i1152).

A summary compilation of recreational water guideline values for freshwater cyanobacteria and cyanobacterial toxins from Australian and international sources is given in Table 9 (based upon Tables A7-1 and A7-2 in Appendix 7 of the Technical Report). This summary indicates that most Australian states have continued to use the NHMRC (2008) guideline of 10 (µg/L) for microcystin, except for SE Queensland who have adopted 2-tier system at the Action level for 5 classes of toxins (microcystin, cylindrospermopsin, anatoxin-a, saxitoxin and nodularin) (Veal *et al*., 2018). International guidelines vary over a relatively wide range. The most recent guidelines, released by WHO (2020) for four classes of toxin have the following values, microcystin: ≥24 µg/L; cylindrospermopsin: ≥6 µg/L anatoxin-a and analogues: ≥59 µg/L and saxitoxins: ≥30 µg/L (Table 9). Definitions of these individual values vary from being defined as 'guidelines', 'provisional guidelines' and 'health-based reference values' (see above). National guidelines in non-US jurisdictions have yet to take a lead from these recently published values and have earlier issued guidelines, usually for microcystin only, in the range of 10 to 25 µg/L.

Guidelines or Action levels in US jurisdictions are highly variable and have a range of definitions based across jurisdictions which make them difficult to compare exactly. The most recent the USEPA (2019a) guidelines published are 'human health recreational ambient water quality criteria' or 'swimming advisories' for 8 µg/L microcystins of 15 µg/L for cylindrospermopsin (Table 9). Many individual US states and jurisdictions have guidelines (Action levels) for microcystins in the range of 6 to >2,000 µg/L. Many states follow the USEPA advisory for cylindrospermopsin of 15 µg/L as an Action level while the most variation is seen for anatoxin-a which range from 1 to 300 µg/L as an Action level.

The number of guideline values published for cyanotoxins by class is in following order: microcystins (12 non-US and 22 US sources) > cylindrospermopsin (4 non-US and 15 US sources) > anatoxin-a (4 non-US and 12 US sources) > saxitoxin (3 non-US and 7 US sources) > nodularin (1, SEQ). Cell counts were used in the guidelines in 12 non-US and 12 US sources (Table 9). The surrogate measurement of chlorophyll-a was used more frequently in non-US sources (5) compared with US sources (2) and biovolume was used only in non-US sources (8). The presence of cyanobacterial scum was used as an Action level in 10 non-US sources and 8 US sources (Table A7-1 and A7-2 in Appendix 7 in the Technical Report).

A collation of recreational water guideline values for marine algae and cyanobacteria from international and Australian sources is given in Table 10. It must be noted that the only published

guidelines values for the marine situation in any jurisdiction were for cell numbers for a small number of specific toxic organisms. No jurisdiction has developed or published a guideline for individual toxins or surrogates other than cell numbers. This table is based upon Table A7-3; Appendix 7 in the Technical Report.

This summary shows that Australian states with marine guidelines (NSW and WA) primarily follow the NHMRC (2008) guideline of ≥10,000 cells/L (Tier 2) for the dinoflagellate *Karenia brevis* and advice for the visible presence of 'moderate', or 'high' numbers of the marine cyanobacterium *Lyngby majuscula*. The only other international guideline for comparison to Australia are the Action levels of >100,000 cells/L – 1,000,000 cells/L (Medium) and >1,000,000 cells/L (High) for *Karenia brevis* from Florida (USA) related to medium and high likelihood or risk of respiratory irritation. These are one to two orders of magnitude greater than the current Australian advice.

This summary of Alert or Action levels within guidelines for both toxin concentrations (µg/L) or cell counts (cells/mL) for freshwater cyanobacteria and cyanobacterial toxins was analysed with regard to their range. This is presented for Australian and international sources (termed non-US) in Table 11 and those from US jurisdictions is given in Table 12.

The summary of Australian and international jurisdictions (Tables 11) shows that the differences in the range of values recommended as the Action level for cyanotoxins (effectively the guideline) were wide but not excessive. They range from 2.5x for microcystin; 3.3x for cylindrospermopsin, 6x for anatoxin-a and with no difference for the recommended saxitoxin Action levels. By contrast the US states (Table 12) show a much wider range of recommended values ranging from 666x for microcystin, 5x for cylindrospermopsin, 300x for anatoxin-a and 25x across saxitoxin Action levels.

It is noteworthy that New Zealand is the only country or jurisdiction to date that specifically considers guidance for the hazard posed by benthic cyanobacteria and their Alert and Action levels are based upon a quantitative visual estimation of coverage of a substrate or production of scum by detachment of benthic cyanobacteria. However, it has been argued by Veal *et al.* (2018) in Queensland that a cyanotoxin-based monitoring program takes into account the production of both the free-floating and benthic cyanotoxins. This assumes however that benthic cyanobacteria produce only one or more of the five cyanotoxins listed the SEQ guidelines, namely microcystins, saxitoxins, cylindrospermopsin, nodularin or anatoxin-a.

Another anomaly is that the New York (2021) guideline specifies a different Action level for microcystins in open water (10 µg/L) compared with shoreline (20 µg/L) but no other jurisdiction distinguishes between different localities within a freshwater body. The reason for this in not known.

Implementation of Guidelines

A range of resources was identified during the search of grey literature. These are considered to have have potential value for agencies and organisations (e.g. state agencies, local government, lake managers, etc.) that are required to implement recreational guidelines or for others that may have to deal with the range of impacts on both humans and animals (e.g. physicians, veterinarians, dog owners, farmers, etc.). A selection of examples of material that may provide useful resources for information and advice is given in Appendix 9 of the Technical Report. The material covers the following topics: local action plans, field identification of cyanobacteria, fact sheets about cyanobacterial blooms, sampling and monitoring advice, and advice for veterinarians, dog owners, physicians, general homeowners, irrigators, and livestock owners.

Table 9: Summary compilation of recreational water guideline values for freshwater cyanobacteria and cyanobacterial toxins from Australian and international sources. In this summary the value at the 'Alert' level is for the issue of a health advisory and the 'Action' level is for a health warning and is effectively the guideline. Where the guideline specifies Microcystin-LR this is stated. Otherwise, it is given as total microcystins. This table is a summary of more comprehensive information covering guidelines and their surrogates given in Appendix 7 of the Technical Report. It is an abbreviation of information in Tables A7-1 and A7-2 (Technical Report). All references are provided in Appendix 7 of the Technical Report.

Source	Toxin	Toxin concentration (µg/L)		Cell count [1.](cells/mL)	
		Alert[2.]	Action[3.]	Alert[2.]	Action[3.]
Australia					
NHMRC 2008	microcystin *Microcystis aeruginosa*		≥10 total microcystins	≥5,000 - <50,000	≥50,000
	cylindrospermopsin	Not given			
	anatoxin-a	Not given			
	saxitoxin	Not given			
NSW Water NSW 2021	microcystin	Not given		>5,000 - <50,000 *Microcystis aeruginosa*	>50,000 *Microcystis aeruginosa*
Queensland SE Qld 2016 Veal *et al.* 2018	microcystin	≥3	≥10 (Tier 1) ≥25 (Tier 2)		
	cylindrospermopsin	≥3	≥10 (Tier 1) ≥25 (Tier 2)		
	anatoxin-a	≥3	≥10 (Tier 1) ≥25 (Tier 2)		
	saxitoxin	≥9	≥30 (Tier 1) ≥75 (Tier 2)		
	nodularin	≥4	≥13 (Tier 1) ≥30 (Tier 2)		
ACT 2014	microcystin *Microcystis aeruginosa*			≥5,000 - <50,000	≥50,000-≤125,000 (Tier 1) ≥125,000 (Tier 2)
Victoria 2021	microcystin *Microcystis aeruginosa*			>50,000 (one location)	>50,000 (many locations)
Tasmania 2011	microcystin	Not given	≥10 total (Tier 1)	>5,000 – 50,000 *M. aeruginosa*	≥50,000 toxic *M. aeruginosa* (Tier 1)
New Zealand 2009	microcystin-LR (toxicity equivalents)	Not given	≥12 total microcystins (child)		
	Benthic	Not given			
Canada 2020	microcystin	Not given	10		50,000 Total cyanobacteria
	cylindrospermopsin	Not given			
	anatoxin-a	Not given			
	saxitoxin	Not given			

Table 9: (continued)

Source	Toxin	Toxin concentration (µg/L)		Cell count [1.](cells/mL)	
		Alert[2.]	Action[3.]	Alert[2.]	Action[3.]
British Columbia 2018	microcystin-LR		>20		
	cylindrospermopsin	Not given			
	anatoxin-a	Not given			
	saxitoxin	Not given			
Czech Republic 2012	microcystin-LR			>20,000	>100,000
	cylindrospermopsin	Not given			
	anatoxin-a	Not given			
	saxitoxin	Not given			
France 2012	microcystin-LR eq		≥25 (± 5%)	>20,000 -100,000 (± 20%)	>100,000 (± 10%)
	cylindrospermopsin	Not given			
	anatoxin-a	Not given			
	saxitoxin	Not given			
Italy 2017	microcystin-LR eq	<20	>20	≥20,000 (± 20%) Total cyanobacteria	>100,000 (± 20%) potentially toxigenic cyanobacteria
	cylindrospermopsin		>20		
	anatoxin-a		>20		
	saxitoxin		Not given		
Netherlands 2017	microcystin-LR eq	Not given[4.]			
Turkey 2017	microcystin-LR eq		>25		20,000 – 100,000 (Tier 1) Scum observed (Tier 2)
	cylindrospermopsin	Not given			
	anatoxin-a	Not given			
	saxitoxin	Not given			
Scotland 2012	microcystin-LR eq			≥20,000	≥100,000
	cylindrospermopsin	Not given			
	anatoxin-a	Not given			
	saxitoxin	Not given			
WHO 2003	microcystin	(2-4) – 20	≥20	>20,000 – 100,000	≥100,000
	cylindrospermopsin				
	anatoxin-a				
	saxitoxin				
WHO 2020	microcystin		≥24		
	cylindrospermopsin		≥6		
	anatoxin-a		≥59		
	saxitoxin		≥30		
Chorus and Testai 2021	microcystin	≤24	>24		
	cylindrospermopsin	≤6	>6		
	anatoxin-a	≤60	>60		
	saxitoxin	≤30	>30		

