Australia's National
Science Agency

Evaluation of the Evidence of the Recreational Water Quality Guidelines

Section: Free-living organisms

Evidence Evaluation

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CSIRO

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

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1 Executive Summary

The National Health and Medical Research Council (NHMRC) commissioned Commonwealth Scientific and Industrial Research Organisation (CSIRO) to conduct a narrative review on free-living organisms in recreational waters. This Evidence Evaluation Report and accompanying Technical Report describe the narrative review of evidence to inform the update to the NHMRC's *Guidelines for Managing Risks in Recreational Water* (2008) (the Guidelines).

The review process was informed by a research protocol developed in collaboration with NHMRC's Recreational Water Quality Advisory Committee (The Committee). The research protocol described the review steps which included a systematic search of several international databases of primary scientific research literature (Scopus, Web of Science, PubMed) using search strings constructed from an extensive key word list. In addition to primary research literature, a search of grey literature was undertaken which included existing recreational water quality guidelines and/or reports.

The search strings were constructed to identify literature citations relevant to a primary research question and seven secondary research questions supplied by NHMRC's Recreational Water Quality Advisory Committee. The primary research question was:

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

The secondary questions were:

- *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?*

The results of the searches in the Scopus and PubMed databases produced 1104 studies. An additional 144 records were identified from other sources via a Google search. Following removal of duplicates there was a total of 991 records progressed to title/abstract screening. A total of 145 full articles were further assessed for inclusion or exclusion with reasons. Following this screening process, 58 records were deemed eligible to answer the Primary Question and/or the Secondary Questions. The full text review was composed of one Guideline, 14 Literature Reviews and 43 Primary Studies.

The review identified the following:

- Multiple *Naegleria fowleri* studies and two *Burkholderia pseudomallei* studies addressed the Primary Question and had links to different recreational water activities (swimming, diving, and water sports) as well as different recreational water types (lakes, ponds, rivers, reservoirs and geothermal waters).
- Recreational water users infected by *Naegleria fowleri* causing primary amoebic meningoencephalitis (PAM) typically resulted in fatality of the individual (96%), with 85% of all PAM

cases occurring during warm, hot, or summer seasons (Gharepure et al., 2021a). Fatal *Naegleria fowleri* cases have occurred in recreational waters with reported water temperatures between 22 °C (Kemble et al., 2012) and >30 °C (Moussa et al., 2013). The median age for *Naegleria fowleri* infections is 14 years old (ranging from 1-month old to 85 years old) with 75% of cases being male and 25% female (Gharepure et al., 2021a). Infections with *Burkholderia pseudomallei* were more common in people with underlying medical conditions, such as diabetes, alcoholism, or chronic renal disease (Inglis and Sousa, 2009).

- Regarding the Secondary questions, multiple studies provided information on potential indicators (and/or surrogates of the risks posed by the *Naegleria fowleri* and *Burkholderia pseudomallei*. For thermophilic *Naegleria fowleri*, the abiotic conditions of water temperature (environmental detections between 16-47 °C, with recorded fatalities at ≥22 °C), salinity (0-1.4% NaCl) and pH (3-11) were cited (Kemble et al., 2012; Lam et al., 2019; Stahl and Olson, 2021) as well as biotic conditions of bacterial food concentrations (Goudot et al., 2012), microbial ecology (Morgan et al., 2016) and potential preferential food sources (Miller et al., 2018) are mentioned. For *Burkholderia pseudomallei*, multiple abiotic factors (dew point, cloud cover, rainfall and max temperature) were potential indicators (Kaestli et al., 2016).
- There was no published information within the review period on the reported frequency of *Naegleria fowleri* in Australian natural water.
- *Burkholderia pseudomallei* is noted to occur in Australian natural waters, mainly in Northern Australia.
- *Naegleria fowleri* occurrence appears to increase on a seasonal basis to coincide with warmer weather and with the abundance of microbial food sources. High salt concentrations decreased the occurrence of *Naegleria fowleri* and *Naegleria fowleri* is not found in saltwater (Lam et al., 2019).
- *Burkholderia pseudomallei* were known to increase with weather conditions such as, dew point, cloud cover and maximum temperature.
- *Naegleria fowleri* exposure pathway is through direct contact with the olfactory mucosa of the upper nasal cavity. No dose response is recorded for *Naegleria fowleri* and risk minimisation is focused on preventing water going into the nose.
- *Burkholderia pseudomallei* exposure pathway is through skin cuts and abrasions, inhalation, and through the eyes. No dose response is recorded for *Burkholderia pseudomallei* and no risk minimisation practices are currently listed.

