Research Protocol

Evidence Review: Cyanobacteria and Algae

FINAL

Report to the Recreational Water Quality Advisory Committee (RWQAC)

This report constitutes Deliverable 2 - Final Research Protocol that describes the rationale for the evidence evaluation, its objectives and the methods that will be used to locate, select and critically appraise studies, and to collect and analyse data from the included studies to support:

2019-20RFQ017 - Evaluation of the Evidence for the Recreational Water Quality Guidelines by the National Health and Medical Research Council (NHMRC)

Section: Cyanobacteria and Algae

Document Information

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Background and Purpose of the Report

The National Health and Medical Research Council (NHMRC) through the Recreational Water Quality Advisory Committee (RWQAC) will update the Guidelines for Managing Risks from Recreational Water (2008) during 2020.

As part of this update a series of Narrative Reviews will be conducted by contractors to gather evidence to answer research questions on microbial risks and chemical hazards as determined by the (RWQAC). The contractors are required to undertake the narrative reviews using a systematic approach according to details provided in the Scope of Services and in C.A.2 (a) Standards.

Australis Water Consulting (AWC) has been engaged to undertake the Narrative Review for the sub-topic of Cyanobacteria and Algae. AWC will deliver a comprehensive evidence review to inform the update to Chapters 6 and 7 of the Guidelines for Managing Risks in Recreational Water (2008).

The purpose of this report by Australis Water Consulting is to provide the following deliverable:

Deliverable 2: Final Research Protocol that describes the rationale for the evidence evaluation, its objectives and the methods that will be used to locate, select and critically appraise studies, and to collect and analyse data from the included studies.

Scope

The scope of this report is to provide the following specified deliverable:

Deliverable 2: Final Research Protocol incorporating feedback from the RWQAC that describes the rationale for the evidence evaluation, its objectives and the methods that will be used to locate, select and critically appraise studies, and to collect and analyse data from the included studies.

The Research Protocol outlined here will support the narrative reviews and resulting reports for cyanobacterial and algae section of the revised Guidelines.

The Scope for the **Research Protocol** as specified in the contract requirements is:

The Contractor is required to develop a Research Protocol to guide the Narrative Review by:
- Drafting a Research Protocol outlining the methodology to be used and developed into a more comprehensive protocol as appropriate. The draft protocol will be circulated to RWQAC and a methodological reviewer.
- Finalising the protocol (and any amendments) (this Report) and seeking approval from ONHMRC, following advice from RWQAC and the methodological reviewer before commencing the review.

Further detailed requirements specified in the contract are:

The protocol will specify the key information needed for another reviewer to replicate the search if needed and as much as possible outline how the evidence will be handled, including:
- Rationale for the review
- Objectives
- A structured search strategy outlining the methods that will be used to locate, select and critically appraise relevant studies, including:

- o confirming the review question
- o delineating the need, if any, for analysis of special population subgroups, e.g. people for whom English is not the first language
- o search terms
- o publication dates
- o language
- o databases to be searched
- o criteria for inclusion and exclusion to be used to select studies for appraisal; and importance (priority rating) of outcomes
- o the methods that will be used to extract, critically appraise, and synthesise the data from included studies.

Research Protocol

Rationale for the review

The context for this review is as follows. 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 and Technical Reports as part of this project are designed to gather, assess, and contribute to the detailed evidence and to provide the rigour to support the above comprehensive information components contained within the Framework and Guidelines.

Objectives

The objectives of the Research Protocol are to outline the methodology to be used to conduct the evidence collection and evaluation to guide the Narrative Review to answer research questions on Cyanobacteria and Algae risks which have been determined by the RWQAC and will be used to update the Guidelines for Managing Risks from Recreational Water (2008).

Guideline Scope and Application

Unlike the *Guidelines for Managing Risks in Recreational Water* (2008), the updated Guidelines will cover the public health risks associated with recreational water quality *only*. This includes human health risks from biological and chemical hazards that affect the quality of recreational water that people might be exposed to. Other risks associated with recreational water use such as physical risks should be considered as part of the risk management planning process while applying the Framework; however, specific guidance on how to manage these risks will not be provided in the Guidelines. In addition, the Guidelines will not cover details on rescue, resuscitation or treatment associated with risks from recreational water quality.

The Guidelines should be applied within the broader context of protecting public health and as such are not intended to be prescriptive given the variety of recreational water settings and climates across Australia. The inclusion of the Framework is intended to allow for structured risk assessment and risk management planning across the wide variety of existing and emerging recreational water environments that Australian risk managers might encounter. This also includes any unique sites that are currently unregulated and may present risks to public health.

Included:
- Risks from microorganisms, cyanobacteria and algae, free-living microorganisms, chemical hazards.

Excluded:
- Risks from sun, heat and cold and other physical hazards associated with recreational water (e.g. drowning, animal attacks)
- Risks associated with exposure to foodstuffs collected from recreational water or its surroundings
- Risks associated with ancillary facilities that are not part of the recreational water environment other than risks that may affect water quality (e.g. toilet facilities in adjacent areas are not considered unless these need to be managed to minimise contamination of the recreational water body)
- Adverse health effects that are not caused by recreational water quality (e.g. seasickness, the 'bends')
- Risks from sand/soil around recreational water bodies (unless disturbances of sand/soil affect water quality); however, the risk management framework should include assessment of these risks.

Definitions

A list of definitions of terms and abbreviations and a Glossary will be developed and provided for the Narrative Review and Technical Report. The following are some of the specific categories of definitions which will be added to in the Narrative Review and Technical Report.

Definitions of Uses and Users of Recreational Water

Recreational water:

Included: Any natural or artificial water bodies without a chlorine disinfectant residual that might be used for recreating including coastal, estuarine, and freshwater environments. Includes public, private, commercial, and non-commercial recreational water sites. Includes unique unregulated sites such as wave pools, ocean- or river-fed swimming pools, artificial lagoons, and water ski parks.