Table 9: (continued)

Source	Toxin	Toxin concentration (µg/L)		Cell count [1.](cells/mL)	
		Alert[2.]	Action[3.]	Alert[2.]	Action[3.]
USEPA 2019a	microcystins		8		
	cylindrospermopsin		15		
	anatoxin-a		Not given		
	saxitoxin		Not given		
Arkansas 2019	microcystins		8		
	cylindrospermopsin		15		
	anatoxin-a		Not given		
	saxitoxin		Not given		
California 2016	microcystins	0.8	6 (Tier 1) 20 (Tier 2)	4,000 (potential toxin producers)	
	cylindrospermopsin	1	4 (Tier 1) 17 (Tier 2)		
	anatoxin-a	Detect (≤1)	20 (Tier 1) 90 (Tier 2)		
	saxitoxin	Not given	Not given		
Colorado 2020	microcystin		8		
	cylindrospermopsin		15		
	anatoxin		15		
	saxitoxin		8		
Connecticut 2019			Not given	>20,000 -<100,000	>100,000
Idaho 2015			Not given		≥100,000 potentially toxigenic taxa (Tier 1) >40,000 (*Microcystis* or P*lanktothrix*) (Tier 2)
Illinois 2019	microcystin		8		
	cylindrospermopsin		15		
	anatoxin-a		Not given		
	saxitoxin		Not given		
Indiana 2020	microcystin	8	20 0.8 (dog)		100,000
	cylindrospermopsin	15	20 1 (dog)		
	anatoxin-a	80	300 0.4 (dog)		
	saxitoxin	8	3 0.05 (dog)		
Iowa 2017	microcystin		20		
	cylindrospermopsin		Not given		
	anatoxin-a		Not given		
	saxitoxin		Not given		
Kansas 2020	microcystin	>4 – ≤ 8	>8 – ≤ 2,000 (Tier 1) >2,000 (Tier 2)	>80,000 – ≤ 250,000	>250,000 – <10,000,000 (Tier 1) >10,000,000 (Tier 2)
	cylindrospermopsin	Not given			
	anatoxin-a	Not given			
	saxitoxin	Not given			

Table 9: (continued)

Source	Toxin	Toxin concentration (µg/L)		Cell count [1.](cells/mL)	
		Alert[2.]	Action[3.]	Alert[2.]	Action[3.]
Massachusetts 2021	microcystin	<14	≥14	>50,000 -<70,000	≥70,000
	cylindrospermopsin		Not given		
	anatoxin-a		Not given		
	saxitoxin		Not given		
Montana 2019	microcystin	8 – 20	>20	20,000 – 100,000	>100,000
	cylindrospermopsin		Not given		
	anatoxin-a	Detect – 20	>20		
	saxitoxin		Not given		
New Jersey 2020	microcystin		3 (Advisory) >20-<2,000 (Warning) >2,000 (Danger)	≥40,000 - 80,000	≥80,000 (Advisory)
	cylindrospermopsin		8		
	anatoxin		27		
	saxitoxin		Not given		
New York 2021	Microcystin		≥10 (open water) ≥20 (shoreline)		
	cylindrospermopsin		Not given		
	anatoxin-a		Not given		
	saxitoxin		Not given		
Ohio 2020 and Ohio River 2021	microcystin		8	≥20,000 - <100,000	≥100,000
	cylindrospermopsin		15		
	anatoxin-a		8		
	saxitoxin		0.8		
Oregon 2019	microcystin		8 0.2 (dog)		
	cylindrospermopsin		15 0.4 (dog)		
	anatoxin-a		15 0.4 (dog)		
	saxitoxin-eq		8 0.02 (dog)		
Pennsylvania 2014	microcystin	6	20		
	cylindrospermopsin	5	20		
	anatoxin-a	80	300		
	saxitoxin-eq	0.8	3		
Rhode Island 2020	microcystin-LR (eq)		4		>70,000
	cylindrospermopsin	Not given			
	anatoxin-a	Not given			
	saxitoxin	Not given			
Utah 2017	microcystin	4 - 2,000	>2,000	20,000 - 10,000,000	>10,000,000
	cylindrospermopsin		>8		
	anatoxin-a	Detection - 90	>90		
	saxitoxin		Not given		

Table 9: (continued)

Source	Toxin	Toxin concentration (µg/L)		Cell count [1.](cells/mL)	
		Alert[2.]	Action[3.]	Alert[2.]	Action[3.]
Vermont 2015	microcystin-LR (eq)		≥6		
	cylindrospermopsin		≥10		
	anatoxin-a		≥10		
	saxitoxin		Not given		
Virginia 2019	microcystin		8		40,000 (*Microcystis* sp) 100,000 (total toxigenic sp)
	cylindrospermopsin		15		
	anatoxin-a		Not given		
	saxitoxin		Not given		
Washington 2008; 2011	microcystin		6		
	cylindrospermopsin		4.5 ug/L		
	anatoxin-a		1 ug/L		
	saxitoxin		75 ug/L		
West Virginia 2018	microcystin	6	20		
	cylindrospermopsin	5	20		
	anatoxin-a	80	300		
	saxitoxin	0.8	3		
Wisconsin 2019	microcystin-LR	10-20	20-2,000 (Tier 1) >2,000 (Tier 2)	20,000-100,000	100,000-10,000,000 (Tier 1) >10,000,000 (Tier 2)
	cylindrospermopsin	Not given			
	anatoxin-a	Not given			
	saxitoxin	Not given			

[1.] Cell count based on all total potentially toxic cyanobacteria unless specified;

[2.] Alert = health advisory;

[3.] Action = health warning/guideline/health advisory; where sources did not distinguish between Alert and Action values the value was listed as Action;

[4.] The Netherlands have not issued toxin concenrations or cell numbers as Alert or Action levels, however they have instead provided surrogates as Alert and Action levels based upon chlorophyll-a and cyanobacterial biovolume. Details are given in Tables A7-1: Appendix 7 in the Technical Report.

Table 10: Collation of recreational water guideline values for marine algae and cyanobacteria from international and Australian sources. Note that the only published guidelines values for the marine situation are for cell numbers of a range of specific toxic organisms. No jurisdiction has developed or published a guideline for individual toxins or surrogates other than cell numbers. This table is based upon Table A7-3; Appendix 7 in the Technical Report.

Country or Jurisdiction	Organism	Cell count [1.]		Comment
		Alert[2.]	Action[3.]	
United States				
Florida Fish and Wildlife Research Institute 2021	*Karenia brevis*	>10,000 cells/L – 100,000 cells/L (LOW)	>100,000 cells/L – 1,000,000 cells/L (MED) >1,000,000 cells/L (HIGH)	LOW, MED and HIGH- respiratory irritation
Australia				
National **NHMRC 2008**	*Karenia brevis*	≤1 cell/mL	>1 - <10 cells/mL (Tier 1) ≥10 cells/mL (Tier 2)	NHMRC 2008 Table 7.3
	Lyngbya majuscula *Pfiesteria* sp.		Present in: Low numbers (Tier 1) High numbers (Tier 2)	'low' and 'high' not defined
Water NSW, 2021.	*Karenia brevis*		10 cells/mL	
	Lyngbya *Pfiesteria*		High numbers	'High' not defined
Western Australia Department of Health, Public Health and Clinical Services, 2021.	*Lyngby majuscula*	Detected	Relative widespread visible presence of algal filaments	NHMRC 2008
	Trichodesmium		Presence of algal scums	NHMRC 2008
	Other cyanobacteria	≥5,000 cells/L	≥15,000 cells/L	
	Karenia brevis	≥5,000 cells/L	≥10,000 cells/L	
	Karenia sp.	≥50,000 cells/L	≥100,000 cells/L	
	Pfiesteria	Detected	Presence of algal scums	NHMRC 2008

1. Cell count based on all total potentially toxic cyanobacteria unless otherwise specified

2. Alert = health advisory

3. Action = health warning/guideline/health advisory; where sources did not distinguish between Alert and Action values the value was listed as Action

Table 11: Range[1] of values given for Alert or Action guidelines for toxin concentration (µg/L) or cell count (cells/mL) from Australian and international sources (excluding USA) that had a guideline value. All references are provided in Appendix 7 of the Technical Report.

Toxin	Toxin concentration (µg/L)						Cell count [2.] (cells/mL)					
	Alert[3.]			Action[4.]			Alert[3.]			Action[4.]		
	minn	max	Difference in range	minn	Max	Difference in range	minn	max	Difference in range	minn	max	Difference in range
Microcystin	2-4 WHO 2003	≤24 Chorus & Testai 2021	12x	>10 NHMRC, SEQ, Tas	25 France, Turkey	2.5x	>5000 ACT, NHMRC, NSW, Tas	>50,000 Vic (one location)	10x	>20,000 Turkey	≥ 100,000 Canada, Czech, Italy, France, WHO 2003, Scotland	5x
Cylindrospermopsin	≥3 SEQ 2019	≤ 6 Chorus & Testai 2021	2x	≥6 Chorus & Testai 2021	>20 Italy	3.3x						
Anatoxin-a	≥3 SEQ 2019	≤ 60 Chorus & Testai 2021	20x	>10 SEQ	>60 Chorus & Testai 2021	6x						
Saxitoxin	≥9 SEQ 2019	≤ 30 Chorus & Testai 2021	3.3x		>30 SEQ, Chorus & Testai 2021	0x						

[1.] For this comparison the minimum value was used when a range was given by a jurisdiction;

[2.] Cell count based on all total potentially toxic cyanobacteria unless otherwise specified;

[3.] Alert = health advisory;

[4.] Action = health warning/guideline/health advisory; where sources did not distinguish between Alert and Action values, the value was listed as Action in this compilation

Table 12: Range of values given for Alert or Action guidelines for toxin concentration (µg/L) or cell count (cells/mL) for Alert level and Action (Tier 1) level across US Federal and State agencies that had a guideline value. The US state associated with a particular value is indicated by its conventional abbreviation. All references are provided in Appendix 7 of the Technical Report. Where a range was given for an Alert or Action the minimum value was used for the comparison made in the table below.

