Draft Research Protocol: Free-living Organisms Narrative Review

Contents

Version control

Version	Author	Submitted to	Date
V1 – draft protocol	Geoff Puzon	ONHMRC	9/11/2020
V2 – final protocol	Geoff Puzon	ONHMRC	16/11/2020

Background and purpose

The National Health and Medical Research Council (NHMRC) is updating the *Guidelines for Managing Risks from Recreational Water* (2008) to ensure that they reflect the best available evidence and are current and relevant for the Australian context. This update of the 2008 Guidelines will enable NHMRC to continue its role of providing advice to jurisdictions on how to manage risks to public health from recreational waters and ensure that recreational water sites are safe to use. The update is being overseen by the Recreational Water Quality Advisory Committee (RWQAC).

The update will be informed by a series of independent evidence reviews. These will provide NHMRC with assurance that this revision of the Guidelines is grounded in the most up-to-date and relevant scientific evidence.

A reviewer has been contracted to undertake a narrative review on the risks to human health from free-living organisms in recreational water. This review will be used to inform the update to Section 8.2.6 of the *Guidelines for Managing Risks in Recreational Water (2008)* and any relevant sections throughout the rest of the document.

The purpose of this report is to provide a draft 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 (Deliverable 1). This will be discussed with RWQAC and finalised to meet Deliverable 2 (Final Research Protocol).

Scope of review

The scope of the narrative review on free-living organisms will be determined by a number of factors as determined by RWQAC, including:

- key definitions
- research questions
- criteria for population, exposure (and comparator) and health outcomes of interest (PECO)
- inclusion/exclusion criteria.

These criteria will be determined by RWQAC as part of the protocol and are outlined below. They are based on a balanced assessment of new evidence, guidelines and standards published since the 2008 Guidelines, stakeholder feedback, Committee members' expertise/knowledge and other sources of information on the continued relevance of the guidelines.

The scope of the review will also be partly determined by the nature of the evidence and the evaluation methods used to answer each research question. Depending on the anticipated size/type of the literature base available there are several options including:

- critically appraising all relevant primary studies
- review all existing reviews
- assess existing guidance to adopt/adapt advice
- a combination of some/all of the above (mixed methods approach).

The methods used to evaluate the evidence for each research question will be predetermined by the reviewer and RWQAC to ensure that the review is conducted within available resources.

Best practice methods for evaluating evidence will be applied as much as practically possible as per the NHMRC Standards for Guidelines. This includes applying systematic and transparent approaches to searching, selecting, reporting and assessing the quality of the body of evidence as outlined in NHMRC's Guidelines for Guidelines online handbook.

Key definitions

The definitions in **Table 1** have been determined by RWQAC to set the scope of the update to the Guidelines. They also determine a number of inclusion and exclusion criteria that will be used to screen the evidence in the narrative review. Additional definitions in the current Guidelines (e.g. primary and secondary contact) will also be used where required.

Table 1: Key definitions	
Free-living microorganisms	Microscopic organisms such as amoeba, saprozoic bacteria and protozoa that can exist independently of other organisms and which are generally considered opportunistic pathogens.
Recreational water	*Included*: Any natural or artificial water bodies without a chlorine disinfectant residual that might be used for recreation including coastal, estuarine and fresh water 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.

Research questions

The following research questions have been developed by RWQAC to guideline the narrative review on free-living organisms:

Primary question:

- What is the risk of any adverse health outcome for water users from exposure to *Naegleria fowleri* or *Burkholderia pseudomallei* in recreational water?

Secondary questions:

- What are the indicators/surrogates of this/these risk/s? (e.g. temperature, thermally polluted, turbidity, faecal indicators and microbial ecology)
- What is the frequency of occurrence of identified health outcomes in Australia? Is there an association with exposure to recreational waters?
- What is known about the occurrence of these organisms in natural waters in Australia?
- What are the conditions associated with increased occurrence? What are the conditions associated with absence of these microorganisms?
- What is known about the exposure pathway for each organism?
- What is known about the dose-response for each organism?
- What are the current practices to minimise or manage this/these risk/s?