Excluded: Aquatic facilities using chemical disinfection including swimming pools, spas, splash parks, ornamental water sites.

Recreational water use:

Included: Any designated or undesignated activity relating to sport, pleasure and relaxation that involves whole body contact or incidental exposure (through any exposure route) to recreational water (e.g. swimming, diving, boating, fishing)

Excluded: Consuming the catch from fishing or foodstuffs collected from recreational water or its surroundings. Therapeutic uses of waters (e.g. hydrotherapy pools). Occupational exposure.

Recreational water users:

Recreators or users of recreational water bodies including:
- the general public including all relevant life stages, ages and states of health other than persons that are explicitly advised to avoid such activities (e.g. for specific medical conditions)
- tourists
- specialist sporting users (e.g. athletes, anglers, kayakers, divers, surfers)
- any groups that may have high exposures to recreational water.

Target audience of the Guidelines:

The Guidelines are intended for end users that will implement the Guidelines (government agencies, local councils, private recreational water managers); however, it is anticipated that there will also be significant public interest. It is anticipated that tailored guidance (e.g. plain English fact sheets or summaries) will be developed for specific groups where necessary.

Structure

The **Research Protocol** has the following components:

- **Research Questions:** Description of the Primary and Secondary Research Questions
- **Population, Exposure, Comparator, Outcome (PECO) Table:** This table summarise the populations to be considered, the potential exposure routes, any comparators and the health outcomes of interest.
- **Search Strategy and Selection of Evidence:** This describes the searching strategy for studies based upon PECO criteria for initial eligibility and collation of publications for review and the screening process of these studies using inclusion and exclusion criteria to select studies to be included in the literature review.
- **Process for Extracting and Presenting Data:** Evidence will be extracted from included studies and evaluated to answer the research questions. This includes development of meta-database tables based around the PECO Tables and other criteria.
- **Process for Critically Appraising the Evidence:** Evidence from included studies will be checked for relevance and suitability to answer the Research Questions which includes assessing the risk of bias and assessing the certainty of the body of evidence.
- **Process for Reporting:** Reporting comprises the Narrative Reviews report based around each research question and the Technical Report containing detailed information about the methods used to undertake the literature reviews.
- **Declared Interests**: This outlines the declared interests of the primary author for this review.
- **Process for Making Amendments to the Protocol**: The process for making any changes to the agreed Research Protocol after the review has commenced.

These components are described in detail below.

Methods

This review is 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 questions to be addressed consist of one primary question and five secondary questions provided in detail in the next section (Table 1). An abbreviated summary of these questions is as follows:

Primary question: What is the risk of adverse health outcomes from exposure to cyanobacteria and algae in recreational water?

Secondary questions:

1. What are the indicators/surrogates of these hazards?
2. What guidelines, guidance and implementation practices are in place in comparable countries to minimise or manage these hazards?
3. What are the specific exposure scenarios that might increase the risk for sub-populations?
4. What is the extent of evidence of adverse effects due to recreational exposure to marine cyanobacteria or algae?
5. What is the evidence for exposure/risk to freshwater benthic cyanotoxin production in Australia?

The review process to answer these questions will include four components. Each component has a different methodological approach selected to optimise information collection and evidence evaluation to answer the type of question. These components are:

1) A conventional systematic search and review of primary studies to address the Primary Question of 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 primary studies and reports derived from targeted literature searches specifically constructed to relate to Secondary Questions 3, 4 & 5. This approach is similar to and a variation of 1).

The justification for this differential approach related to the different questions is provided in the next section.

Research Questions

The Research Questions (primary & secondary) are provided in Table 1.

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

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.

Methodological Approach related to Research Questions

Primary Question

The approach taken to answer the Primary Question will be a conventional systematic search and review of primary studies and reports. This will follow the procedures outlined in subsequent sections of this protocol: constructing a structured literature search based around the PECO criteria; searching for and selecting publications in multiple literature databases; screening these for suitability for full review based upon inclusion and exclusion criteria and critical assessment and appraisal of studies for risk of bias and evaluation of evidence quality and certainty assessment.

Analysis of the primary Research Question: "*What is the risk of any adverse health outcome for water users from exposure to cyanobacteria or algae in recreational water?*" indicates it can be broken down into components or elements (Table 2) which are related to the PECO Table categories (Table 3).

Table 2: Elements of the primary research question and relevant coverage in the PECO analysis.

Element	PECO Category where the element is included
Risk	Not directly referenced in the PECO, but is a measure and consequence of the interaction of the other elements
Adverse Health Outcome	Outcomes

Water users – general population and sub-groups	Population
Exposure – freshwater, benthic & marine cyanobacteria; and algae	Exposure
Recreational Water	Not addressed directly, but the identified medium associated with water users and the pathway for recreational exposure
Cyanobacteria and Algae	Exposure components

This analysis of these elements in the context of the PECO shows there are no deficiencies or inconsistencies related to the Primary Question developed for this study.

Secondary Questions

The secondary questions (Table 1) seek to identify a range of supplementary information required to provide context to assist in the development and application of sound revised guidelines. These relate to the use of surrogates/indicators for monitoring hazards (Q 1); examples of other guidelines and guidance practices (Q 2); exposure scenarios for sub-populations (Q 3); evidence related to exposure to marine cyanobacteria and algae (e.g. *Lyngbya majuscula*) (Q 4); and sourcing of additional evidence for hazards and risks posed by benthic cyanobacteria (Q 5).

Secondary Questions 3, 4 & 5 will be assessed by minor variations on the comprehensive search described for the Primary Question. This will involve incorporating additional search terms into strings to cover for example marine cyanobacteria and algal types (Q4) and specific toxins and benthic cyanobacteria (Q5). In addition, evidence of potential adverse health outcomes for sensitive sub-groups will specifically include reference to Aboriginal and Torres Strait Islander indigenous peoples in Australia to address (Q3).