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Glossary

CSIRO	Commonwealth Scientific and Industrial Research Organisation
NHMRC	National Health and Medical Research Council
OHAT	Office of Health Assessment and Translation
The Committee	NHMRC Recreational Water Quality Advisory Committee
PAM	Primary Amoebic Meningoencephalitis
PECO	Population, Exposure (Comparator), Outcome

2 Introduction

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 NHMRC's Recreational Water Quality Advisory Committee (the Committee).

Free-living organisms may present a risk to recreational water users. As part of this review two specific free-living organisms, *Naegleria fowleri* and *Burkholderia pseudomallei*, were investigated for their potential links and risks to recreational water users. *Naegleria fowleri* is a free-living amoeba found in freshwaters globally. *Naegleria fowleri* causes a highly fatal disease, Primary Amoebic Meningoencephalitis (PAM) and has been known to be associated with fatalities linked to recreational water activities. *Burkholderia pseudomallei* is a gram-negative bacterium found in the soil and water. *Burkholderia pseudomallei* is endemic to Norther Australia and other tropical regions. Infections with *Burkholderia pseudomallei* cause the disease melioidosis and can range from minor infections to fatalities. Understanding the presence, risks and associated factors for these two organisms is important to inform the latest recreational water quality guidelines to ensure safety for the public users.

A research protocol to guide the review of the evidence was developed was developed by Commonwealth Scientific and Industrial Research Organisation (CSIRO) in consultation with NHMRC's Recreational Water Quality Advisory Committee (the Committee). The research protocol set out the methods to be used for the review including the research questions, population groups, health outcomes of interest, and a structured search and evaluation strategy. This Evidence Evaluation Report summarises the methodology used to find and select the studies and the findings of the literature search and evaluation process. It synthesises the results of key studies identified in the evaluation process into evidence statements and assesses this body of evidence taking into consideration its strengths and limitations.

2.1 Purpose and objectives of review

The purpose of the free-living organisms review is 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. This review, undertaken using a systematic approach, aims to provide NHMRC with an independent body of evidence to assure that the revision of the Guidelines is based on the most up-to-date and relevant scientific literature.

3 Methodology

3.1 Review Period

Papers and reports published from 2004 until 2021 were considered for inclusion in this review. The selection of this date ensured the inclusion of relevant studies and reports published since the last review for the *Guidelines for Managing Risks in Recreational Water* (2008). Search results were restricted to English language publications only.

3.2 Definitions

Key definitions as outlines in the protocol were used to define the scope of the review.

Table 3.1 Key definitions

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 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.

3.3 Research Questions

The research questions that form the basis of this review were developed by the NHMRC Recreational Water Quality Advisory Committee (the Committee). There was one primary question and seven secondary questions.

3.3.1 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?

3.3.2 Secondary questions

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

3.4 Search Strategy and Selection of Evidence

The databases searched for this review were PubMed®, Scopus® and Web of Science™. PubMed® was used due to its coverage of biomedical journals and its capacity for advanced searching. Scopus® was used due to its coverage of life sciences, health sciences, physical sciences, social sciences and humanities. Web of Science™ was used to identify academic journals, conference proceedings and publications from a range of organisations. Keywords used for the database searches and search strings are listed in the Technical Report (Table 2.3, 2.4 & 2.5).