Population, Exposure (Comparator), Outcomes

The following advice has been scoped out and provided by RWQAC to inform the evidence review:

Table 2: Population, Exposure (Comparator), Outcome (PE(C)O) table

Element	Criteria

Population	Population groups that are relevant to the Guidelines:
	• The general population
	• Specific subpopulations:
	  ○ Elderly
	  ○ Infants and children
	  ○ Pregnant women
	  ○ 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.
	  ○ Subgroups with unusual exposure patterns making them more susceptible (e.g. athletes, people or age-groups practicing energetic water-based activities or using recreational water for cultural ablution purposes) due to larger volumes of water ingested and/or inhaled, different frequency of exposure etc.

Exposure (and comparator)	Free-living microorganisms of interest (through all routes of exposure, compared to no exposure): • *Naegleria fowleri* • *Burkholderia pseudomallei* Include circumstances that lead to elevated exposures (e.g. sediment concentrations and exposure, settings with incidences of thermal pollution)
Outcomes	Relevant human health outcomes of interest: For *Naegleria fowleri:* • primary amoebic meningoencephalitis (PAM) • all other adverse health outcomes For *Burkholderia pseudomallei*

	• melioidosis • all other adverse health outcomes

Narrative review process

The narrative review for free-living organisms will be carried out using the following key steps:

1. Search and select evidence
2. Extract relevant data
3. Critical appraisal of the evidence
4. Synthesis of findings
5. Reporting

As mention in the **Scope** section, the quantity/types of evidence available will determine what type of evidence will be used to answer each research question and how it will be evaluated.

The following approaches will be used in this review depending on the research questions and subject to approval by RWQAC (see **Table 3**):

- **primary studies, reports or other types of direct evidence/data**: each study will be assessed separately against criteria that can be used to evaluate the quality and certainty of the evidence.
- **existing systematic/ literature reviews**: the process used by the authors to review the cited primary studies will be assessed against set criteria to determine how trustworthy the conclusions of the review are.
- **existing guidelines/guidance/advice**: the processes used by the agency/organisation to develop the guideline/advice will be assessed against set criteria to determine how robust the advice is.
- **mixed methods approach**: when a combination of the above is anticipated, the quality of each type of evidence will be assessed separately as described above and the results combined for analysis afterwards.

Table 3: Methods to evaluate evidence for each research question	
Primary Question: What is the risk of any adverse health outcome for water users from exposure to *Naegleria fowleri* or *Burkholderia pseudomallei* in recreational water?	Mixed methods approach – primary studies/reports and any existing review/guidance that contains relevant data to address the question
Secondary Questions: What are the indicators/surrogates of this/these risk/s?	Review of reviews only
What is the frequency of occurrence of identified health outcomes in Australia? Is there an association with exposure to recreational waters?	Mixed methods approach
What is known about the occurrence of these organisms in natural waters in Australia?	Mixed methods approach
What are the conditions associated with increased occurrence? What are the conditions associated with absence of these microorganisms?	Mixed methods approach
What is known about the exposure pathway for each organism?	Mixed methods approach

What is known about the dose-response for each organism?	Mixed methods approach
What are the current practices to minimise or manage this/these risk/s?	Review existing guidance only

The methods used for each research question will be predetermined before the review commences based on prior knowledge of the literature base by the reviewer and RWQAC. As outlined in **Table 3**, most of the research questions listed for this review are anticipated to be addressed using all relevant evidence. While the preference will be to use primary Australian studies and reports, international evidence including any reviews or guidance documents will be considered if relevant. However, the reviewer and RWQAC may determine that a different approach may be more appropriate, such as:

- reviewing existing reviews when the literature search results in an extensive body of studies and the workload of the reviewer will be unmanageable.
- reviewing existing guidelines or guidance to assess current practices to manage risk – reports evaluating the effectiveness of current practice may also be included.

In the event that the approach might need to be varied after the literature search commences, the reviewer will consult with RWQAC and ONHMRC to decide which approach to take. Final decisions will be made by RWQAC and any changes will be recorded as required in the Technical Report.