Secondary Questions 1 & 2 will be addressed by a different methodological approach which has been selected to optimise information collection and evidence evaluation to answer the question type. The approaches to be adopted will be:

Question 1)   A review of selected reviews in addition to the search for the primary question will be conducted to address Secondary Question 1. related to the indicators/surrogates of hazards posed by cyanobacterial toxins.

The reason for undertaking a review of selected reviews is as follows. It is not regarded as time and resource-effective to structure a specific additional search to review monitoring of cyanobacteria and algae to investigate the use of surrogates for monitoring specific cyanotoxins more widely. This is because monitoring of cyanobacteria in natural waters is a very extensive research and management topic for lake, reservoir and river management and is not restricted to monitoring toxic cyanobacteria and associated cyanotoxins. As such a broad search and review is likely be so extensive and time-consuming that it is not an efficient use of resources for the purpose of specifically answering the secondary question.

The approach to gathering information to address this question will depend upon the eventual form of the Guidelines and guidance that are developed. The question makes the reasonable assumption

that guidelines will be provided for classes of specific cyanotoxins (e.g. microcystins, cylindrospermopsins, saxitoxins, anatoxin-a). While these are the most relevant potential and well-characterised toxin hazard posed by cyanobacteria in recreational exposure situations, there is evidence (not strong) and discussion that there can be adverse health outcomes from exposure to large quantities of cyanobacterial material. This has been alluded to in the context of reports of respiratory and skin effects from unspecified material within or associated with cell mass. The secondary question then assumes that a range of surrogates may offer an alternative to monitoring for specific toxins. A preliminary scan of recent relevant literature reviews related to the topic of monitoring approaches for cyanobacterial biomass and toxins shows that the alternative monitoring techniques that may include, but not be limited to:

- Cells counts

- Biovolume (usually derived from cell counts)

- Chlorophyll a and specific accessory pigments (usually with *in vivo* fluorescence techniques)

- Toxin-producing genes (molecular techniques)

This scan produced three highly relevant and cited recent reviews that cover the topic, and which indicate the scope and quantity of literature associated with this topic:

Srivastava *et al* (2013): contains 188 references focussed on the full range of monitoring techniques listed above.

Zamyadi *et al* (2016): focussed mainly on fluorescent probe techniques which are more specifically for the detection and quantification of cyanobacterial cells as opposed to cyanotoxins.

Padisak *et al* (2020) is an authoritative review of laboratory analyses of cyanobacteria and water chemistry that covers the recent developments in monitoring relevant to toxic cyanobacteria.

In addition to the review of these selected reviews, key studies from their bibliographies may also be added for review and information will also be obtained from studies captured in the search carried out to answer the primary question. The primary question search in any case should also provide a large quantity of relevant evidence to address this secondary question in a satisfactory way in the context of recreational exposure studies.

Question 2)   A review of examples of guidelines, guidance, and implementation practices in place in comparable countries will be carried out from grey literature searches obtained from the websites from a range of national organisations and agencies and local jurisdictional agencies (i.e. states) to address Secondary Question 2.

The search will be structured to gather and extract information on guidelines/guidance from other countries and sub-jurisdictions in addition to Australian states.

Population, Exposure, Comparator, Outcome (PECO) Table

The PECO table is provided in Table 3. This PECO table developed by the RWQAC is regarded as sound and comprehensive.

Table 3: PECO for the Narrative Review: Cyanobacteria and Algae (provided by RWQAC).

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)

Search Strategy and Selection of Evidence

The strategy to be adopted to find and select the evidence for review involves the following sequential steps.

Search Terms

Search terms and search-string combinations will be defined based upon the PECO Table and the Research Questions. The arrangement of search terms will be based around Search "Concepts". The example provided here is for the topic of *Pelagic Freshwater Cyanobacteria and Algae* to demonstrate how an advanced search is constructed using the PubMed database. This is regarded as the most advanced and complex type of search and will demonstrate the approach which will be followed for other searches with appropriate modifications for each database.

The approach for this advanced search combines the three (3) defined Concepts: Freshwater **Cyanobacteria/algae/toxins**, **recreational, health** with the Boolean AND operator. These concepts are placed in a "Logic Grid" which is used to define the combination of search term key words and likely synonyms. Terms are initially tested within the PubMed database to check if they are indexed MeSH terms or supplementary terms. These terms and other non-MeSH synonyms are combined to comprise the search string for each concept (Table 4). The string search for each concept is searched individually and then the resultant searches are then combined to generate the end-result output for screening.

Table 4: Logic Grid for construction of an advanced search for the Primary Question: "What is the risk of any adverse health outcome for water users from exposure to cyanobacteria or algae in recreational water?".

Concepts to be searched in combination (based upon the suggested search terms and PECO Table)		
FW Cyanobacteria/algae/toxins AND Recreational AND Health		
Keys words and variants to be searched for each of these concepts prior to the combination of searches		
cyanobacteria	recreation	health
blue-green algae	recreational	health effects
algae	swimming	health outcome/s
cyanobacterial bloom/s	bathing	disease
algal bloom/s	wading	illness/es
harmful algal blooms	paddling	symptoms
HAB/s		gastrointestinal
cyanotoxin/s	boating	nausea
neurotoxin/s	sailing	vomiting
hepatotoxins/s	wind surfing	diarrhea
microcystin/s	water skiing	pneumonia-like symptoms
saxitoxin/s	fishing	fever
cylindrospermopsin/s	kayaking	headache
anatoxin-a	canoeing	hay fever-like
nodularin/s	jet-skiing	flu-like
		skin rash/es

Cylindrospermopsis raciborskii		skin irritation
Raphidiopsis		eye irritation
Microcystis		pruritis
Dolichospermum circinale		dermatologic
Anabaena circinalis		allergic reaction/s
Nodularia spumigena		neurotoxicity
Lyngbya wollei		neurologic/al
"total cyanobacteria"		hepatotoxicity
		dermal irritation
		allergic reaction/s
		inhalation-related symptoms
		induction of asthma
		shortness of breath
		exposure
		oral
		inhalation
Note: This table is an example to illustrate the structure of the logic grid and does not include Index and MeSH terms and wildcard terms (*) which will be added during development of the final search string combinations for each concept		

It is anticipated that four separate searches will be required to fully cover the four topics listed for review to update the guidelines. These are:

- 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).