3.4.1 Inclusion and exclusion criteria

The following advice was provided by the Committee to inform the evidence review:

Table 3.2 Population, Exposure, Comparator, Outcome table

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

3.4.2 Inclusion and exclusion criteria

Key terms including those listed in the key definitions and PECO were used to determine if studies were eligible for inclusion in the review.

Publications were screened by title and abstract using the defined key terms to determine if they were included or excluded from the review.

When a reviewer was unsure of the inclusion/exclusion of a publication at title and abstract screening, full text publications were screened to determine eligibility.

3.5 Evidence Collection

3.5.1 Classification of evidence

To assist in the literature assessment, citation search results were classified into two broad categories:

(i) primary studies that were largely peer-reviewed journal articles

(ii) existing guidelines that were mainly regulatory guidelines or technical guidance publications produced by federal and state agencies in support of regulatory compliance goals. Such literature is also commonly included in the classification “grey literature”, which refers to literature produced by organisations other than conventional academic journal publishers. Published reviews that also considered a body of evidence were included in this category.

3.5.2 Existing guidelines and reviews

Searches for grey literature were used to identify reports and news articles, conference papers, journal articles, factsheets, publications and statistics from government health websites, and articles from journals articles/online publications published by organisations (method described in the Technical Report, Section 2.2.4). Each document was evaluated for its relevance based on the inclusion and exclusion criteria (Technical Report, section 2.2.8) related to the primary and secondary questions and excluded if not relevant.

3.5.3 Primary studies

Searches for primary studies (method described in Technical Report, Section 2.2) with the modification of the Keywords to include the term "water" in the list of Exposure terms (Technical Report, Table 2.3) were used to identify research articles and review articles. After the searches, duplicate records were removed and evaluated for relevance (inclusion or exclusion) based on the inclusion and exclusion criteria related to the primary and secondary questions (Technical Report, section 2.2.8). Articles were excluded after initial review due to a lack of relevance and after review of the abstract and full text. The remaining documents which met the quality criteria were included in the review. The methodological quality of individual studies was assessed using an adaptation of the OHAT risk of bias tool (OHAT, 2019). The documents were quality assessed following the outlined process (Technical Report, Section 2.3 and 2.4) and included in the Technical Report, Section 4, Tables 4.2-4.5.

3.5.4 Assessment of included evidence (by types)

Existing guidelines and reviews

The methodological quality of existing guidelines was assessed using administrative and technical criteria in the assessment tool shown in the Technical Report Appendix 1. The criteria listed in the tool were based on common domains that have been evaluated in several existing tools for assessing guidelines and systematic reviews (e.g. AGREE tool: Brouwers, Kerkvliet, et al., 2016; AGREE Next Steps Consortium, 2017). Based on the responses in the form, a decision was made on whether that guideline should be included or excluded from the review.

In addition to this formal quality assessment approach, the close inspection of the full text document was used to identify evidence contained in the document that did not satisfactorily contribute to answering the primary and/or secondary research questions. Where that was the case, the document was classified as "Quality satisfactory but content not relevant (or obsolete)" and excluded on relevance.

Primary studies

All primary studies for inclusion were assessed for potential Risk of Bias. The methodological quality of individual studies was assessed using an adaptation of the OHAT risk of bias tool (Technical Report Appendix 2) (OHAT, 2019). Studies were evaluated on applicable risk of bias questions based on study design. The rating or answer to each risk of bias question was 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 (--)

Studies that were determined to have a high risk of bias or serious concerns with study quality were excluded from the review. Their removal was recorded with justification in the PRISMA Flow Diagram.

Conflicts of interest and funding data from the study characteristics tables were 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 were serious overall concerns, these were noted under 'Other sources of bias' in Technical Report Appendix 1. The outcome of the risk of bias assessments are presented in the in Section 4.2 together with a discussion of the overall quality of each study.

3.6 Data extraction

Data were extracted from individual studies using standardised data extraction forms designed for each class of literature. Samples of the data extraction forms are presented in the Technical Report.