Toxin	**Toxin concentration (µg/L)**						**Cell count [1] (cells/mL)**					
	Alert[2.]			Action[3.]			Alert[2.]			Action[3.]		
	minn	max	Difference in range	minn	Max	Difference in range	minn	max	Difference in range	minn	max	Difference in range
Microcystin	0.8 CA	<14 MA	17.5x	3 NJ	>2,000 UT	666x	4,000 CA	80,000 KA	20x	40,000 VA	10,000,000 UT	250x
Cylindrospermopsin	1.0 CA	15 IN	15x	4.0 CA	20.0 IN, OH, PA, WV	5x						
Anatoxin-a	<1.0 CA	80 IN, PA, WV	80x	1.0 PA, WA	300 IN, OH, PA, WV	300x						
Saxitoxin	<0.8 PA, WV	0.8	0x	3 IN, PA, OH, WV	75 WA	25x						

[1.] Cell count based on all total potentially toxic cyanobacteria unless otherwise specified;

[2.] Alert = health advisory;

[3.] Action = health warning/guideline/health advisory; where sources did not distinguish between Alert and Action values, the value was listed as Action in this compilation

US state abbreviations: CA California, IN Indiana, KA Kansas, MA Massachusetts, OH Ohio, PA Pennsylvania, UT Utah, VA Virginia, WA Washington, WV West Virginia

5.1.4.3 Secondary Question 3

What are the specific exposure scenarios that might increase risk for sub-populations (e.g. infants playing in shallow waters in presence of benthic mats, water skiers/beach goers inhaling aerosolised cells/toxins) and how are these managed by other organisations?

This question was addressed by a combination of sources from the search for the Primary Question and the information related to guidelines development from the grey literature searches for the secondary questions.

Most guidelines recognise that children are a sensitive sub-population with regard to recreational exposure. The USEPA (2019a) states "recreating children are likely to spend more time in direct contact with waters and measured incidental ingestion data while swimming show that children between 6 and 11 years ingest on average more water than older children and adults. Also, children ages 5 to 11 years tend to spend more time in the water compared to younger and older life stages".

Similarly, Chorus and Welker (2021) summarised the approach and rationale to account for the higher potential exposure of children in guideline development in the recently released WHO (2020) guidelines as follows: "For recreational exposure, the corresponding GV proposed (GV (recreation)) takes into account the higher total exposure of children due to their increased likelihood of longer playtime in recreational water environments and accidental ingestion. The default bodyweight of a child and the volume of water unintentionally swallowed are 15 kg and 250 mL, respectively (WHO, 2003), and these are used to calculate the GV (recreation). The same NOAEL or LOAEL and UFs applied for the GV (short-term) are used to calculate the GV (recreation)"

In line with these approaches the majority of guideline derivations use the body weight and water ingestion rate based upon children (Appendix 6: Technical Report). However, the values used are variable and as indicated in Secondary Question 2, the weight for a child aged 6 to 11 years old used in the derivations for all four cyanotoxins, ranged from 15 kg (NZ, 2009; NHMRC, 2008; WHO, 2020) to 35 kg (Massachusetts, 2021).

In recent feedback on USEPA Draft Guidelines the Mississippi River Collaborative stated that the "draft guidelines may not adequately protect sensitive groups, such as immunocompromised people, people with liver or liver and kidney disease, people with nervous system disorders, pregnant women, nursing mothers, and the elderly". Further, the data on red blood cell acanthocytes observed in animal studies of cylindrospermopsin suggest that individuals that suffer from anemia (e.g., hemolytic or iron-deficiency) might be a potentially sensitive population (USEPA, 2019b). The USEPA responded that "Sensitive populations are taken into account in the derivation of the toxicity values for microcystins and cylindrospermopsin. Specifically, an uncertainty factor (UF) is applied to account for variability in the human population. No information was available to characterize inter-individual and age-related variability in the toxicokinetics or toxicodynamics among humans." The collation of the derivations of TDI or RfD (Appendix 6: Technical Report) showed generally consistent use of UF's of 10 for intra- and 10 for inter-species variability for microcystin, anatoxin-a and cylindrospermopsin However, the UF's for conversion of LOAEL to NOAEL, life-time exposure and/or database limitations were highly variable for microcystin and cylindrospermopsin (Appendix 6: Technical Report). For example, the UF's for database limitations for cylindrospermopsin ranged from 3 (Oregon, 2019; WHO, 2020) to 10 (California, 2016 Tier 1; Washington, 2011). Similarly, Australia used an UF of 10 for carcinogenicity concerns for microcystins (NHMRC, 2008) while Canada stated an UF for life-time exposure was not necessary since types of exposure are short-term (Health Canada, 2020).

The literature review for the Primary Question showed numerous studies have targeted sub-groups who are considered more vulnerable than the general population. Marine studies on aerosolised algal toxins (principally brevetoxins) have focussed on asthmatics (Bean *et al.*, 2011; Cheng *et al.*, 2010; Fleming *et al.*, 2005, 2007, 2009; Kirkpatrick *et al.*, 2011; Milian *et al.*, 2007) since they were considered the most vulnerable beach users. Another marine study investigated impacts on lifeguards (Backer *et al.*, 2005) since their prolonged occupational exposure would increase their vulnerability. Another occupational group targeted was fisher-people in the study of *Pfiesteria* exposure over four years in Chesapeake Bay (Morris Jr. *et al.*, 2006). While children are acknowledged as a vulnerable subgroup an almost equal number of marine studies (excluding case reports and those studies where age was not specified) had only subjects $\geq$ 18 y (7 studies) as those with subjects $\leq$ 18 y (8 studies). Only one freshwater study had only subjects $\geq$ 18 y and 6 studies with subjects $\leq$ 18 y. In Stewart *et al.* (2006) a subgroup of <12 y was identified. No studies have targeted only children.

The most important primary study identified from the searches that clearly identifies the increased risk to small children from exposure to toxic cyanobacteria in a recreational water situation is the case study by Vidal *et al.,* (2017). This study reports on a family (3 adults and a 20-month-old child) who were exposed to an algal bloom while bathing at a beach in Uruguay. A few hours after the last exposure all family members developed diarrhea. While the adults soon recovered the child's symptoms continued for 5 days until she was admitted to a hospital intensive care unit. A liver transplant was performed on the child 20 days after the hospital admission. This study provides extensive details about the medical outcome for this case of severe exposure. Despite the water sampling associated with the study potentially not being at the exact location as the exposure, the detection of microcystins in the explanted liver provided good evidence of exposure. The study provided good evidence of the potential for exposure of a small child playing in shallow water and exposed to toxic cyanobacteria for a relatively short period leading to severe illness and an extreme medical outcome.

Organisations manage the increased risk for these sub-populations in multiple ways. Firstly, within the development of regulations, risk is accounted for by the approach of selecting body weight and water ingestion volumes relevant to children and by the use of uncertainty factors in guideline derivation (see Secondary Question 2). Secondly, agencies use a range of strategies to guide and influence the behaviour of recreational water users to avoid the hazard. Options for this range from informing users by creating awareness and enabling individual responses to bloom situations to temporarily banning waterbody use for the duration of the bloom (Chorus and Testai, 2021).

5.1.4.4 Secondary Question 4

What is the extent of evidence of adverse effects due to recreational exposure to marine cyanobacteria or algae (e.g. skin irritation due to Lyngbya majuscula or inhalation-related symptoms due to cells/toxins aerosolised by wave action, boats, jet-skis, etc.)? Are there any existing guidelines that address these exposure risks?

The extent of evidence of adverse effects due to recreational exposure to marine cyanobacteria or algae was addressed comprehensively as part of the review of primary freshwater and marine studies (See Section 5.1.2 and results related to marine studies in Section 3.4.1 of the Technical Report). This precluded the requirement for any additional specific searches to address this to answer Secondary Question 4.

As discussed in the Methods Section 2.3 in the Technical Report, the development of the Primary Question search protocol was based around logic grids that were constructed to capture all relevant studies to answer this question for both the marine and freshwater environments. The combined

search included terms relevant to all freshwater, marine, and benthic algae and cyanobacteria (all known potentially toxic genera), and all associated freshwater and marine toxins.

As discussed in Section 5.1.2 the search identified 22 primary marine studies which were comprised of 12 cohort, 4 observational and 6 case studies (see Table 4). The majority of these studies (12/22: 55%) related to exposure to brevetoxins, often via aerosols from the marine dinoflagellate *Karenia brevis* associated with red tides in Florida, USA. Three studies were related to dermal effects associated with exposure to the marine cyanobacterium *Lyngbya majuscula*, of which two were Australian studies in Queensland (Osborne et al., 2007; and Osborne and Shaw, 2008). The remainder of the primary studies were mostly case studies where exposure and the agent or organism was either poorly or not characterised.

All of these marine primary studies were assessed for study quality by risk of bias assessment and found to have a range of sources of bias. They were considered as having significant weaknesses in study quality across multiple bias domains. The conclusion for these marine studies (as for the freshwater studies) was that the body of evidence overall was rated as having a "definitely high risk of bias" (see Section 5.1.2). Despite this the review has clearly identified a range of studies that reported adverse human health outcomes ranging from respiratory, gastro-intestinal and irritation effects from exposure to marine algae and their toxins in recreational waters (see Table 18 in the Technical Report).