The concepts and key word string searches will be similar for each of these searches apart from the substitution of specific cyanobacterial types and toxins associated with marine and benthic organisms. It is anticipated that the animal exposure search as a health outcomes indicator for human exposure will also have a similar structure and will capture information for pelagic and benthic cyanobacteria and algae.

Databases

The databases to be searched include PubMed, Scopus and Science Direct.

PubMed is regarded as the primary search database for this review due to its coverage of biomedical journals and capacity for advanced searching.

Scopus claims to be the world's largest abstract and citation database of peer-reviewed literature. It is very broad based, covering thousands of journals in the life sciences, the social sciences and humanities, the physical sciences, and the health sciences.

Google Scholar may be searched for validation purposes, but it not regarded as a primary search data base for advanced searches as it does not support running complex Boolean logic search strategies.

Publication Dates

The review will consider papers and reports published from 2006 onwards. This allows for the Guidelines update to include relevant new evidence and information since the publication of last revision of the Guidelines in 2008.

Language

Search results will be restricted by language to English language publications only. In the event that that RWQAC should decide that a non-English publication should be included, translation of this publication will be arranged by ONHMRC.

Inclusion and Exclusion Criteria

The criteria to be applied to select studies for downloading for full review are summarised in Table 5.

Table 5: Inclusion/exclusion criteria applied to select studies for full review.

Inclusion
Searched studies are anticipated to closely match search concepts and elements developed from the PECO criteria (population, exposure, outcomes). Amongst these the priorities for inclusion are:
Systematic Reviews – particularly those with reviews of evidence
All matching Australian Studies
Primary studies with quantitative evidence of exposure to the specified cyanobacteria and/or cyanotoxins resulting in measured health outcomes (positive or negative)
Relevant international studies
Peer-reviewed as a preference
Case Reports
International, national or state/provincial government agency documents containing recreational exposure guidelines and/or guidance. These may be grey literature – and may not be peer-reviewed
Exposures include recreational activities in untreated natural waters such as rivers, lakes, ponds, or man-made reservoir
All studies reporting benthic cyanobacteria in recreational water situations
Exclusion
Duplicates for the three (3 databases) searches
Studies with exposure to unspecified cyanotoxins
Studies with exposure to cyanobacteria not identified to species level as per exposure list
No clear or weak evidence of exposure to cyanotoxins or cyanobacteria in recreational water
Studies with illness acquired from treated recreational water (swimming pools, spas, hot tubs)
Non-peer reviewed studies after review of the abstract or summary

Search Protocol and Validation Methods

The search protocol will contain the following steps and processes to ensure the search is validated:

1. Keys words will be tested in an initial search

2. Key words will be assessed within the database index and the MeSH library for inclusion as Index and MeSH terms in the search strings. The initial search will be tested in combination with the selected Index and MeSH terms to ensure they are defined correctly.
3. Key word alternatives of Index terms will be included separately to capture recent publications not yet indexed in the database
4. The strings will adopt truncated terms with wildcards for plurals variants: e.g. alga* for algae, algal. For simplicity these are not shown in Table 4.
5. Key words and phrases from the concept synonyms table will searched for in titles and abstracts of articles (i.e. the [tiab] functionality) to ensure that material that has only recently been added to the database and does not yet have MeSH terms included will be captured.

Screening methods

The search protocol to be used with advanced search capability in PubMed makes use of tested Index and MeSH terms in the string search ensure the search does not "explode" to generate large numbers of irrelevant results. This will be combined with restricting the search for key words and phrases to Titles and Abstracts only (e.g. [tiab] functionality in advanced searches in PubMed) which will contain the search results to highly relevant publications only.

Quality Check

The primary means of quality checking the compiled searches will be by cross checking bibliographies of selected key publications to determine any examples of omissions or missed papers from the primary database searches.

Three examples of key publications are provided here and more may be included from the compiled search.

Chorus and Testai (2020). Recreational and occupational exposure. Chapter in Toxic Cyanobacteria in Water, 2nd edition. WHO has recently endorsed a review entitled *Recreational and Occupational exposure to Cyanobacteria* as part the update for the 2nd edition to the *Book Toxic Cyanobacteria in Water* (1st Edition published in 1999: Chorus and Bartram, Editors). This book will be published in 2020 and the comprehensive bibliography provides an initial valuable synthesis of the literature and is regarded as the most comprehensive current review that is closely related to the topic for the NHMRC review.

Ibelings *et al* (2014). Harmful Algae 40: 63-74 – This is a recent trusted authoritative review related to cyanotoxin risk assessment & guidance in multiple countries.

Backer, L. C. (2015) Toxins, 7: 1048-1064 - This is a recent trusted authoritative review of harmful algal bloom events and suspected adverse health effects in the US.

In addition, the compiled included search will be checked for inclusion of relevant papers by the following authors: Stewart I.; Shaw G.; Backer L.C.; Hilborn, E.D. These authors are regarded as leaders in research in this field over the time period of interest. Equivalent lists of highly regarded and cited authors will be developed to quality check the comprehensiveness of searches related to Marine Cyanobacteria and Algae.

Grey literature will be searched using three approaches:

1. A range of national organisations will be searched for relevant grey literature directly on their websites. These will include but not be limited to UK Health Protection Agency, United States Environmental Protection Agency, Centers for Disease Control and Prevention, World Health Organisation, United Nations Environment Programme, European Environment Agency, Umweltbundesamt, and Canada Environmental.
2. The topic will be searched by Google searches based around the concepts and key word to capture relevant guidance or advisory documents from State/Provincial health or environmental authorities.
3. Citation searching of key papers will be used as a key source of grey literature on the topic

Records of the search procedure, organisations and reports sourced will be documented for the Technical Report.