Where the reviewer is the author of a primary study that is retrieved in the literature search, other members of the review team who are not listed as authors will carry out the screening and critical appraisal of the relevant study. In the event that all members of the review team are authors of the study, a subgroup of RWQAC members may screen and evaluate the study. The findings will then be returned to the review team to assess certainty of the body of evidence. The details of this process will be recorded in the Technical Report.

Process for searching and selecting evidence

The following process will be used to search the literature and decide what evidence to include:

- **Literature search**: At least two databases will be searched using pre-agreed search terms combined into different combinations of search strings. The search strings will be used to analyse key words, titles and abstracts of publications available in the databases. Several different searches may be required to identify the evidence needed to answer all of the research questions. An online search for grey literature including existing guidance will also be undertaken using a selection of key search terms.
- **Validation:** An initial series of literature searches will be undertaken to test the proposed search strategy and refinements made as needed. A quality check will also be undertaken at an early stage by cross-checking the search results for key publications (as determined by the reviewer and RWQAC at **Appendix A**). If the reviewer notes serious omissions, the search strings might need to be modified to ensure that they are picking up the key publications.
- **Screening**: The results of the literature searches will be consolidated and analysed for duplicates, and the title and abstracts screened against inclusion/exclusion criteria. If the reviewer is unsure if the study meets inclusion criteria based on analysis of the title/abstract, the study should be flagged for full analysis of the full-text article to determine whether it meets criteria.
- **Retrieving publications**: Digital copies of any studies that have been found to meet inclusion criteria or require further hand-searching will be retrieved and compiled in a database.

- **Reporting**: Details of the search and selection process will be recorded in the Technical Report.

Some literature searches are so broad that even using combinations of key terms to narrow it down, thousands of papers might be retrieved for screening. Amendments to the protocol during the search process may be applied as needed to tighten the scope and keep the project within available resources.

Options for tightening the search results can include:

- adding further inclusion/exclusion criteria
- prioritising outcomes to be considered
- prioritising organisms of interest
- changing the method of the narrative review, e.g. conducting a review of reviews to reduce the number of publications that need to be appraised
- adopt/adapting existing guidance/guidelines (screening criteria still apply).

The reviewer will consult with RWQAC and the relevant subgroup through ONHMRC about any suggested changes to the literature search. Any final decisions by RWQAC will be recorded in the final report.

Search terms

The search terms in **Table 4** will be used to find evidence to answer the research questions based on the PECO elements. The key terms will be developed into search string combinations that will be used across all selected databases for consistency. The search string combinations, order of searching and outcomes (number of studies found, number of duplicated, number of excluded studies and justification) will be reported using a PRISMA flow diagram (Moher et al. 2009). This process will be reported in the Technical Report.

Databases

The databases that will be searched include PubMed and Scopus. The search strings used across the databases will be consistent using the key terms outlines above. The search strings will be provided in the Technical Report.

Publication date

Publications from 2004 onwards will be retrieved in this literature search. The selection of this date is to ensure the inclusion of relevant studies that have been published since the last review for the *Guidelines for Managing Risks from Recreational Water* (2008).

Language

English publications will be retrieved in this literature search. In the event that RWQAC decides that a non-English publication should be included, translation of this publication will be arranged by ONHMRC.