3.7 Process for assessing the body of evidence

Overview

The evidence collected and appraised for each research question was grouped by study type and outcome where possible and summarised in an Evidence Summary table that assigned the level of certainty (or confidence) in that body of evidence. Due to the different nature and quality of evidence between existing guidelines and primary studies different approaches were required to review and evaluate the body of evidence for each class of literature. The assessment methodology for each literature class is described in the following sections.

3.7.1 Assessment of the body of evidence – primary studies

The evidence collected and appraised for each research question was grouped by study type and outcome where possible and summarised in an Evidence Summary table that assigned the level of certainty (or confidence) in that body of evidence. Due to the different nature and quality of evidence between guidelines and primary studies different approaches were required to review and evaluate the body of evidence for each type of literature.

3.7.2 Assessment of the body of evidence – existing guidelines

The evidence collected and appraised for each research question was grouped by study type and outcome where possible and summarised in an Evidence Summary table that assigned the level of certainty (or confidence) in that body of evidence. Due to the different nature and quality of evidence between guidelines and primary studies, different approaches were required to review and evaluate the body of evidence for each type of literature.

4 Literature search results

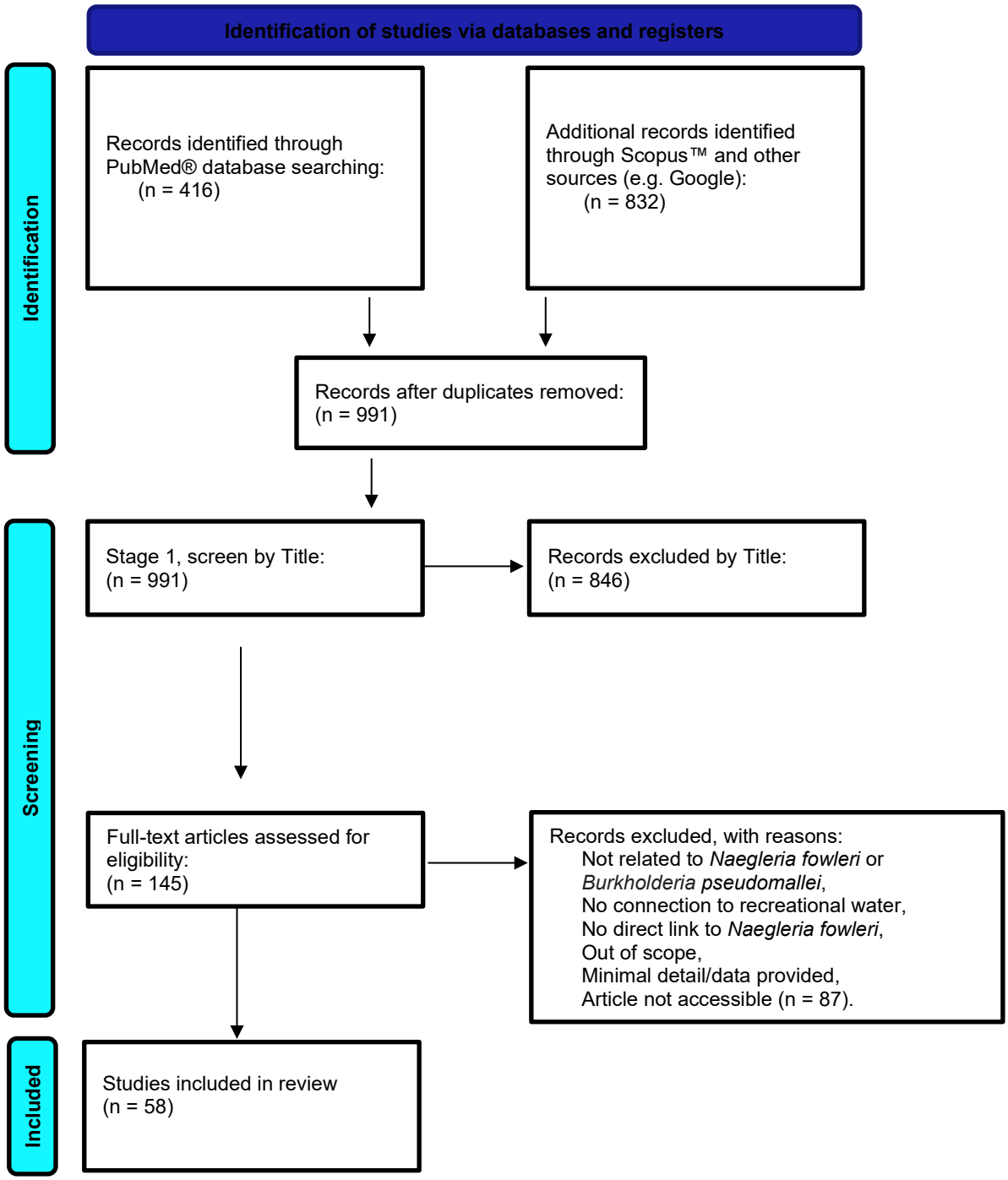
4.1 Existing guidelines/reports and reviews