Documentation of Search

The search process will be fully documented giving the search strategy, the sequence and progression of iterative searches and their results. The statistics of the searches will be recorded preferably by screen shot capture or alternatively by direct copy and paste for inclusion in the Technical Report. The outcome of the searches and screening process will be presented in a PRISMA Flow Diagram (Moher et al, 2009).

Retrieval of Publications

Publications and reports will be obtained via the University of Adelaide Library or from open access literature databases where available. Publications and reports downloaded will be collated into a literature database using EndNote reference management software. EndNote will also be used to manage bibliographies and references when writing the Narrative Review and Technical Report. The version to be used is EndNote V9.3.3.

Process for Extracting and Presenting Data

Data will be extracted from each paper for full review and presented in summary 'Metadata' files. These will be compiled in Excel and will have searchable filters. These files are both a compilation and analysis table which are principally designed to record details of study type and design, exposure categories and reported outcomes and include the contents of the PECO criteria. The units used in all data will be checked and converted where required to achieve consistency. One table is required for the freshwater cyanobacteria and algae publications and one for the marine cyanobacteria and algae studies. The tables will be formatted for presentation as Word Tables in the Technical Report.

A draft example of a Metadata File table for freshwater pelagic cyanobacteria and algae is given in Table 6 (see **Appendix 1**). A draft list of definitions for filter terms are provided in Tables 7 (see **Appendix 1**).

The Metadata compilation tables are a valuable resource to record contact of studies in a consistent manner and guide the initial analysis. Their further value is as a legacy resource from the project, which can be readily interrogated using the filters to pull out studies into groups related to different

categories of exposure (cyanobacteria and toxin types), water body types, types of health outcomes, etc.

These Metadata tables are not an analysis tool for Risk of Bias and results assessment and evidence quality, and this will be achieved in more specific tables related to evidence evaluation for each research question. These will be developed and provided in a Word format.

Process for Critically Appraising the Evidence

Primary studies will be used to answer the primary research question using a narrative review approach. One reviewer will perform this assessment.

Studies selected for full review will be critically appraised for relevance and suitability for the update of the NHMRC guidelines. This appraisal consists of both assessing the risk of bias of individual studies and assessing the certainty of the body of evidence.

The studies included in this Narrative Review are anticipated to cover a range of types of evidence including existing guidelines or guidance, comprehensive reviews, and peer-reviewed primary studies. The process of evaluation differs for each type of studies and is summarised as follows:

- Guidelines or Guidance: assessment will be made of how that guideline was developed.
- Comprehensive reviews: assessment will be made of how the authors reviewed the evidence.
- Primary studies: evidence will be assessed separately against criteria that can be used to evaluate how trustworthy the results (see sections below).

Assessment of the risk of bias (study quality) of individual studies

Definitions used here are provided by NHMRC as follows:

- "**Bias** refers to factors that can systematically affect the observations and conclusions of a study and cause them to be different from the truth"
- "**Risks of bias** are the likelihood that features of the study design will give misleading results" Reference: https://www.nhmrc.gov.au/guidelinesforguidelines/develop/assessing-risk-bias

The methodological quality of individual studies will be assessed using an adaptation of the OHAT risk of bias tool (**Appendix 2**) (OHAT, 2019). Studies will be evaluated on applicable risk of bias questions based on study design. The rating or answer to each risk of bias question will be selected on an outcome basis from four options:
- definitely low risk of bias (++)
- probably low risk of bias (+)
- probably high risk of bias (-)
- definitely high risk of bias (--).

Data used to assess risk of bias will be extracted using existing approaches/templates such as those available in the OHAT Handbook (OHAT, 2019), from the CASP website (*Reference*) or the appendices of the US EPA (draft) methodological framework (*Reference*) depending on study type. Study types that do not have an existing template (such as monitoring studies) can be assessed against the usual risk of bias domains using questions such as those outlined in the OHAT framework Table 5 (OHAT, 2019) where applicable.

Studies that are determined to have a high risk of bias or serious concerns with study quality can be excluded from the review. Their removal will be recorded with justification in the PRISMA flow diagram.

Conflicts of interest and funding data from the study characteristics tables will be considered when assessing whether these might have affected any of the risk of bias domains (e.g. selection of comparators, selective reporting of results). If there are serious overall concerns, these will be noted under 'Other sources of bias' in Appendix 2.

The outcome of the risk of bias assessment will be presented in the Evidence Evaluation Report, together with a discussion of the overall quality of each study. Full details of each assessment will be provided in the Technical Report.

Once a determination of risk of bias for each domain has been made, a visual summary of the risk of bias ratings for the included studies can be prepared and used in the next stage of the critical appraisal process to determine overall risk of bias across the body of evidence (see the *OHAT Handbook: Table 9* (OHAT, 2019) and **Appendix 3**).

A template for questions for assessing the risk bias in studies in this review based upon the PECO Table is provided in Table 8. These questions may be further refined and updated after the search and review of full reports and papers in underway.

Table 8: Template for questions to be addressed for assessing risk of bias in individual studies presented in terms of the PECO categories

Population
How was selection of the population to be studied performed?
Was the population exposure group suitably segmented by health/atopic status, age, sensitive groups?
Exposure
How was exposure defined/assessed? i.e.   • Was exposure clearly categorised as full or partial immersion contact, non-contact?   • Was the exposure route clearly categorised for example as oral vs inhalation ingestion; dermal.
Was the sampling or monitoring program systematic and adequate to clearly document exposure (e.g. quantitative data on cyanobacteria or algae, suitable surrogates, quantitative toxin data, high level taxonomic identification)? i.e.   • Was the sampling and monitoring sufficiently close to the exposure zone?   • Was there insufficient sample replication?   • Was there recognition and accounting for spatial variance?   • Were the cyanobacteria and/or algal types and numbers confirmed by credible high-level taxonomic identification and quantitation methods?   • Were cyanotoxins identified and quantified by appropriate methods?
Is there sufficient confidence in confirmation or matching of exposure with adverse health outcomes/no outcomes (no significant time lags were observed between sampling/monitoring for cyanobacteria/cyanotoxins and exposure/health effects reports)?
Is there evidence that exposure may be relevant to occupational situations/categories? (only include where the study is clearly valuable in terms of protecting a sub-group)
Outcomes

Was the adverse health impact diagnosis confirmed medically (clinically) or were they records of self-reported cases of illness?
Study Design/Methods (Additional assessments)
Is the sample size of exposed individuals suitable for the study design and satisfactory for meaningful assessment?
Are there suitable control groups (non-exposed)? How were the control subject group members selected (i.e. derived from the same overall population as the case group enabling appropriate comparison and minimising selection bias)?
Were appropriate statistical methods applied for type of data?