In relation to existing guidelines that address these exposure risks the grey literature search for guidelines found only four recreational water quality guidelines for marine algae and cyanobacteria were found. No guidelines for marine algal or cyanobacterial toxins (see Section 5.1.4.2). It is important to note that no national or local jurisdiction has yet to develop any guidelines for specific marine toxins for recreation water quality in the marine environment. The four existing guidelines consisted of cell number guidelines for the dinoflagellate *Karenia brevis* from Florida, USA, and cell number guidelines for dinoflagellates and various marine cyanobacteria from three Australian sources (NHMRC, 2008; Water NSW and Western Australian Department of Health) (see Table 10). None of these guidelines included any other surrogates or indicators in addition to cell counts.

In addition, all four sources for marine recreational guidelines for cyanobacterial toxins provided no information about derivation of the guideline values and these were all based on cell counts only. Guidelines provided for *Karenia brevis* in these four sources had a 50-fold range in the Alert guideline ($\leq$ 1,000 cells/L, NHMRC - $\geq$ 50,000 cells/L, WA) and 100-fold range in the Tier 1 Action guideline (>1,000 cells/L, NHMRC - $\geq$ 100,000 cells/L, WA). A qualitative guideline was given for *Lyngbya* and *Pfiesteria* in all three Australian jurisdictions. Western Australia also provided a qualitative guideline for *Trichodesmium* and values for other cyanobacteria (see Table 10).

As discussed in Section 5.1.2, an issue with implementation of these guidelines is the use of qualitative terms such as 'low' or 'high' numbers (NHMRC, 2008) and 'Relative widespread visible presence of algal filaments' (WA, 2021) that are not defined and hence open to interpretation by the authorities responsible for implementation.

5.1.4.5 Secondary Question 5

Much of the evidence for freshwater benthic cyanotoxin production in Australia is anecdotal and often linked to dog deaths following swimming in water bodies (e.g. at least 4 dog deaths in Lake Burley Griffin). It would be useful to try to collate the grey literature evidence to provide a clearer picture of the extent of any risk.

This secondary question relating to animal deaths, in particular dog poisonings and benthic cyanobacteria, was addressed by the analysis of studies captured in the literature search for the primary question (See Results Sections 3.4.1 and 3.4.2 in the Technical Report). These studies captured were regarded as providing potentially higher quality evidence which related exposure to both toxin and cyanobacterial types to dog poisonings. In most case the studies were accompanied by comprehensive veterinary assessment of adverse health outcomes which was also regarded as being superior to information from anecdotal grey literature reports.

The health assessment and outcomes from primary animal studies are summarised in Section 3.4.2 in the Technical Report along with the overall breakdown of outcomes for the entire body of primary studies captured by the primary question search. The search produced twenty-five papers on animal studies, principally related to dogs, and 18 of these were included as primary studies. A detailed description of these 18 primary source papers for the animal literature is given in Table A10-1: Appendix 10 in the Technical Report.

As outlined in Table 16: Section 3.4.1 in the Technical Report, the breakdown of the 18 primary animal studies found that 9 reported exposure to benthic cyanobacteria, 6 to planktonic cyanobacteria (1 marine), 1 to a mixture of cyanobacteria and 2 did not report the habitat type. Most of the studies were from the USA (8), followed by New Zealand (3), the Netherlands (2) and 1 each from Canada, Finland (marine), France, Germany and Switzerland. The exposure scenario was predominantly direct immersion with one direct non-immersion and one unspecified. Most of the studies reported ingestion as the exposure pathway with one also reporting dermal exposure. The range of adverse health outcomes for animals encompassed a similar range of symptoms to reports from human exposure including gastrointestinal (GI), irritation, or neurotoxicity symptoms.

The animal primary studies also included a relatively high number (14/18: 78%) that recorded death as the endpoint (see Table 19: Section 3.4.1 in the Technical Report). Since death was commonly the outcome it was possible, in post-mortem examination of the animals to measure cyanobacteria and/or cyanotoxins in the liver (Gugger *et al.*, 2005; Simola *et al.*, 2012) or stomach (Fastner *et al.*, 2018; Puschner *et al*., 2008; ibid, 2010; Wood *et al*., 2007). In other cases, cyanobacteria and/or cyanotoxins were measured in dog vomit (Lurling *et al*., 2013) or faecal material (Rankin *et al*., 2013). These measurements provided evidence for strong association between the exposure to cyanobacteria and the observed health outcomes for the animals.

The evidence suggested that animals are susceptible to poisoning by cyanotoxins and can become very ill, or potentially die, due to exposure in recreational water environments. The primary route of exposure to these toxins is through ingestion. Ingestion occurs when pets and wildlife drink water from a cyanobacteria-contaminated lake or pond, lick their fur after swimming, or eat dried cells that accumulate along the shoreline (Oregon Health Authority, 2019). It is not clear whether dogs in particular are any more sensitive than other animals or that they simply have opportunities for exposure to high concentrations. Exposure in dogs is unpredictable because they may consume both scum at the shoreline and drying algal mats that wash up on shore. They are also exposed by cleaning cyanotoxin-containing material from their coats after being in the water.

Since dogs are at risk of being poisoned and deaths have been confirmed due to CyanoHABs in the US, the states of Oregon and Indiana have developed dog-specific guideline values for cyanotoxins in recreational water. The Indiana (2020) guideline specifies an Action level for microcystin (0.8 μg/L), cylindrospermopsin (1 μg/L), anatoxin-a (0.4 μg/L) and saxitoxin (0.05 μg/L) specifically for dogs. These guideline values range from 20 times to 750 times lower than the guideline value given for the same

toxin for human exposure. The Oregon guidelines are 0.2 µg/L for microcystins, 0.4 µg/L for both cylindrospermopsin and anatoxin-a and 0.02 µg/L for saxitoxin. The Oregon Health Authority (2019) does not use these dog-specific values as the basis for public health advisories. Rather, they are offered as a resource to veterinarians and veterinary associations to use as appropriate, when treating dogs believed to have been exposed to cyanotoxins.

While the Californian guidelines do not give dog-specific values they note that microcystin, anatoxin-a and cylindrospermopsin are potent and very fast-acting toxins that have been responsible for numerous deaths of domestic animals and wildlife (California Government, 2019). They note that dogs and livestock are susceptible to acute cyanotoxin poisoning at water concentrations that are below the Tier 1 level (6 µg/L, 20 µg/L and 4 µg/L for microcystins, anatoxin-a and cylindrospermopsin, respectively) due to high exposures in animals. They suggest the Action trigger level (0.8 µg/L, 1 µg/L and 1 µg/L, for microcystins, anatoxin-a and cylindrospermopsin, respectively) should be used for the protection of dogs and livestock from microcystin and anatoxin-a poisoning (California Government, 2019).

The significance and risk posed by benthic cyanobacteria to both humans and animals is summarised in the recent guide related to all aspects of toxic cyanobacteria in water published on behalf of WHO. In this document Ibelings *et al*., (2021) state that: "The health risk that benthic cyanobacteria proliferations pose to humans is still relatively unknown. There have been numerous cases of domestic and wildlife poisoning following the ingestion of cyanobacterial mats (Quiblier *et al*., 2013; McAllister *et al*., 2016). Anecdotal reports of human illness after recreating in streams containing cyanobacterial proliferations are documented, but conclusive evidence is lacking. As long as the mats are attached to the substrate, the risks of human ingestion are probably limited. However, detached mats often accumulate at the banks of rivers, streams, and lakes, where animals are much more likely to consume them (Quiblier *et al*., 2013; McAllister *et al*., 2016, Wood *et al.,* 2020). Dogs may be attracted to them by the smell of the decaying material, and numerous cases of dog deaths have been documented, sometimes with cyanobacterial cells and cyanotoxins found in their stomachs (Wood *et al*., 2007; Fastner *et al*., 2018). For some species, "free" toxin, that is, dissolved in water, can be detected in lake and stream water, although the concentrations are usually well below drinking-water guideline values (Wood *et al*., 2018). Assessing risks for human health is challenging in situations where deaths of pets and wildlife have been observed, while the water appears clear and toxin concentrations in the water are low or nondetectable. In such situations, it is best to inform users about the situation, to show what the mats look like and to advise avoiding contact with floating or beached benthic material".

5.1.5 Additional and Supplementary Searches

Several additional supplementary searches were carried out to explore evidence related to potential adverse health effects the cyanobacterial components Endotoxins/LPS and the amino acid, β-methylamino-L-alanine (BMAA) in a recreational exposure setting. A specific search was also carried out to assess of relevance of this topic to public health of Australian indigenous people/s. These are summarised for further consideration by the Committee.

5.1.5.1 Endotoxins/LPS

A supplementary search for Endotoxins/LPS (based upon narrow search terms) was developed to combine with the Recreation/al and Health outcomes concept developed for the full combined searches for the primary question in PubMed® (see Section 3.3.1 and Table 14; Technical Report). The results for this combined search (see Section 4.3.1) were low and returned only 170 studies/papers and these were of very limited or no relevance to environmental exposure to Endotoxins/LPS in recreational water situations. These 170 results were screened based upon titles and 6 studies were

selected that related to Endotoxins/LPS in natural water and potential for human exposure and adverse health outcomes. These studies were further reviewed and narrowed down to only two relevant studies that mentioned cyanobacteria and Endotoxins/LPS: Berg *et al*., (2011) and Lévesque, *et al*., (2016). The details of this screening and the studies is given in Section 3.3.1 in the Technical Report. The search indicated that there is limited evidence available for the assessment of the potential significance of cyanobacterial lipopolysaccharides and their relevance for adverse human health effects in a recreational water exposure setting.