Searches for grey literature identified a total of 144 documents in including, reports and news articles, conference papers, reports by World Health Organisation, journal articles, factsheets, publications and statistics from government health websites, and articles from journals articles/online publications published by organisations. One item was suggested by the Committee. Each document was evaluated for its relevance based on the inclusion and exclusion criteria (Technical Report section 2.2.8) related to the primary and secondary questions and excluded if not relevant. This process identified one additional document for inclusion. The document was quality assessed following the outlined process (Technical Report Section 2.3 and 2.4) and listed in Technical Report Table 4.1 for inclusion in the assessment of evidence.

4.2 Primary studies

Searches for primary studies identified a total of 1104 publications (416 by PubMed® search and 688 by Scopus®). The primary studies were combined with the 144 grey literature review. After duplicate records were removed and a total of 991 documents were evaluated for relevance to the primary and secondary questions (based on the inclusion and exclusion criteria) (see Technical Report section 2.2.8). A total of 846 articles were excluded after screening by Title due to a lack of relevance (e.g. not related to *Naegleria fowleri* or *Burkholderia pseudomallei*, no connection to recreational water, no direct link to *Naegleria fowleri*, minimal detail/data provided), with 145 subjected to additional scrutiny. Following abstract and full text review, 87 articles were excluded with reasons listed (Technical Report Section 7, Tables 7.1-7.3). A single reverse quantitative microbial risk assessment (QMRA) paper was excluded at this point due to the original manuscript being retracted. The documents were quality assessed following the outlined process (Technical Report Section 2.3 and 2.4) and listed in the Technical Report Section 4, (Tables 4.2-4.5) and included in the assessment of evidence. A total of 58 documents met the quality criteria for inclusion in the review. An additional two documents were identified through searching other sources and were included with the primary studies.

Figure 1 PRISMA diagram



A full list of included guidelines and included studies in the Evidence Evaluation Report is provided in Section 4 of the Technical Report.

5 Assessment of included evidence

5.1 Suitability of existing guidelines and reviews for adoption /adaptation

5.1.1 Critical appraisal of included Guidelines and literature reviews on *Naegleria fowleri*

A critical appraisal of relevant included primary studies and guidelines by was conducted by CSIRO, the findings of which are included in this report. Existing guideline publications were assessed by CSIRO and the NHMRC project team against an Assessment Tool developed specifically for water projects. Included primary studies were assessed for risk of bias and certainty where possible using existing tools and frameworks used in similar contracted reviews (e.g. OHAT risk of bias tool - OHAT, 2019).

Data relevant to answering the research questions was extracted by CSIRO from included publications and summarised for consideration by NHMRC and the Committee.