Table 4: Literature search keys words and variants			
Population terms	**Recreational water terms**	**Exposure terms**	**Outcome reporting terms**
general population	recreation/al water use	free-living microorganisms	primary amoebic meningoencephalitis (PAM)
elderly	primary/secondary contact	free-living amoebae	meliodosis
children	swimming	surface water pathogens	
infant/s	bathing	pathogen	health
pregnant/pregnancy	wading	amoeba	health effects
susceptible/vulnerable	paddling	ameba	health outcome/s
immunocompromised	water sports	amoebae	adverse effects
athlete/s	boating	amebae	waterborne disease/s
recreational water user/s	sailing/sailboating	thermophilic	recreational water infections
recreator/s	body boarding/surfing	*Naegleria fowleri*	disease
tourists	wakeboarding	*Burkholderia pseudomallei*	infection
Aboriginal	wind surfing		illness/es
Torres Strait Islander	water/jet skiing	aerosol/s	symptoms
indigenous	fishing	sediment/s	gastrointestinal
(*check Lowitja library terms*)	anglers/angling	sand	nausea
	kayaking	water quality	vomiting
Study type terms	canoeing	exposure	diarrhea
study	rowing	oral	diarrhoea
review	snorkelling	ingestion	accidental faecal discharge
epidemiology	scuba divers/diving	inhalation	pneumonia-like symptoms
epidemiological	surfers/surfing	dermal	fever
systematic review	kite boarding/surfing	aural	headache
narrative review	parasailing	ocular	hay fever-like
literature review	pentathlon	nasal	flu-like
randomised controlled trial	triathlon	aerosols	skin rash/es
cohort		climate change	skin irritation
case report	recreational water	storm events	eye irritation
case-control	fresh/salt water/marine		pruritis
cross-sectional	beach/es	**Measurement terms**	dermatologic
diagnostic test study	river/s	temperature	allergic reaction/s
recreational guidelines	lake/s	monitoring	neurotoxicity
guidelines	dam/s	direct pathogen monitoring	neurologic/al
report	hot spring/s	non-microbial indicator/s	hepatotoxicity
jurisdiction/al	reservoir/s	sampling	dermal irritation
legislation	catchment/s	indicator/s	allergic reaction/s
	coast/al	surrogate/s	inhalation-related symptoms
	estuary	source tracking	induction of asthma
	shoreline	source vulnerability	shortness of breath
	riverbank	dose-response	meningitis
	water park/s	outbreak/s	
	stormwater	risk/s	
	rural	risk factors (physical, chemical, biological)	

		environmental conditions water quality conditions analytical methods	

Note: does not include Index and MeSH terms and wildcard terms (*). Also might need to use English and US spelling.

Study type

Depending on the method chosen to address each research question, the review will consider:

- primary human studies evaluating the risk of disease from exposure to natural waters. This includes any randomised controlled studies, cohort studies, case-control and cross-sectional studies that meet the selection criteria.
- reports/papers on the occurrence of the target organisms in natural waters.
- systematic/literature reviews of recreational water quality risk monitoring and management.
- existing recreational water quality guidelines/reports (national and international). A list of reports supplied by RWQAC is included in **Appendix A**.
- grey literature, reports and guidelines from the websites of reputable international and national agencies that will be helpful for assessing the nature of current guidance focusing on warm climates (e.g. WHO, US EPA, State and Commonwealth Departments of Health, State EPAs, environmental agencies of OECD member countries where such documents are available in English). If any agencies are directly contacted for copies of publications this will be documented in the Technical Report.

Peer-reviewed publications are preferred. Animal and in vitro studies will be excluded.

In the absence of Australian data, international studies may be included.

In the absence of data on indicators and surrogates (e.g. faecal indicator bacteria) for the target organisms when monitoring recreational water quality, studies examining indicators and surrogates used in engineered systems may be considered.

Screening against inclusion/exclusion criteria

The following criteria will be used to determine which studies are relevant for inclusion in the review:

- key definitions in **Table 1**
- PECO elements in **Table 2**
- criteria applicable to study types as outlined above and determined by the method chosen.

Publications will be screened by title and abstract using a selection of key terms to determine whether they are relevant and should be included or excluded from the review. If the reviewer is still unsure after looking at the title and abstract, the full text publication will then be screened for relevance.

If the reviewer is the author of the study being screened, a member of the review team who is not listed as an author will screen the study against inclusion/exclusion criteria. A subgroup of RWQAC members may screen the study if there is no one on the review team available to carry out this task.

Presentation of search results

Search strings and results of the literature searches and the screening process will be documented in the Technical Report. A PRISMA flow diagram (Moher et al. 2009) will provide details on the number of publications that were found and how many were excluded. Tables of citations retrieved from the searches and justification for their inclusion/exclusion will be recorded in the Technical Report.