A review by Welker (2021) in the recent guide related to all aspects of toxic cyanobacteria in water published on behalf of WHO (Chorus and Welker, 2021), provides a comprehensive assessment of the significance of cyanobacterial lipopolysaccharides and the relevance of these compounds for adverse human health effects. This review covered the general characteristics of bacterial LPS; what is known about their bioactivity; methodological problems associated with measuring cyanobacterial LPS and possible exposure routes. Welker (2021) pointed out that the terms "LPS" and "endotoxin" are often used as synonyms in the literature, but not always. This review outlined that lipopolysaccharide (LPS) are part of the outer membrane of most Gram-negative prokaryotes, including enteric bacteria and also cyanobacteria and there is evidence that LPS-like compounds can be found in green algae. It also indicated that there is a large body of literature available on the structure, composition of LPS and their association with adverse health effects, generally focusing on heterotrophic bacteria of clinical relevance.

Welker (2021) noted that: "to date, no study has unambiguously related cyanobacterial LPS to adverse health effects in mammals, including humans *in vivo*, like has been demonstrated for microcystin toxicity". He pointed out that: "In most studies that imply an association between observed adverse human health effects and cyanobacterial LPS, this is based more on associative argumentation than on conclusive evidence." In summary he concluded that: "based on the current knowledge, accumulated in several decades of research, cyanobacterial LPS are not likely to pose health risks to an extent known from toxins like microcystins or cylindrospermopsins, in particular, when considering plausible exposure pathways".

Welker (2021) reviewed one of the relevant papers captured in the search run here (i.e. Lévesque *et al*., 2016). In relation to this study Welker concluded that "the observed health effects consisted of generally mild gastrointestinal symptoms not requiring medical examination... and… the statement made in the title is not well supported by the presented data".

5.1.5.2 BMAA

The amino acid, β-methylamino-L-alanine (BMAA), which may be found in cyanobacteria, was not initially included in the specific list of known toxins of interest in the PECO table for review. It was included after discussion with the Committee and added to the Cyanobacteria/Algae/Toxins concept searches developed to answer the primary question.

BMAA was also searched for in an abbreviated supplementary search with a limited range of terms for cyanobacteria to determine the extent of literature on this compound, although this search was not necessarily directed to capture health effects. This supplementary search was carried out in the PubMed® database only. This was regarded as sufficient to explore the relationship and extent of literature for this topic in the context of this review.

The supplementary search for the potentially toxic amino acid BMAA combined with a limited range of terms for cyanobacteria to determine the extent of literature on this compound is given in Section 3.3.2 and specifically in Table 15 in the Technical Report.

The specific individual search for BMAA terms (5 terms only) returned 399 results (from 2006-2020). The combined cyanobacteria and BMAA search returned 234 results for (2006-2020). This combined result of 234 suggested that the association of BMAA with cyanobacteria is a recent popular research topic and approximately 60% of the publications from 2006 that mentioned BMAA also mentioned cyanobacteria (234 from 399).

It must be noted this search return was for the terms "cyanobacteria" and "BMAA" found in titles and abstracts only, and the relevance of this for the public health hazard of BMAA can only be confirmed by a detailed assessment of these publications. This search was regarded as satisfactory to assess the extent of literature on this topic for information of the Committee.

The significance of the compound for human health is currently controversial and is addressed in recent comprehensive review by Chernoff *et al.,* (2021). This review is relevant as it is also contained in the recent guide related to all aspects of toxic cyanobacteria in water published on behalf of WHO (Chorus and Welker, 2021).

This review stated that "the nonproteinogenic amino acid, β-methylamino-L-alanine (BMAA), has been postulated to be a cause of neurodegenerative diseases that affect large numbers of people" and points out that a number of inconsistencies must be clarified before its role in human disease can be assessed with more certainty. These include discrepancies introduced by incorrect BMAA analysis where the nonspecific analytical techniques such as liquid chromatography fluorescence detection (LC-FLD) has been widely used for quantification of BMAA in environmental and human tissue samples rather than more reliable mass-specific analytical methods (e.g. LC-MS/MS). In addition, the authors contend that there is a lack of clear evidence for the "BMAA-neurodegenerative disease hypothesis at the present time". The authors concluded that research efforts on BMAA should be balanced with regard to those on the other cyanotoxins and identified that "the key question that needs to be answered first is whether the proposed toxic effects of BMAA can be confirmed in health-relevant dose ranges" (Chernoff *et al.,* 2021).

5.1.5.3 Assessment of the Significance of the Topic for Indigenous Health

The searches for this review were combined with an indigenous search term string to determine the relevance of this topic to public health of Australian indigenous people/s.

A search string for Indigenous peoples based upon terms for indigenous groups associated with specific regions, states and territories and indigenous health services had been developed for other research purposes by the University of Adelaide library. This string was combined with two full combined searches in PubMed® repeated at two different times with a five-month interval between in November 2020 and April 2021. This represented an initial search and a validation search as was used for the other full combined searches to answer the primary question. Details of the searches and the results are given in Section 4.3.3 and Section 3.3.3 of the Technical Report.

This search was tested only within PubMed® as the low number results were regarded as a sufficient to indicate that there is limited or no published literature on this topic in conventional databases. The outcome was that no results were found from the searches that related to indigenous studies or health outcomes and the Primary Question. This was regarded as a sufficient indication that there is limited or no published literature on this topic in conventional databases.

6 Conclusions

6.1 Primary Question

What is the risk of any adverse health outcome for water users from exposure to cyanobacteria or algae in recreational water?

The literature search and subsequent screening identified 51 primary studies to further assess for answering the Primary Question. From these studies, however, only the human exposure studies were included for further assessment of study quality by risk of bias assessment. These were comprised of 11 freshwater and 22 marine studies.

The freshwater studies consisted of 5 cohort, 3 observational and 3 case studies. The marine studies consisted of 12 cohort, 4 observational and 6 case studies. There were two Australian investigations in the freshwater primary studies, and both were epidemiological studies related to exposure to cyanobacteria in recreational waters (Pilotto *et al.,* 1997; and Stewart *et al*., 2006). There were also only two Australian-based investigations within the marine primary studies. These were both related to health effects associated with exposure to the marine cyanobacterium *Lyngbya majuscula* in Queensland (Osborne *et al.*, 2007; and Osborne and Shaw, 2008).

The risk of bias assessment is designed principally for the assessment of the validity of studies for the evaluation of clinical outcomes. The type of studies reviewed here were either field-based observational and case studies, or cohort studies associated with environmental contaminants, so not all of the usual bias domains were applicable.

The conclusion from the risk of bias assessment was that there was a clear and consistent pattern in the types of bias in all of the marine and freshwater studies assessed that led to weaknesses overall in study quality and in the resulting body of data. The majority of the studies suffered from shortcomings in some of the major bias domains including:

- failing to include suitable comparators or control groups
- not considering potential confounders (i.e., factors or causes for adverse outcomes other than cyanobacteria, algae or toxins)
- not adequately accounting for exposure characterisation for these organisms and compounds in an environmental setting
- many studies had a reliance on self-reporting as part of outcome assessment.

These limitations in design reflect that none of the studies reviewed were designed as randomised control trials or similar clinical trials. Only about 50% of both the freshwater and marine and studies were cohort studies, with the remainder being observational and case studies.

Consequently, all of the primary studies assessed for study quality by risk of bias assessment were regarded as having significant weaknesses in study quality across multiple bias domains. The conclusion was that the body of evidence overall was rated as having a “definitely high risk of bias”. This led to the conclusion that there was insufficient confidence in the studies. As a consequence, there was insufficient information to determine if there were any further reasons to upgrade the certainty of the overall body of evidence from ‘very low certainty’ using the GRADE system.

These shortcomings considered together led to the conclusion that there was insufficient confidence in the findings of the available studies. It is worth noting that methods and approaches for systematic reviews of environmental health evidence is still an area of research and development, and further

modification of the available frameworks and tools is beyond the scope of services required for this review.

Despite this, the review has clearly identified a limited range of studies that reported adverse health outcomes, ranging from respiratory, gastro-intestinal and irritation effects, from exposure to freshwater cyanobacteria and marine algae and their toxins in recreational waters. These are summarised in Table 13 below.

Many of these studies, as for most of the primary studies reviewed, suffered from design deficiencies related to lack of control groups, confounding, exposure characterisation for either organism types, toxins or associated biomarkers that did not correspond with the exact exposure site and time. There were also limitations with regard to the type and degree of health assessment. This is indicated and supported by an assessment of certainty/confidence in the evidence based upon the risk of bias assessment.

A high-level summary of findings for the Primary Question is given in Table 14 below.

Table 13: Selected examples of primary studies that show a relationship between quantitative exposure to freshwater cyanobacteria and/or cyanotoxins; and marine algae and/or their toxins and adverse health outcomes. Each study has an indication of the certainty of the evidence based upon its risk of bias assessment. Further comprehensive details of results for these individual studies are given in Tables 3 and 4 (Section 5.1.2).