Table 5.1 Summary Table of Assessment of included literature reviews for *Naegleria fowleri*

Administrative and Technical Criteria	N30	N31	N32	N33	N34	N36	N37	N38	N39	N42
Overall guidance/advice development process										
Are the key stages of the organisation's advice development processes compatible with Australian processes?	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Are the administrative processes documented and publicly available?	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Was the work overseen by an expert advisory committee? Are potential conflicts of interest of committee members declared, managed and/or reported?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Are funding sources declared?	N	Y	N	N	Y	N	N	N	N	N
Was there public consultation on this work? If so, provide details.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Is the advice peer reviewed? If so, is the peer review outcome documented and/or published?	Y	Y	Y	Y	N	Y	Y	Y	Y	N
Was the guidance/advice developed or updated recently? Provide details.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N
Evidence review parameters										
Are decisions about scope, definitions and evidence review parameters documented and publicly available?	Y	Y	N	N	N	N	Y	Y	Y	N
Is there a preference for data from studies that follow agreed international protocols or meet appropriate industry standards?	Y	Y	Y	Y	Unclear	Y	Y	Y	Y	N
Does the organisation use or undertake systematic literature review methods to identify and select data underpinning the advice? Are the methods used documented clearly?	N	Unclear	N	N	N	N	N	N	Partial	N
If proprietary/confidential studies or data are considered by the agency, are these appropriately described/recorded?	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Are inclusion/exclusion criteria used to select or exclude certain studies from the review? If so, is justification provided?	N	N	N	N	N	N	N	N	Y	N

Administrative and Technical Criteria	N30	N31	N32	N33	N34	N36	N37	N38	N39	N42
Does the organisation use or adopt review findings or risk assessments from other organisations? What process was used to critically assess these external findings?	N	N	N	N	N	N	N	N	N	Y
Can grey literature such as government reports and policy documents be included?	Y	Y	Y	N/A	N/A	N/A	Y	Y	Y	Y
Is there documentation and justification on the selection of a toxicological endpoint for use as point of departure for health-based guideline derivation?	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Evidence search										
Are databases and other sources of evidence specified?	N	Y	N	N	N	N	N	N	Y	N
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)?	N	N/A	N/A	N/A	N/A	N	N	N	N	N
Is it specified what date range the literature search covers? Is there a justification?	N	Y	N	Y	N	N	N	N	Y	N
Are search terms and/or search strings specified?	N	N	N	N	N	N	N	N	N	N
Are there any other exclusion criteria for literature (e.g. publication language, publication dates)? If so, what are they and are they appropriate?	N	N	N	N	N	N	N	N	N	N
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?	N	N	N	N	N	N	N	N	N	N
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.	N	N	N	N	N	N	N	N	N	N
Does the organisation assess the overall certainty of the evidence and reach recommendations? If so, provide details.	N	N	N	N	N	N	N	N	N	N
Derivation of health-based guideline values*										
Is there justification for the choice of uncertainty and safety factors?	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Are the parameter value assumptions documented and explained?	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Are the mathematical workings/algorithms clearly documented and explained?	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Does the organisation take into consideration non-health related matters to account for feasibility of implementing the guideline values (e.g. measurement attainability)?	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Is there documentation directing use of mechanistic, mode of action, or key events in adverse outcome pathways in deriving health-based guideline values?	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
If expert judgement is required, is the process documented and published?	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Is dose response modelling (e.g. BMDL) routinely used?	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Has the organisation's policy for dealing with substances for which a non-threshold mode of action may be applicable in humans been articulated and recorded?	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Y
If applicable: For carcinogens, what is the level of cancer risk used by the organisation to set the health-based guideline value?	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Comments*										
Useful for answering primary research question?	Partially	Partially	Partially	Partially	Partially	Partially	Partially	N	Y	N

Administrative and Technical Criteria	N30	N31	N32	N33	N34	N36	N37	N38	N39	N42
Useful for answering secondary research questions?	Partially	Partially	Partially	Partially	Partially	Partially	Partially	Y	Y	Partially
Include in review	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**

Study ID for Table 4.3

N30: Bright, K.R., Gerba, C.P. Review: Occurrence of the pathogenic amoeba *Naegleria fowleri* in groundwater. Hydrogeol J 25, 953–958 (2017).