Assessment of the certainty of the body of evidence

A process based on the OHAT (2019) approach to using the GRADE system will be used to assess the certainty of a body of evidence. The GRADE system to assess the certainty of the evidence as recommended by NHMRC is described at:
https://www.nhmrc.gov.au/guidelinesforguidelines/develop/assessing-certainty-evidence.

Evidence streams for each research question will be tabulated together by outcome if possible. It is anticipated that the summary tables will include evidence streams for multiple studies and grouped together to present evidence for the four topics listed for review to update the guidelines. These are: 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).

An overall certainty rating will be assigned to each evidence stream after the domains used to assess certainty in the GRADE framework are applied to the body of evidence: overall risk of bias across studies, unexplained inconsistency, imprecision, indirectness, publication bias. Under the GRADE system, the overall quality of the evidence for an outcome is categorised as high, moderate, low or very low.

Each evidence stream will be assigned an initial certainty rating similar to that described in the OHAT Handbook (OHAT, 2019). For example, evidence from randomised controlled trials is initially graded as high certainty and evidence from observational studies is initially graded as low certainty. If there are any study types that do not have an initial rating, an appropriate initial rating will be determined by the reviewer in a similar manner to the approach used in OHAT (2019).

The certainty of the evidence can be downgraded or upgraded from the initial rating if any of the conditions in the Table 9 (below) are met. If none are met, the initial certainty rating is retained. These domains are explained in more detail in the OHAT (2019). Conflicts of interest and funding sources will also be considered as a reason to downgrade if there are serious concerns that these have influenced the findings from the body of evidence.

Table 9: Approach used to downgrade or upgrade the certainty of the evidence from the initial rating (Based upon Figure 6 in the OHAT (2019)).

Reasons to Downgrade	Reasons to Upgrade
• **Risk of bias** - Serious or very serious concerns about study quality across the body of evidence (reliability) (see **Appendix 2**) • **Unexplained inconsistency** - Important inconsistency of results across the included studies that can't be explained by study design • **Indirectness** - Some or major uncertainty about directness (relevance to the research question that is being answered) • **Imprecision** - Imprecise or sparse data • **Publication bias** - High probability of reporting bias (selective reporting of results across the body of evidence that might skew results)	• **Consistency** - Strong or very strong evidence of association based on consistent evidence from two or more observational studies, with no plausible confounders • **Magnitude of effect** - Very strong evidence of association based on direct evidence with no major threats to validity • **Dose-response** - Evidence of a dose-response gradient • **Residual confounding** - All plausible confounders would have reduced the effect • **Other reasons** – any topic-specific reasons as determined by experts in the field

The results of the certainty assessment process will be tabulated in a similar manner to that described in the OHAT (2019) framework (**Appendix 3**). Where a conclusion is unable to be made by the reviewer around any of the domains (e.g. inconsistency and imprecision may be difficult to ascertain with the kind of evidence that will be included in the review) this will be recorded as 'not applicable' or 'unknown'. Tables summarising the results for each outcome will be included in the Evidence Evaluation Report and the full evidence profiles will be included in the Technical Report.

Process for Reporting

Reporting for this review will comprise two documents – the Evidence Evaluation Report (Narrative Reviews) based around each research question; and the Technical Report containing detailed information about the methods used to undertake the literature reviews.

The Evidence Evaluation Report is structured to systematically address each research question and be informed by the evidence evaluation. This report will follow the format and content required by the NHMRC as follows:

- **Executive Summary**
- **Introduction** and Background: including definitions of key terms, outcome measures, abbreviations, rationale for review and objectives
- **Methodology:** brief overview only, with a reference to full details provided in the Technical Report
- **Results:** a summary of results for each research question, main findings, document characteristics. The results section will present outcome data presented in the included studies and will be extracted and will be presented in an evidence summary table as appropriate, along with the overall certainty rating for those results. Draft evidence

statements outlining how these results address the relevant research questions will be prepared. The evidence statements will take into account the extent and strength/limitations of the evidence.

- **Discussion:** including strengths and limitations of the studies as per the evidence statements provided in the Results section, comparison of existing literature, a discussion of gaps in the evidence (if identified during the evaluation of the evidence) and a suggestion of areas for further research
- **Conclusions**
- **References**
- **Appendices**

The Methodology for the Evidence Evaluation report is a concise record of the approach and procedures used for the Search Strategy and Selection of Evidence, the Process for Extracting and Presenting Data and the Process for Critically Appraising the Evidence. The Results section will likewise comprise concise summaries of the outcome from each step in the Methodology. This will primarily be presented in tables.

The Technical Report is a stand-alone supplementary document that contains comprehensive information about the full detail of methods used to undertake the literature reviews. This information is more comprehensive than that contained in the Evidence Evaluation report. The purpose of the Technical Report is to allow the Evidence Evaluation report to be as concise as possible, and also to serve as a comprehensive reference to all methods, supplementary and ancillary information regarding the process of the Review.