Retrieval of publications

Once screening of titles and abstracts has taken place against inclusion/exclusion criteria has taken place, full-text publications of included studies and any that require further screening will be obtained by the reviewer through their library services. These publications will be collated with citation information in a suitable literature database (e.g. Endnote) and stored in secure backup storage. Digital copies of the publications included in the final reports will provided to ONHMRC.

Process for extracting and presenting data

Information required to answer the research question will be extracted using a template such as that provided at **Appendix B** to facilitate data collection. The information extracted will depend on the agreed research questions, the PECO criteria, the evidence related to inclusion, the study methodology and evidence strength and limitations. This information will be used to assess study quality and to compare to other studies.

RWQAC have also requested that the following data is extracted from studies to help in decision-making:

- environmental and water quality conditions associated with reported results
- analytical method for all environmental results.

If infection through recreational water in a study has been confirmed but a route of exposure for infection is not reported (e.g. aerosols, submersion, splashing), a multiple exposure pathway will be assumed and reported in the template.

Any studies found to not be relevant at this stage can be excluded from further assessment, with this process reported in the PRISMA diagram and in the Technical Report alongside other excluded studies.

Individual data summaries for each included primary study will be provided in the Technical Report.

Process for critically appraising the evidence

The evidence used to answer the research questions will require different methods to assess whether it is relevant and trustworthy before it can be used. Different methods are described below for assessing primary studies or for assessing existing guidance or reviews. Existing tools that may be used or modified in each process are described.

Process for assessing individual studies

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

If the reviewer is the author of the study being assessed, a member of the review team who is not listed as an author will assess the study for risk of bias. A subgroup of RWQAC members may assess the study if there is no one on the review team available to carry out this task.

Risk of bias (study quality) assessment of individual studies

The methodological quality of individual studies will be assessed using an adaptation of the OHAT risk of bias tool (**Appendix C**). 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, from the CASP website or the appendices of the US EPA (draft) methodological framework depending on study type (US EPA 2019). 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 where applicable. A list of study type definitions is provided in **Appendix D** and the reviewer must verify that the reported study type is correct before using the appropriate template.

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 and **Appendix E**).

Process for assessing the body of evidence

The evidence collected and appraised for each research question will be grouped by study type and outcome if possible and summarised in an Evidence Summary table that will have an assignment of the certainty (or confidence) in that body of evidence.

Assessment of the body of evidence

A process based on the OHAT approach to using the GRADE system will be used to assess the certainty of a body of evidence. Evidence streams for each research question will be tabulated together by outcome if possible. 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 Table 8. 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 Table 8.

The certainty of the evidence can be downgraded or upgraded from the initial rating if any of the conditions in **Table 5** are met (see Figure 6 in the OHAT Handbook). If none are met, the initial certainty rating is kept. These domains are explained in more detail in the OHAT Handbook. 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 5 *(adapted from Figure 6 in the OHAT Handbook)*

Reasons to Downgrade ↓	Reasons to Upgrade ↑
• **Risk of bias** - Serious or very serious concerns about study quality across the body of evidence (reliability) (**Appendix E**) • **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 framework (**Appendix F**). 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.

Presentation of results

A summary of the methodology used to find and select the studies and the findings of the critical appraisal process will be included in the Evidence Evaluation Report. Full details will be provided in the Technical Report.

Outcome data presented in the included studies 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. The evidence statements will be considered by RWQAC, who may provide advice on their revision.

Process for assessing existing guidance or reviews

Due to the large volume of evidence that might be found when undertaking the systematic literature search, several secondary research questions will be addressed instead using a review of existing guidance or reviews.

Similar search strategies to those used to search and select primary studies will be used to identify existing guidance and reviews. In addition, grey literature such as jurisdictional reports and guidance will be provided by RWQAC members and assessed by reviewers.

Critical appraisal of existing guidance and reviews

The methodological quality of the existing guidelines or reviews will be assessed using an adaptation of the tool provided in **Appendix G**. The criteria listed in the tool are based on common domains that are evaluated in several existing tools for assessing guidelines and systematic reviews (e.g. AGREE tool). Criteria that are deemed appropriate/inappropriate for a research topic or evidence type (guideline process v reviews) will be removed or added as needed. One reviewer will be performing the assessment.