N31: Capewell LG, Harris AM, Yoder JS, Cope JR, Eddy BA, Roy SL, Visvesvara GS, Fox LM, Beach MJ. Diagnosis, Clinical Course, and Treatment of Primary Amoebic Meningoencephalitis in the United States, 1937-2013. J Pediatric Infect Dis Soc. 2015 Dec;4(4):e68-75.

N32: Cooper, Amanda Marie PA-C; Aouthmany, Shaza MD; Shah, Kruti MD; Rega, Paul P. MD, FACEP. Killer amoebas: Primary amoebic meningoencephalitis in a changing climate. Journal of the American Academy of Physician Assistants 32(6):p 30-35, June 2019.

N33: Cope JR, Ali IK. Primary Amebic Meningoencephalitis: What Have We Learned in the Last 5 Years? Curr Infect Dis Rep. 2016 Sep;18(10):31. doi: 10.1007/s11908-016-0539-4. PMID: 27614893; PMCID: PMC5100007.

N34: De Jonckheere JF. The impact of man on the occurrence of the pathogenic free-living amoeboflagellate *Naegleria fowleri*. Future Microbiol. 2012 Jan;7(1):5-7.

N36: Grace E, Asbill S, Virga K. *Naegleria fowleri*: pathogenesis, diagnosis, and treatment options. Antimicrob Agents Chemother. 2015 Nov;59(11):6677-81.

N37: Heggie TW. Swimming with death: *Naegleria fowleri* infections in recreational waters. Travel Med Infect Dis. 2010 Jul;8(4):201-6.

N38: Stahl LM, Olson JB. Environmental abiotic and biotic factors affecting the distribution and abundance of *Naegleria fowleri*. FEMS Microbiol Ecol. 2021 Jan 1;97(1):fiaa238.

N39: Yoder JS, Eddy BA, Visvesvara GS, Capewell L, Beach MJ. The epidemiology of primary amoebic meningoencephalitis in the USA, 1962-2008. Epidemiol Infect. 2010 Jul;138(7):968-75.

N42: Department of Health, Western Australia. (2019). *Naegleria* Response Protocol for drinking water supply systems.

5.1.2 Critical appraisal of included Guidelines and literature reviews on *Burkholderia pseudomallei*

A critical appraisal of the relevant included primary studies and guidelines was conducted by CSIRO, the findings of which are included in this report. Existing guideline publications were assessed by CSIRO and the NHMRC project team against an Assessment Tool developed specifically for water projects. Included primary studies were assessed for risk of bias and certainty where possible using existing tools and frameworks used in similar contracted reviews (e.g. OHAT risk of bias tool - OHAT, 2019).

Data relevant to answering the research questions was extracted by CSIRO from included publications and summarised for consideration by NHMRC and the Committee.

Table 5.2 Summary Table of Assessment of included literature reviews for *Burkholderia pseudomallei*

Administrative and Technical Criteria	B11	B12	B13	B14	B15
Overall guidance/advice development process					
Are the key stages of the organisation's advice development processes compatible with Australian processes?	N/A	N/A	N/A	N/A	N/A
Are the administrative processes documented and publicly available?	N/A	N/A	N/A	N/A	N/A
Was the work overseen by an expert advisory committee? Are potential conflicts of interest of committee members declared, managed and/or reported?	Y	Partially	N	Partially	N
Are funding sources declared?	N	Y	N	Y	N
Was there public consultation on this work? If so, provide details.	N/A	N/A	N/A	N/A	N/A
Is the advice peer reviewed? If so, is the peer review outcome documented and/or published?	Y	Y	Y	Y	Y
Was the guidance/advice developed or updated recently? Provide details.	N/A	N/A	N/A	N/A	N/A
Evidence review parameters					
Are decisions about scope, definitions and evidence review parameters documented and publicly available?	Partially	Partially	Partially	Partially	Partially
Is there a preference for data from studies that follow agreed international protocols or meet appropriate industry standards?	Unknown	Unknown	Unknown	Unknown	Unknown
Does the organisation use or undertake systematic literature review methods to identify and select data underpinning the advice? Are the methods used documented clearly?	N	N	N	N	N
If proprietary/confidential studies or data are considered by the agency, are these appropriately described/recorded?	N	N/A	N/A	N/A	N/A