Freshwater Studies
Pilotto *et al*., (1997) *Health effects of exposure to cyanobacteria (blue-green algae) during recreational water-related activities*. (Study #5; Table 3)
This Australian epidemiological prospective cohort study examined specific exposure to cyanobacteria in recreational situations. Dominant types across all sites included a wide range of types including *Microcystis aeruginosa*, *Microcystis* sp., *Anabaena* sp., *Aphanizomenon* sp., and *Nodularia* spumigena. No toxin identification or quantification was done by a chemical analytical technique. Total cell counts were used for the analysis to correlate to symptom occurrence rates. Symptoms assessed and recorded during the study included vomiting or diarrhoea, cold and flu-like symptoms, mouth ulcers, eye irritation, ear irritation, skin rash and fever. Seven days after exposure there was a significant trend of increasing symptom rates with increasing duration of exposure. Participants exposed to > 5,000 cells/mL for >1 h had a significantly higher symptom occurrence rate than the unexposed. The authors concluded that symptom occurrence was associated with duration of contact with water containing cyanobacteria, and with cyanobacterial cell density. Certainty of Evidence: Low due to high risk of bias in Confounding bias and Detection bias (exposure characterisation and outcome assessment).
Vidal *et al*., (2017) *Recreational exposure during algal bloom in Carrasco Beach, Uruguay: A liver failure case report.* (Study #8; Table 3)
The study reported recreational exposure during a severe algal bloom in Uruguay and was notable for confirming a liver failure case report associated with exposure of a 20-month-old child. This paper reports on a family (3 adults and a 20-month-old child) who were exposed to an algal bloom while bathing and all family members developed diarrhea. While the adults soon recovered a liver transplant was required to be performed on the child 20 d after the hospital admission. Histological studies and microcystin determination were performed on the explanted liver. During the exposure period blooms of mainly *Microcystis* with very high microcystin levels (mean 2.9 mg/L and max 8.2 mg/L). The analysis of MCs revealed the presence of two microcystin toxins which was considered to confirm the role of microcystins in the development of hepatitis in this this child. Certainty of Evidence: Low due to high risk of bias in Selection bias (comparison groups), Confounding bias and Detection bias (exposure characterisation).
Giannuzzi *et al*., (2011) *An acute case of intoxication with cyanobacteria and cyanotoxins in recreational water in Salto Grande Dam, Argentina*. (Study #9; Table 3)
In this case report a 19-year-old man who was accidentally immersed in an intense *Microcystis* sp. later began to experience GI symptoms, malaise, nausea, vomiting and muscle weakness. His condition worsened and he was hospitalized and diagnosed with a liver disorder. Water samples were collected for a quantitative phytoplankton and toxin analysis on the same day and place where the patient was immersed within 4 h of the incident. Total phytoplankton ranged between 33,680 and 35,740 cells/mL. The most abundant species was *Microcystis wesenbergii*, with cell numbers between 30,600 and 31,600 cells/mL. *Microcystis aeruginosa* was also detected in the range of 3,080–4,100 cells/mL. High levels of Microcystin-LR were detected in water samples (48.6 ± 15 µg/L). The authors indicated that that this is the first report an acute case of cyanobacterial poisoning in Argentina due to an accidental exposure of a person to a cyanobacterial bloom with confirmation of the presence of cyanotoxins. Certainty of Evidence: Moderate due to high risk of bias in Confounding bias.

Table 13: (continued)

Marine Studies
Backer *et al*., (2003) *Recreational exposure to aerosolized brevetoxins during Florida red tide events*. (Study #1; Table 4)
This cohort study reports interviews and pulmonary function tests with a group of people potentially exposed to aerosolised toxins of *Karenia brevis*. Nasal-pharyngeal (nose and throat) swabs for cytologic evaluation of epithelial and inflammatory cells and brevetoxin analyses were taken from participants before and after going to the beach. At one site on a high-exposure day people reported an increase in lower respiratory symptoms and a significant increase in reports of upper respiratory symptoms on a moderate exposure day. The authors found an inflammatory response in over 33% of these participants and did not find any clinically significant changes in pulmonary function test results. Certainty of Evidence: Low due to high risk of bias in Confounding bias and Detection bias (exposure characterisation and outcome assessment)
Fleming *et al*., (2005) *Initial evaluation of the effects of aerosolized Florida red tide toxins (brevetoxins) in persons with asthma*. (Study # 4; Table 4)
The cohort study followed asthmatics before and after going to the beach with and without exposure to *Karenia brevis* red tide. Cell counts were made in water samples and brevetoxins were measured in water and air samples. For the exposure days the brevetoxin in the air ranged from <LOD to 36.57 ng/m^3 and in the seawater from 3.31 – 14.01 µg/L. Participants were significantly more likely to report symptoms and have measurable respiratory impairment symptoms after the red-tide exposure event. The study claims to be the first to show objectively measurable adverse health effects from exposure to aerosolized red tide toxins in persons with asthma. Certainty of Evidence: Low due to high risk of bias in Selection bias (control groups), Confounding bias, Detection bias (exposure characterisation and outcome assessment) and Selective Reporting bias (outcome assessment).
Lin *et al*., (2016) *A prospective study of marine phytoplankton and reported illness among recreational beachgoers in Puerto Rico, 2009*. (Study #8; Table 4)
This study is a large prospective cohort study of the relationship between phytoplankton cell counts and self-reported illnesses following recreational exposure at beach over 26 days in Puerto Rico. Water samples were analysed for phytoplankton cell counts. Daily total phytoplankton cell counts ranged from 346 to 2,012 cells/mL (median, 712 cells/mL). The category with the highest (≥ 75th percentile) total phytoplankton cell count was associated with eye irritation, followed by rash, eye irritation and earache in that order. The conclusion was that there was an association between recreational exposure to total marine phytoplankton cell counts and eye irritation, respiratory illness, earache, and rash at a tropical beach in the absence of a visible algal bloom. Certainty of Evidence: Low due to high risk of bias in Selection bias (control groups), Confounding bias, Detection bias (exposure characterisation and outcome assessment) and Selective Reporting bias (outcome assessment).

Table 13: (continued)

Marine Studies
Milian *et al.*, (2007) *Reported respiratory symptom intensity in asthmatics during exposure to aerosolized Florida red tide toxins.* (Study #9; Table 4)
This was a study of 97 asthmatics before and after going to the beach (>1 h) with and without exposure to *Karenia brevis* red tide events. *Karenia brevis* cell counts were measured in seawater and brevetoxins were measured in seawater and air. Asthmatics reported increased respiratory symptom intensity after 1-h exposure, while no change in respiratory symptom intensity was reported during non-exposure. The study reported that both *K brevis* cells and brevetoxins were also present during what was defined as the non-exposure study periods: "the *K. brevis* cell counts in this area of the Gulf of Mexico were between < 1,000 and 6,000 cells/L, and the concentrations of brevetoxins in the water ranged from < 0.01 to 0.20 µ m/L. The concentrations of brevetoxins in the aerosol did not exceed 0.2 ng/m^3 but were often much lower. During exposure study periods, there were *K. brevis* cell counts between 14,000 and 200,000 cells/L in the water; the concentrations of brevetoxins in the water ranged from 0.50 to 29.20 µ m/L; and the concentrations of brevetoxins in the aerosol from 0.02 to 76.6 ng/m^3 (with higher levels during direct onshore winds)". There was approximately an order of magnitude difference in the exposure agent between exposed and non-exposed periods, which may suggest a threshold, however the importance of this is unknown. Certainty of Evidence: Low due to high risk of bias in Selection bias (control groups), Confounding bias, Detection bias (exposure characterisation and outcome assessment).
Backer *et al.*, (2005) *Occupational exposure to aerosolized Brevetoxins during Florida red tide events: Effects on a healthy worker population.* (Study #12; Table 4)
In this study lifeguards were required to perform spirometry tests and reported symptoms before and after exposure and non-exposure to a red tide comprised of the dinoflagellate *Karenia brevis* and brevetoxins which were measured in seawater and air. The group of lifeguards reported more upper respiratory symptoms during the exposed periods. Compared with non-exposure periods the lifeguards reported more upper airway but not lower airway discomfort during the red tide exposure periods. Certainty of Evidence: Low due to high risk of bias in Selection bias (control groups), Confounding bias, Detection bias (exposure characterisation and outcome assessment).

Table 14: Primary Question – High-Level Summary of Findings

Primary Question: ***What is the risk of any adverse health outcome for water users from exposure to cyanobacteria or algae in recreational water?***
Search Results and Study Types
• The literature search identified 51 primary studies to assess for the Primary Question. From these, 11 freshwater and 22 marine studies involving human exposure (33 studies) were further assessed for study quality by risk of bias assessment. The freshwater studies consisted of 5 cohort, 3 observational and 3 case studies and the marine consisted of 12 cohort, 4 observational and 6 case studies. • There were two Australian investigations which were epidemiological studies in the freshwater primary studies (Pilotto *et al.,* 1997; Stewart *et al*., 2006). and two Australian-based investigations within the marine primary studies (Osborne *et al.*, 2007; Osborne and Shaw, 2008).
Quality of Studies
• All of the primary studies assessed for study quality by risk of bias assessment were regarded as having significant weaknesses in study quality across multiple bias domains.
Quality of Body of Evidence
• The risk of bias assessment concluded that the body of evidence overall was rated as having a "definitely high risk of bias". These shortcomings considered together led to the conclusion that there was insufficient confidence in the findings of the available studies. • There was insufficient information to determine if there were any further reasons to upgrade the certainty of the overall body of evidence from 'very low certainty' using the GRADE system.
Evidence of adverse health outcomes from exposure in recreational water
• The review clearly identified a limited range of studies where exposure to freshwater cyanobacteria and marine algae and their toxins in recreational waters caused adverse health outcomes ranging from respiratory, gastro-intestinal and irritation effects. • Selected examples of some of the primary studies that were notable for showing a relationship between exposure to freshwater cyanobacteria and/or cyanotoxins, and marine algae and/or their toxins and adverse health outcomes were: Freshwater Studies: Pilotto *et al*., (1997), Vidal *et al*., (2017), Giannuzzi *et al*., (2011). Marine Studies: Backer *et al*., (2003), Fleming *et al*., (2005), Lin *et al*., (2016), Milian *et al.*, (2007), Backer *et al*., (2005). • Many of these studies, as for most of the primary studies reviewed, suffered from design deficiencies related to a lack of control groups, confounding, inadequate exposure characterisation for either organism types, toxins or associated biomarkers that did not correspond with the exact exposure site and time. There were also limitations with regard to the type and degree of health assessment.