The Technical report will also follow the format and content proposed by NHMRC as follows:
- the research questions
- the search strategy used to identify and retrieve studies
- the process for selecting studies (i.e. inclusion/exclusion criteria)
- the methodology used to critically appraise the literature and the quality assessment of included studies
- the methods used for data extraction
- the methods used to critically appraise and synthesise the data of included studies
- the methods used to analyse and summarise the results of included studies
- the methods used for any calculations and explanatory text for any assumptions if used
- documentation of the declared interest(s) of the author(s) of each paper
- a description of how comments from the independent methodological review of the draft research protocol were addressed.

Declared Interests

The Author of this Review (Associate Professor 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 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 reviewer has professional scientific relationships with several members (three members) of the RWQAC 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

Process for Making Amendments to the Protocol

If amendments are required to the Protocol after the Review commences, the proposed changes will be communicated to ONHMRC (Water Team) for review and endorsement by RWQAC if needed. Any agreed amendments will be documented in the Technical Report.

References

Backer, L. C., Manassaram-Baptiste, D., LePrell, R. and Bolton, B., (2015) Cyanobacteria and algae blooms: review of health and environmental data from harmful algal bloom-related illness surveillance system (HABISS) 2007-2011. Toxins, 7: 1048-1064

Chorus, I and Bartram, J., (1999). Toxic Cyanobacteria in Water. A guide to their public health consequences, monitoring and management. E&FN Spon publishers, London.

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NHMRC, (2008). Guidelines for Managing Risks in Recreational Water. National Health and Medical Research Council, Canberra, Australia.

OHAT, (2019). Handbook for Conducting a Literature-Based Health Assessment Using OHAT Approach for Systematic Review and Evidence Integration. Office of Health Assessment and Translation (OHAT). Division of the National Toxicology Program, National Institute of Environmental Health Sciences.

APPENDIX 1

Table 6: Draft example of information to be compiled from each paper into a Metadata Excel file for freshwater pelagic cyanobacteria and algae.

General information	Study ID	Filters to be applied in Excel sheet
	Date template completed	
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	State State Journal/report Yes/no State State/ not provided State/ not provided
Study characteristics	Aim/objectives of study	Not in Excel database
	Study type/design	Epidemiological/Field/Lab
	Epidemiological study type	Randomised cohort study - RCoh Cohort study - Coh Case-control - C-Con Cross-sectional - C-Sec
	Study duration Lag Time	Specify length of exposure Yes/no
	Type of water source/water body	Lake/reservoir/river/farm dam
Population characteristics	Population/s studied	Human/animal Men/women/both Wild/domestic
	Selection criteria for population	Healthy/compromised/unspecified
	Subgroups reported	Elderly/Adults/children/infants/unspecified
	Size of study	Number in each study
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Causal organism/ toxin(s)	See above Indirect contact/ direct immersion/ direct non-immersion ingestion, respiration, dermal Organism identified - yes/no Specify organism name(s) on separate line Monitoring - cell counts, chlorophyll, biovolume/biomass Surrogate measurement – yes/no Surrogate - specify Toxin(s) identified - yes/no Toxin type - microcystins, cylindrospermopsins, saxitoxins, anatoxin-a, nodularin Toxin quantification – yes/no Toxin analysis - instrument, immunoassay/other Analytical precision
	Comparison group(s) - Unexposed identified	Yes/No
Study methods	Water quality measurement used	See above

	Method of microorganism isolation and enumeration (if applicable)	
	Water sampling methods (monitoring, surrogates)	See above
	Water Monitoring program type (e.g. part of local agency program or custom designed for the study	Routine/study specific
Results (for each outcome)	Definition of outcome	Health impact - gastro-intestinal, neurological, respiratory, skin irritation, allergic/not defined
	How outcome was assessed	Health assessment – self-reported/medically diagnosed
	Method of measurement	Quantitative/qualitative
	Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Same as size of study
	Lag in health outcome reporting	Was follow-up immediate, within 2-3 days, after a week, etc
Statistics	Statistical methods used	State
	Details of statistical analysis (if any)	To be provided in Word document.
	Relative risk/odds ratio, confidence interval?	
Author's conclusion	Interpretation of results	Not relevant for Excel database. To go into evidence appraisal tables
	Assessment of uncertainty (if any)	
Guidance or Guidelines	Contains guidelines	Yes/No
	Jurisdiction	National/Local
	Guidance based upon primary evidence review	Yes/No/Unknown
	Guidance has been subject to review/consultation	Yes/No/Unknown
Reviewer comments	Results included/excluded in review (if applicable)	
	Notes on study quality e.g. gaps, methods	Not relevant for Excel database. To be provided in evidence appraisal tables

Tables 7: Draft list of definitions for filter terms applied in Metadata Excel file for freshwater pelagic cyanobacteria and algae (Table 6).

FILTER TERM	DEFINITION
Article Type	
Journal	
Report	
Study Type	
Epidemiological	Epidemiological study type: Randomised cohort study - RCoh Cohort study - Coh Case-control - C-Con Cross-sectional - C-Sec
Field	Study of animals or humans exposed from contact with natural water.
Study Duration	
Lag time	Any time delay between sampling & quantification of cyanobacteria and/or toxins and reports/measurement of health outcome.
Study Location	
Lab	Study of animals or humans exposed in the laboratory
Lake	Inland water body not used for provision of potable water.
Reservoir	Inland water body used for provision of potable water.
River	Flowing body of water.
Farm dam	Water body used for provision of water for stock or irrigation.
Population	
Elderly	Adult humans > xxx years old.
Adults	Adult humans 18 to xxxx years old.
Children & Adolescents	Humans 2 to <18 years old.
Infants	Humans <2 years old.
Healthy	Humans with no identified pre-existing health condition(s) prior to exposure.
Compromised	Humans with identified pre-existing health condition(s) prior to exposure.
Wild	Undomesticated animal.
Domestic	Domesticated animal.
Exposure	
Indirect contact	Subject exposed to aerosol. This would include activities such as fishing from a boat or jetty, kayaking, walking along edge of waterbody etc.
direct immersion	Subject fully immersed, including head in water body. This would include activities such as swimming, windsurfing, surfing, etc.
direct non-immersion	Subject not fully immersed in water body. This would include activities such as wading, fly-fishing, etc.
ingestion	Cyanobacteria and/or toxins are ingested orally.
inhalation	Cyanobacteria and/or toxins are ingested by inhalation
dermal	Cyanobacteria and/or toxins are exposed to subject through skin or eye contact.
Cyanobacterial Quantification	
cell counts	Quantification of cyanobacteria/algae by any method of counting cells.
chlorophyll	Quantification of cyanobacteria/algae by measuring chlorophyll.
biomass	Quantification of cyanobacteria/algae by any method for counting cell biomass.
Toxin Type and Measurement	
Toxin type	Microcystins, cylindrospermopsins, saxitoxins, anatoxin-a, nodularin, LPS endotoxins.
instrument	Identification of toxin by analytical instrumentation such as GCMS, LCMS