Presentation of results

A summary of the methodology used to find and select existing guidance/reviews and the findings of the critical appraisal process of the included guidance/reviews will be included in the Evidence Evaluation Report. Full details will be provided in the Technical Report.

Outcome data presented in the guidelines/review will be extracted and will be presented in a results tables (evidence summary table) or figures as appropriate. Any important limitations of the existing guidance/reviews will be described. Draft evidence statements outlining how the existing guidance/reviews address the relevant research questions will be prepared. The evidence statements will take into account the extent and strength of the evidence. The evidence statements will be considered by RWQAC, who may provide advice on their revision.

Process for reporting

The Evidence Evaluation report will consist of sections including the following:

- 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
- Discussion: including strengths and limitations of the studies, 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
- Conclusion
- References

- Appendices
- References

The Technical Report will contain detailed information about the methods used to undertake the literature reviews that would otherwise make the Evaluation Report difficult to read (e.g. lists of excluded studies, pages of search strings, individual study report tables). Similarly to the Evidence Evaluation report, the Technical Report should describe the methodology used; however, this should be done in full detail, including but not limited to:

- 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 Authors of this review have the following declared interests:

Geoffrey Puzon	
Interest	**Interest Details**
Funded water research and non-funded research activities	The reviewer is currently engaged at CSIRO and conducts funded research project with Water Corporation WA. The reviewer also participates in an active research collaboration focused on *Naegleria fowleri* in natural and engineered water sources/systems with Montana State University and United State Geological Survey. The researcher is an active member of the American Water Works Association Standard Methods committee for Naegleria fowleri, the Australian Water Association, Water Research Australia and the International Water Association. The reviewer has ongoing discussions with national and international individuals/organisations (CDC-USA, University of Georgia, Baylor University, and Texas Commission on Environmental Quality).
Anna Kaksonen	
Interest	**Interest Details**

Funded water research and non-funded research activities	The reviewer is currently engaged at CSIRO and conducts funded research project with Water Corporation WA, mining companies, waste sector and NSW Environmental Trust. The researcher is an active member of the Australian Water Association and Water Research Australia. The reviewer also serves on editorial boards for the Nature Publishing Group, MDPI and is an Advisory Board member of Western Australian Minerals Research Institute.
Guobin Fu	
Interest	**Interest Details**
Funded water research and non-funded research activities	The reviewer is currently engaged at CSIRO and conducts funded research project with Victorian and NSW state governments. The researcher is an active member of the Australian Water Association and Water Research Australia. The reviewer is also on the editorial board of multiple scientific journals.
Tom Walsh	
Interest	**Interest Details**
Funded water research and non-funded research activities	The reviewer is currently engaged at CSIRO and conducts funded research project with Water Corporation WA. The reviewer also participates in an active research collaboration focused on *Naegleria fowleri* in natural and engineered water sources/systems with Montana State University and United State Geological Survey. The researcher is a member of the Australian Water Association and Water Research Australia. The reviewer has ongoing discussions with national and international individuals/organisations (CDC-USA, University of Georgia, Baylor University, and Texas Commission on Environmental Quality).

Process for amending the protocol

Amendments to the research protocol once it has been accepted will need to be agreed upon by RWQAC. Proposed changes will be communicated to ONHMRC for review and endorsement by the technical subgroup and RWQAC as needed. These amendments will be documented in the Technical Report.

References

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Appendix A – List of publications provided by RWQAC

Appendix B – Data extraction template

Example data extraction template for primary studies

General information	Study ID	
	Date template completed	
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	
Study characteristics	Aim/objectives of study	
	Study type/design	
	Study duration	
	Type of water source/water body	
Population characteristics	Population/s studied	
	Selection criteria for population	
	Subgroups reported	
	Size of study	
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s)	
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	

Appendix C – Risk of bias tool

Risk of bias assessment tool for individual studies (*adapted from OHAT RoB tool – see Table 5 in OHAT Handbook for relevant questions for each study type*)

Study ID: **Study Type:** (check study definition)	**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 (++)	++