6.2 Secondary Question 1 - Indicators/Surrogates

The surrogates that are used widely for monitoring cyanobacteria and cyanotoxins are cyanobacterial cell counts, biovolume and the measurement of chlorophyll-a and phycocyanin pigments. The surrogate most-commonly used in guidelines is cell counts followed by chlorophyll-a and biovolume. Phycocyanin is not used in any guideline.

While cell counts are widely used in guidelines, a significant drawback for this measurement is the potentially long delay required for providing results due to the time required for sample collection and processing. Another disadvantage of cell count measurement is associated with the diversity in the range of shapes and sizes of cyanobacterial cells (Wood et al., 2008 in Health Canada, 2020). This can result in very large differences in estimates of cyanobacterial biovolume and hence toxin quantity for equivalent cell count values of different species. In addition, the high variability in toxin cell quotas (toxin content per cell) between individual clones within natural populations is a major source of uncertainty. These factors are all potential limitations for the use of cell counts as a surrogate for cyanotoxin monitoring.

Cyanobacterial biovolume is a more accurate indicator of cyanobacterial biomass than total cell counts. Cyanotoxin concentrations have also been found to relate more directly to cellular biomass than to cell numbers. The World Health Organization (WHO) have discontinued the use of cell numbers in the setting of guidance or Alert Levels for recreational exposure in their most recently issued guidelines and moved to the use of biovolumes. This change reflects experience that the use of cell number thresholds may lead to undue restrictions of recreational use if the dominant cyanobacteria are species with very small cells. This is because toxin concentrations relate to biomass rather than cell numbers.

Chlorophyll-a has frequently been used as an index for eutrophication. It can be used as part of a cyanobacterial alert system to trigger further investigation and action. The use of monitoring by pigment fluorescence, of either chlorophyll or phycocyanin, can potentially be useful to provide continuous and real time data of cyanobacterial hazards. This is particularly the case when using on-line probes and after calibration for the local population.

Molecular methods for monitoring of microorganisms in environmental samples can be used to generate information on the presence of potential toxins in short time frames. These methods detect specific genes that identify cyanobacterial species as well as the presence of the toxin-producing genes. It is suggested that these molecular methods have a role as a screening tool to determine the presence of cyanobacterial species and to provide an indication of the potential for toxin production, particularly as the use of the technology becomes more widespread.

It must be noted that none of the surrogates will provide an indication of free dissolved toxin in water that has been released or liberated from cells. This can be substantial after a bloom has collapsed and will be unknown unless toxin is measured directly.

Irrespective of which method is used, it is strongly recommended that all surrogate measurements need to be locally calibrated against toxin concentration.

6.3 Secondary Question 2 - Guidelines/Guidance and Implementation

Guideline Derivations: The review of the published guidelines found that the majority of cyanotoxin guidelines have been derived following a conventional regulatory model using experimental animal studies rather than human exposure data derived from field studies. This approach uses laboratory animal toxicological studies with pure compounds or characterised cyanobacterial extracts combined with an uncertainty or safety factor approach to determine TDIs or RfDs and subsequent use of

allocation factors. The rationale for adopting the animal model approach is related to the overall limitations of interpreting and applying data from the available human exposure studies. The collation and assessment of all available derivations for cyanotoxin guidelines in different jurisdictions highlighted the wide variation in approach which resulted in the observed differences in final guideline values. These variations included the choice of animal model, different approaches to calculation of the TDI or RfD, through to the choice of uncertainty factors applied to these studies and the use of local conventions for body weight, water ingestion volumes and duration of exposure.

Guidelines and Guidance: The review found recreational water quality guidelines for freshwater cyanobacteria and cyanobacterial toxins for 42 jurisdictions. These were from 17 jurisdictions that represented international and national agencies and 25 jurisdictions within the USA, which were assessed separately. Across these jurisdictions and by class the most frequently issued guideline was for microcystin (34), followed by cylindrospermopsin (19), anatoxin-a (16), saxitoxin (10) and nodularin (1). In relation to surrogates or other indicators, chlorophyll-a was used in 7 guidelines and biovolume was used in 8 guidelines. The presence of cyanobacterial scum was used as an Action level within 18 guidelines. The most authoritative recent guidelines with comprehensive assessments and supporting information are those released by WHO (2020), and the USEPA (2019a).

The review found that most Australian states have continued to use the NHMRC (2008) guideline of 10 (µg/L) for microcystin, except for SE Queensland who have adopted 2-tier system at the Action level for 5 classes of toxins (microcystin, cylindrospermopsin, anatoxin-a, saxitoxin and nodularin) (Veal *et al*., 2018). International guidelines vary over a relatively wide range. The most recent guidelines released by WHO (2020) for four classes of toxin (defined variously as 'guidelines', 'provisional guidelines' and 'health-based reference values') have the following values, microcystin: $\geq$24 µg/L; cylindrospermopsin: $\geq$6 µg/L anatoxin-a and analogues: $\geq$59 µg/L and saxitoxins: $\geq$30 µg/L. National guidelines in non-US jurisdictions have yet to take a lead from these recently published values and have earlier issued guidelines, usually for microcystin only, in the range of 10 to 25 µg/L.

Guidelines or Action levels in US jurisdictions are highly variable and have a range of definitions based across jurisdictions which make them difficult to compare exactly. The most recent the USEPA (2019a) guidelines published are 'human health recreational ambient water quality criteria' or 'swimming advisories' for 8 µg/L microcystins of 15 µg/L for cylindrospermopsin. Many individual US states and jurisdictions have guidelines (Action levels) for microcystins in the range of 6 to >2,000 µg/L. Many states follow the USEPA advisory for cylindrospermopsin of 15 µg/L as an Action level while the most variation is seen for anatoxin-a which range from 1 to 300 µg/L as an Action level.

The range of guidelines were assessed to extract an 'Alert' and 'Action' level for comparative purposes. The summary of Australian and international jurisdictions shows that the differences in the range of values recommended as the Action level (effectively the guideline) for cyanotoxins were wide but not excessive. They range from 2.5x for microcystin; 3.3x for cylindrospermopsin, 6x for anatoxin-a and with no difference for the recommended saxitoxin Action levels. By contrast, the US states show a much wider range of recommended values ranging from 666x for microcystin, 5x for cylindrospermopsin, 300x for anatoxin-a and 25x across saxitoxin Action levels.

New Zealand is currently the only country or jurisdiction that specifically considers guidance for the hazard posed by benthic cyanobacteria.

The review of recreational water guideline values for marine algae and cyanobacteria from international and Australian sources found that the only published guidelines values for the marine situation in any jurisdiction were for cell numbers for a small number of specific toxic organisms. No

jurisdiction has developed or published a guideline for individual toxins or surrogates other than cell numbers.

This review found that Australian states with marine guidelines (NSW and WA) primarily follow the NHMRC (2008) guideline of ≥10,000 cells/L (Tier 2) for the dinoflagellate *Karenia brevis* and advice for the visible presence of 'moderate', or 'high' numbers of the marine cyanobacterium *Lyngby majuscula*. The only other international guideline for comparison to Australia are the Action levels of >100,000 cells/L – 1,000,000 cells/L (Medium) and >1,000,000 cells/L (High) for *Karenia brevis* from Florida (USA) related to medium and high likelihood or risk of respiratory irritation. These are one to two orders of magnitude greater than the current Australian advice.

Implementation: A range of resources was identified during the searches that has potential value for agencies and organisations that are required to implement or provide advice around recreational water guidelines.

6.4 Secondary Question 3 - Exposure Scenarios and Risk for Sub-populations

The specific exposure scenarios leading to an increased risk for sub-populations that have been identified include infants playing in shallow waters in the presence of cyanobacterial blooms, and exposure of sub-groups such as asthmatics and workers such as lifeguards on beaches. These groups are considered more vulnerable than the general population when exposed to aerosolised marine algal or cyanobacterial toxins.

Organisations manage the increased risk for these sub-populations in multiple ways. Firstly, within the development of regulations, risk is accounted for by the approach of selecting body weight and water ingestion volumes relevant to children and by the use of uncertainty factors in guideline derivation (see Secondary Question 2). Secondly, agencies use a range of strategies to guide and influence the behaviour of recreational water users to avoid the hazard. Options for this range from informing users by creating awareness and enabling individual responses to bloom situations, to temporarily banning waterbody use for the duration of the bloom.

6.5 Secondary Question 4 - Evidence of Adverse Effects from Marine Cyanobacteria and Algae

The review found 22 primary studies regarding evidence of adverse health effects due to recreational exposure to marine cyanobacteria. Most of these studies (12/22: 55%) related to exposure to brevetoxins, often via aerosols from the marine dinoflagellate *Karenia brevis* associated with red tides in Florida, USA. There were three studies related to dermal effects associated with exposure to the marine cyanobacterium *Lyngbya majuscula*, of which two were Australian studies in Queensland. All of these marine primary studies were assessed for study quality by risk of bias assessment and found to have a range of sources of bias. They were considered as having significant weaknesses in study quality across multiple bias domains.

In relation to existing guidelines that address these exposure risks, only four recreational water quality guidelines for marine algae and cyanobacteria were found. No guidelines for marine algal or cyanobacterial toxins were found. It is important to note that no national or local jurisdiction has yet developed any guidelines for specific marine toxins for recreational water quality in the marine environment. The four existing guidelines consisted of cell number guidelines for the dinoflagellate *Karenia brevis* from Florida, USA, and cell number guidelines for dinoflagellates and various marine cyanobacteria from three Australian sources.