immunoassay	Identification of toxin by antibody measurement
other	Identification of toxin by any other technique other than instrument or immunoassay.
Outcome	
Health impact	Specify gastro-intestinal, neurological, respiratory, skin irritation, allergic or not specified.
Health assessment – self-reported/medically diagnosed/none	Specify if assessment was done. If done, was it self-reported by follow up from exposed subjects or whether it was medically diagnosed using valid observational assessment criteria e.g. dermatological measurement of skin reaction following exposure.
Health assessment measurement	Specify whether assessment was made quantitatively or qualitatively.
Guidance or Guidelines	
Contains guidelines	Provides or derives recreational water guidelines & guidance
Jurisdiction	Issued nationally or by local/state/provincial health authorities
Guidelines based upon primary evidence review	Guidance based upon a primary evidence review with citations
Guidance has been subject to review/consultation	Guidance/guidelines have been circulated for consultation (as per NHMRC, US EPA, Canadian EPA, etc.)

APPENDIX 2

Risk of bias assessment tool for individual studies (adapted from OHAT RoB tool – see Table 5 in OHAT Handbook (OHAT, 2019) for details on relevant questions for each study type). This tool will be adapted for the Review based upon the study type.

Study ID: Study Type:	Yes/No Unknown N/A	Notes	Risk of bias rating (--/-/+/++)
Selection bias			
Was administered dose or exposure level adequately randomized?			
Was allocation to study groups adequately concealed?			
Did selection of study participants result in appropriate comparison groups?			
Cofounding bias			
Did the study design or analysis account for important confounding and modifying variables? *			
Performance Bias			
Were experimental conditions identical across study groups?			
Were the research personnel and human subjects blinded to the study group during the study?			
Attrition/Exclusion Bias			
Were outcome data complete without attrition or exclusion from analysis?			
Detection Bias			
Can we be confident in the exposure characterization? *			
Can we be confident in the outcome assessment? *			
Selective Reporting Bias			
Were all measured outcomes reported? *			
Other Sources of Bias			
Were there no other potential threats to internal validity (e.g., statistical methods were appropriate, and researchers adhered to the study protocol)? *			

*Key questions for all study types (including any non-human or non-animal studies like monitoring or modelling data)

Risk of bias rating: Definitely low risk of bias (--) `--` | Probably low risk of bias (-) `-` | Probably high risk of bias (+) `+` | Definitely high risk of bias (++) `++`

Overall risk of bias (body of evidence by study type) adapted from OHAT Handbook (OHAT, 2019)

Research Question: *e.g. What is the risk of adverse health outcomes from exposure to cyanobacteria and algae in recreational water?*

Outcome: *e.g. gastrointestinal illnesses*

Risk of Bias Question	Case report					Case-Control study					Cohort study					Other			
	Study 1	Study 2	Study 3	Study 4	Study 5	Study 6	Study 7	Study 8	Study 9	Study 10	Study 11	Study 12	Study 13	Study 14	Study 15	Study 16	Study 17	Study 18	Study 19
Randomization																			
Allocation concealment																			
Confounding (design/analysis)	++	+	++	++	++	+	++	++	++	++	+	++	++	+	−	−	−	−	++
Unintended exposure	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Identical experimental conditions	++	++	+	+	++	++	++	++	++	+	++	+	++	++	++	++	++	++	++
Adhere to protocol	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Blinding of researchers during study																			
Missing outcome data	−	+	++	++	−−	−	+	−	−	+	−−	−	−	+	++	+	++	+	++
Assessment of confounding variables	+	+	++	++	++	−	+	+	++	++	+	+	+	++	++	−	+	+	++
Exposure characterization	++	−	+	+	−	−	+	+	−	−	−	+	+	+	+	+	+	−	+
Outcome assessment	+	+	+	+	+	+	++	+	+	−	++	+	+	+	+	+	+	+	+
Blinding of outcome assessors	+	+	+	+	++	+	+	+	+	+	+	+	−−	+	++	+	+	+	+
Outcome reporting	+	+	+	++	−−	+	+	+	+	−	+	+	−−	+	+	+	++	−	+

Key:

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

APPENDIX 4

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	*Serious, not serious, unknown* Describe trends, key questions, issues	*Serious, not serious, not applicable* Describe results in terms of consistency, explain apparent inconsistency (if it can be explained)*	*Serious or not serious* Discuss use of upstream indicators or populations with less relevance, any time-related exposure considerations (see OHAT RoB tool)	*Serious, not serious, unknown* Discuss ability to distinguish treatment from control, describe confidence intervals (if available)	*Detected, undetected, unknown* Discuss factors that might indicate publication bias (e.g., funding, lag)	*Large, not large, unknown* Describe magnitude of response	*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, not applicable (NA)* Describe cross-species, model, or population consistency	*Yes or no* Describe any other factors that increase confidence in the results	***High, moderate or low*** List reasons for downgrading or upgrading
Research question: *e.g.* What is the risk of adverse health outcomes from exposure to cyanobacteria and algae in recreational water?											
Outcome 1. *e.g. gastrointestinal illness*											
e.g. human case control studies (5 studies) Low to moderate certainty											
Outcome 2:											