Appendix D – Study type definitions

Study Type	CASP https://casp-uk.net/glossary/	Cochrane More study design definitions at https://community.cochrane.org/glossary
Case Control study	A case-control study is an epidemiological study that is used to identify risk factors for a medical condition. This type of study compares a group of patients who have that condition with a group of patients that do not have it, and looks back in time to see how the characteristics of the two groups differ.	A study that compares people with a specific disease or **outcome** of interest (cases) to people from the same **population** without that disease or outcome (**controls**), and which seeks to find associations between the outcome and prior exposure to particular risk factors. This design is particularly useful where the outcome is rare and past exposure can be reliably measured. Case-control studies are usually **retrospective**, but not always.
Case study	A case study is in depth analysis and systematic description of one patient or group of similar patients to promote a detailed understanding of their circumstances.	A study reporting observations on a single individual.
Case series	-	A study reporting observations on a series of individuals, usually all receiving the same **intervention**, with no **control group**.
Cohort study	An observational study in which a group of people with a particular exposure (e.g. a putative risk factor or protective factor) and a group of people without this exposure are followed over time. The outcomes of the people in the exposed group are compared to the outcomes of the people in the unexposed group to see if the exposure is associated with particular outcomes (e.g. getting cancer or length of life).	An **observational study** in which a defined group of people (the cohort) is followed over time. The **outcomes** of people in subsets of this cohort are compared, to examine people who were exposed or not exposed (or exposed at different levels) to a particular **intervention** or other factor of interest. A **prospective** cohort study assembles **participants** and follows them into the future. A **retrospective** (or historical) cohort study identifies subjects from past records and follows them from the time of those records to the present. Because subjects are not allocated by the investigator to different interventions or other exposures, **adjusted analysis** is usually required to minimise the influence of other factors (**confounders**).
Cross-over study/trial	In a cross-over trial two (or more) treatments are tested one after another in the same group of patients. Generally, the order in	A type of **clinical trial** comparing two or more **interventions** in which the participants, upon completion of the course of one treatment, are switched to another. For example, for a comparison of treatments A

	which each patient receives the treatments is decided by chance.	and B, the **participants** are randomly allocated to receive them in either the order A, B or the order B, A. Particularly appropriate for study of treatment options for relatively stable health problems. The time during which the firs **interventions** is taken is known as the first period, with the second intervention being taken during the second period.
Longitudinal study	A study of the same group of people at more than one point in time. (This type of study contrasts with a cross-sectional study, which observes a defined set of people at a single point in time.)	-
Observational study	In research about diseases or treatments, this refers to a study in which nature is allowed to take its course. Changes or differences in one characteristic (e.g. whether or not people received a specific treatment or intervention) are studied in relation to changes or differences in other(s) (e.g. whether or not they died), without the intervention of the investigator. There is a greater risk of selection bias than in experimental studies.	A study in which the investigators do not seek to intervene, and simply observe the course of events. Changes or differences in one characteristic (e.g. whether or not people received the **intervention** of interest) are studied in relation to changes or differences in other characteristic(s) (e.g. whether or not they died), without action by the investigator. There is a greater risk of **selection bias** than in **experimental studies**.
Prospective study	This is a measure of the proportion of people in a population who have a disease at a point in time, or over some period of time.	In evaluations of the effects of healthcare **interventions**, a study in which people are identified according to current **risk** status or exposure, and followed forwards through time to observe **outcome**. **Randomised controlled trials** are always prospective studies. **Cohort studies** are commonly either prospective or **retrospective**, whereas **case-control studies** are usually retrospective. In **Epidemiology**, 'prospective study' is sometimes misused as a synonym for cohort study.
Randomised Controlled Trial	Randomised controlled trial (RCT) is a trial in which participants are randomly assigned to one of two or more groups: the experimental group or groups receive the intervention or interventions being tested; the comparison group (control group) receive usual care or no treatment or a placebo. The groups are then followed up to see if there are any differences between the results. This helps in assessing the effectiveness of the intervention.	An experiment in which two or more interventions, possibly including a control intervention or no intervention, are compared by being randomly allocated to participants. In most trials one intervention is assigned to each individual but sometimes assignment is to defined groups of individuals (for example, in a household) or interventions are assigned within individuals (for example, in different orders or to different parts of the body).