6.6 Secondary Question 5 - Evidence for Risk from Benthic Cyanobacteria and Cyanotoxins

The review found a large body of evidence from primary studies that confirmed the relationship between dog deaths and exposure to both freshwater benthic and planktonic cyanobacteria. Most of the studies reported ingestion as the exposure pathway, with one also reporting dermal exposure. A high proportion of the animal primary studies recorded death as the endpoint, so it was often possible, by veterinary post-mortem examination, to provide strong evidence for a causal link between the exposure to cyanobacteria and the observed health outcomes for the animals. The evidence suggested that animals are susceptible to poisoning by cyanotoxins and can become very ill, or potentially die, due to exposure in recreational water environments. It is not clear whether dogs are any more sensitive than other animals or that they simply have opportunities for exposure to very high concentrations. Exposure in dogs is unpredictable because they may consume both scum at the shoreline and drying algal mats that wash up on shore. Anecdotal evidence indicates that dogs may be attracted to consume cyanobacteria benthic mat material due to its strong odour. They are also exposed by cleaning cyanotoxin-containing material from their coats after being in the water.

A high-level summary of findings for the Secondary Questions is given in Table 15.

Table 15: Secondary Questions – High-Level Summary of Findings

Secondary Question 1: Indicators/Surrogates *What are the indicators/surrogates of this/these hazard/s? What are the advantages and disadvantages of using surrogates versus monitoring specific toxins?*
• Surrogates that are used widely for monitoring cyanobacteria and cyanotoxins are cyanobacterial cell counts, biovolume and the measurement of chlorophyll-a and phycocyanin pigments • The surrogate most-commonly used in guidelines is cell counts followed by chlorophyll-a and biovolume. Phycocyanin is not used in any guideline • Although cell counts are widely used in guidelines, they have disadvantages that are potential limitations as a surrogate for cyanotoxin monitoring. These include: o the potentially long delay required for providing results due to the time required for sample collection and processing o The diversity in the range of shapes and sizes of cyanobacterial cells can result in large differences in estimates of cyanobacterial biovolume and hence toxin quantity for equivalent cell count values of different species o the high variability in toxin cell quotas (toxin content per cell) between individual clones within natural populations is a major source of uncertainty • Cyanobacterial biovolume is a more accurate indicator of cyanobacterial biomass than total cell counts • Pigment monitoring by fluorescence (of either chlorophyll or phycocyanin) can be useful to provide continuous and real time data of cyanobacterial hazards. • Molecular methods for monitoring of microorganisms in environmental samples can be used to generate information on the presence of potential toxins in short time frames. • None of the surrogates will provide an indication of free dissolved toxin in water that has been released from cells. • It is recommended that all surrogate measurements need to be locally calibrated against toxin concentration.

Table 15: (continued)

Secondary Question 2: Guidelines/Guidance and Implementation *What guidelines, guidance and implementation practices are in place in comparable countries to minimise or manage this/these hazards and risks/s?*
Guidelines and Guidance • The majority of cyanotoxin guidelines have been derived with a conventional regulatory model using experimental animal studies rather than human exposure data from field studies. • The reason for this relates to the overall limitations of interpreting and applying the data of variable quality from the human exposure studies • There is wide variation in the approach used in different jurisdictions for derivation of cyanotoxin guidelines which results in significant differences in final values • The review found recreational water quality guidelines for freshwater cyanobacteria and cyanotoxins for 42 jurisdictions, comprised of 17 jurisdictions from international and national agencies and 25 jurisdictions within the USA • Across these jurisdictions the most frequently issued guideline was for microcystin (34), followed by cylindrospermopsin (19), anatoxin-a (16), saxitoxin (10) and nodularin (1) • In relation to surrogates, chlorophyll-a was used in 7 guidelines and biovolume in 8 guidelines • The most recent guidelines released by WHO (2020) for four classes of toxin (defined variously as 'guidelines', 'provisional guidelines' and 'health-based reference values') have the following values - microcystin: $\geq$24 µg/L; cylindrospermopsin: $\geq$6 µg/L anatoxin-a and analogues: $\geq$59 µg/L and saxitoxins: $\geq$30 µg/L • The most recent the USEPA (2019a) guidelines published are 'human health recreational ambient water quality criteria' or 'swimming advisories' for 8 µg/L microcystins of 15 µg/L for cylindrospermopsin • New Zealand is currently the only country or jurisdiction that specifically considers guidance for the hazard posed by benthic cyanobacteria ***Implementation*** • A range of resources was identified that have potential value for agencies required to implement recreational water guidelines
Secondary Question 3: Exposure Scenarios and Risk for Sub-populations *What are the specific exposure scenarios that might increase risk for sub-populations (e.g. infants playing in shallow waters in presence of benthic mats, water skiers/beach goers inhaling aerosolised cells/toxins) and how are these managed by other organisations?*
• The specific exposure scenarios that might lead to an increased risk for sub-populations include infants playing in shallow waters in the presence of cyanobacterial blooms, and exposure of sub-groups such as asthmatics and workers such as lifeguards on beaches • These groups are considered more vulnerable than the general population when exposed to aerosolised marine algal or cyanobacterial toxins • Organisations manage the increased risk multiple ways: o firstly, within the development of regulations, risk is accounted for by often selecting body weight and water ingestion volumes relevant to children o secondly, agencies use a range of strategies to guide recreational water users to avoid the hazard

Table 15: (continued)

Secondary Question 4: Evidence of Adverse Effects from Marine Cyanobacteria and Algae *What is the extent of evidence of adverse effects due to recreational exposure to marine cyanobacteria or algae (e.g. skin irritation due to Lyngbya majuscula or inhalation-related symptoms due to cells/toxins aerosolised by wave action, boats, jet-skis, etc.)? Are there any existing guidelines that address these exposure risks?*
• The review found 22 primary studies regarding evidence of adverse health effects due to recreational exposure to marine cyanobacteria • Most of these studies related to exposure to brevetoxins, often via aerosols from the marine dinoflagellate *Karenia brevis* associated with red tides in Florida, USA • There were three studies related to dermal effects associated with exposure to the marine cyanobacterium *Lyngbya majuscula*, of which two were Australian studies from Queensland • In relation to existing guidelines that address these exposure risks, only four recreational water quality guidelines for marine algae and cyanobacteria were found • No national or local jurisdiction has yet developed any guidelines for specific marine toxins for recreational water quality in the marine environment • The four existing guidelines consisted of cell number guidelines for the dinoflagellate *Karenia brevis* from Florida, USA, and cell number guidelines for dinoflagellates and various marine cyanobacteria from three Australian sources
Secondary Question 5: Evidence for Risk from Benthic Cyanobacteria and Cyanotoxins *Much of the evidence for freshwater benthic cyanotoxin production in Australia is anecdotal and often linked to dog deaths following swimming in water bodies (e.g. at least 4 dog deaths in Lake Burley Griffin). It would be useful to try to collate the grey literature evidence to provide a clearer picture of the extent of any risk.*
• The review found a large body of evidence from primary studies that confirmed the relationship between dog deaths and exposure to both freshwater benthic and planktonic cyanobacteria • Most of the studies reported ingestion as the exposure pathway, with one also reporting dermal exposure • A high proportion of the animal primary studies of dogs recorded death as the endpoint and it was often possible by veterinary post-mortem examination to provide strong evidence for a causal link between the exposure to cyanobacteria and the observed health outcomes • It is not clear whether dogs are any more sensitive than other animals or that they simply have opportunities for exposure to very high concentrations

6.7 Additional and Supplementary Searches

6.7.1.1 Endotoxins/LPS

The supplementary search for Endotoxins/LPS related to the Primary Question indicated that there is limited evidence for the assessment of the potential significance of cyanobacterial lipopolysaccharides to determine their relevance for adverse human health effects in a recreational water exposure setting.

6.7.1.2 BMAA

The supplementary search for the potentially toxic amino acid BMAA, combined with terms for cyanobacteria to determine the extent of literature on this compound, returned a moderate number of publications (399 results; 2006-2020). These were not screened or considered separately from the assessment undertaken to answer the Primary Question for the review. The significance of the compound for human health is currently controversial.

6.7.1.3 Assessment of the Significance of the Topic for Indigenous Health

The searches for this review were combined with an indigenous search term string to determine the relevance of this topic to public health of Australian indigenous people/s. The outcome was that no results were found that related to indigenous studies or health outcomes and the Primary Question.

7 Declared Interests

The author of this review (Michael D Burch) has the following declared interests:

Interest	**Interest Details**
NHMRC	The reviewer was involved in the development of the previous version of the NHMRC guidelines (The Guidelines for Managing Risks in Recreational Water. 2008). This was initially as a volunteer member of the steering Committee and subsequently as chair of the Committee (2004-2006).
Visiting Associate Professor in the School of Biological Sciences in the Faculty of Sciences at the University of Adelaide	The reviewer participates in research projects with university staff and students; publishes journal articles with University affiliation. This includes publications on cyanobacteria and algae.
Director, Australis Water Consulting Pty Ltd.	The reviewer is the Director and Principal of an Australian water consulting company that provides advice on water management and research management to a range of Australian and international clients, including government agencies, water authorities, research Institutions, Universities and local government organisations.
Professional association with members of the NHMRC Recreational Water Quality Advisory Committee (RWQAC) (the Committee)	The reviewer has professional scientific relationships with several members (three members) of the Committee which has included joint research and producing joint publications at different times over the last 30 years.
Member of Water Research Australia through affiliation with the University of Adelaide, and as a consultant.	The reviewer provides professional and scientific advice to Water RA staff on research project design and management. This may be as a consultancy on a normal commercial basis.
The reviewer is a joint author on the following paper which was included in the review: Pilotto, L. S., Douglas, R. M., Burch, M. D., Cameron, S., Beers, M., Rouch, G. J., Robinson, P., Kirk, M., Cowie, C. T., Hardiman, S., Moore, C. and Attewell, R. G. (1997). Health effects of exposure to cyanobacteria (blue-green algae) during recreational water-related activities. Australian and New Zealand Journal of Public Health 21, 562-566.	The study by Pilotto *et al.*, (1997) was included in the review although it was outside the date range specified (2006-2021). This was because it was a highly relevant Australian epidemiological study designed at the time to gather information to inform exposure to toxic cyanobacteria in recreational water environments.

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