Appendix E – Assessing certainty: overall risk of bias

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

Research Question: *e.g. What is the risk to human health from microbial sources in recreational water?*	**Case report**					**Case-Control study**					**Cohort study**					**Other**			
Outcome: *e.g. gastrointestinal illnesses* **Risk of Bias Question**	**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	--

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

Appendix F – Summary of findings template

Summary of findings – body of evidence (*adapted from OHAT Handbook*)

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 are the risks to human health from microbial sources in recreational water exposure?*											
Outcome 1. *e.g gastrointestinal illness*											
e.g. human case control studies (5 studies) Low to moderate certainty											
Outcome 2:											

Appendix G – Criteria for assessing existing guidance or reviews

Administrative and technical criteria for assessing existing guidance or reviews

Criteria have been colour-coded to assess minimum requirements as follows: 'Must have', 'Should have' or 'May have'

	Criteria	Y/N/?/NA	Notes
	Overall guidance/advice development process		
	Are the key stages of the organisation's advice development processes compatible with Australian processes?		
	Are the administrative processes documented and publicly available?		
	Was the work overseen by an expert advisory committee? Are potential conflicts of interest of committee members declared, managed and/or reported?		
	Are funding sources declared?		
	Was there public consultation on this work? If so, provide details.		
	Is the advice peer reviewed? If so, is the peer review outcome documented and/or published?		
	Was the guidance/advice developed or updated recently? Provide details.		
	Evidence review parameters		
	Are decisions about scope, definitions and evidence review parameters documented and publicly available?		
	Is there a preference for data from studies that follow agreed international protocols or meet appropriate industry standards?		
	Does the organisation use or undertake systematic literature review methods to identify and select data underpinning the advice? Are the methods used documented clearly?		
	If proprietary/confidential studies or data are considered by the agency, are these appropriately described/recorded?		
	Are inclusion/exclusion criteria used to select or exclude certain studies from the review? If so, is justification provided?		
	Does the organisation use or adopt review findings or risk assessments from other organisations? What process was used to critically assess these external findings?		
	Can grey literature such as government reports and policy documents be included?		
	Is there documentation and justification on the selection of a toxicological endpoint for use as point of departure for health-based guideline derivation?		

	Evidence search		
	Are databases and other sources of evidence specified?		
	Does the literature search cover at least more than one scientific database as well as additional sources (which may include government reports and grey literature)?		
	Is it specified what date range the literature search covers? Is there a justification?		
	Are search terms and/or search strings specified?		
	Are there any other exclusion criteria for literature (e.g. publication language, publication dates)? If so, what are they and are they appropriate?		
	Critical appraisal methods and tools		
	Is risk of bias of individual studies taken into consideration to assess internal validity? If so, what tools are used? If not, was any method used to assess study quality?		
	Does the organisation use a systematic or some other methodological approach to synthesise the evidence (i.e. to assess and summarise the information provided in the studies)? If so, provide details.		
	Does the organisation assess the overall certainty of the evidence and reach recommendations? If so, provide details.		
	Derivation of health-based guideline values		
	Is there justification for the choice of uncertainty and safety factors?		
	Are the parameter value assumptions documented and explained?		
	Are the mathematical workings/algorithms clearly documented and explained?		
	Does the organisation take into consideration non-health related matters to account for feasibility of implementing the guideline values (e.g. measurement attainability)?		
	Is there documentation directing use of mechanistic, mode of action, or key events in adverse outcome pathways in deriving health-based guideline values?		
	What processes are used when expert judgement is required and applied? Is the process documented and published?		
	Is dose response modelling (e.g. BMDL) routinely used?		
	What is the organisation's policy for dealing with substances for which a non-threshold mode of action may be applicable in humans? Has the policy been articulated and recorded?		
	If applicable: For carcinogens, what is the level of cancer risk used by the organisation to set the health-based guideline value?		