	Administrative and Technical Criteria	B11	B12	B13	B14	B15
	Are inclusion/exclusion criteria used to select or exclude certain studies from the review? If so, is justification provided?	N	N	N	N	N
	Does the organisation use or adopt review findings or risk assessments from other organisations? What process was used to critically assess these external findings?	Unknown	N	N	N	N
	Can grey literature such as government reports and policy documents be included?	Y	N	N	N	N
	Is there documentation and justification on the selection of a toxicological endpoint for use as point of departure for health-based guideline derivation?	N/A	N/A	N/A	N/A	N/A
	Evidence search					
	Are databases and other sources of evidence specified?	N	N	N	N	N
	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)?	N	N	N	N	N
	Is it specified what date range the literature search covers? Is there a justification?	N	N	N	N	N
	Are search terms and/or search strings specified?	N	N	N	N	N
	Are there any other exclusion criteria for literature (e.g. publication language, publication dates)? If so, what are they and are they appropriate?	N	N	N	N	N
	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?	N	N	N	N	N
	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.	N	N	N	N	N
	Does the organisation assess the overall certainty of the evidence and reach recommendations? If so, provide details.	N	N	N	N	N
	Derivation of health-based guideline values*					
	Is there justification for the choice of uncertainty and safety factors?	N/A	N/A	N/A	N/A	N/A
	Are the parameter value assumptions documented and explained?	N/A	N/A	N/A	N/A	N/A
	Are the mathematical workings/algorithms clearly documented and explained?	N/A	N/A	N/A	N/A	N/A
	Does the organisation take into consideration non-health related matters to account for feasibility of implementing the guideline values (e.g. measurement attainability)?	N/A	N/A	N/A	N/A	N/A
	Is there documentation directing use of mechanistic, mode of action, or key events in adverse outcome pathways in deriving health-based guideline values?	N/A	N/A	N/A	N/A	N/A
	If expert judgement is required, is the process documented and published?	N/A	N/A	N/A	N/A	N/A
	Is dose response modelling (e.g. BMDL) routinely used?	N/A	N/A	N/A	N/A	N/A
	Has the organisation's policy for dealing with substances for which a non-threshold mode of action may be applicable in humans been articulated and recorded?	N/A	N/A	N/A	N/A	N/A
	If applicable: For carcinogens, what is the level of cancer risk used by the organisation to set the health-based guideline value?	N/A	N/A	N/A	N/A	N/A
	Comments*					
	Useful for answering primary research question?	Partially	Partially	Partially	Partially	Partially
	Useful for answering secondary research questions?	Partially	Partially	Partially	Partially	Partially
	Include in review	**Y**	**Y**	**Y**	**Y**	**Y**

Study ID for Table 4.4

B11: Merritt AJ, Inglis TJJ. The Role of Climate in the Epidemiology of Melioidosis. Curr Trop Med Rep. 2017;4(4):185-191.

B12: Stephens DP, Thomas JH, Ward LM, Currie BJ. Melioidosis Causing Critical Illness: A Review of 24 Years of Experience From the Royal Darwin Hospital ICU. Crit Care Med. 2016 Aug;44(8):1500-5.

B13: Foong YC, Tan M, Bradbury RS. Melioidosis: a review. Rural Remote Health. 2014;14(4):2763. Epub 2014 Oct 30.

B14: Hsueh PT, Huang WT, Hsueh HK, Chen YL, Chen YS. Transmission Modes of Melioidosis in Taiwan. Trop Med Infect Dis. 2018 Feb 28;3(1):26.

B15: Inglis TJ, Sousa AQ. The public health implications of melioidosis. Braz J Infect Dis. 2009 Feb;13(1):59-66.

5.2 Risk of bias assessment of primary studies

5.2.1 Risk of bias of included primary studies for *Naegleria fowleri*

The included primary studies were assessed for risk of bias using an adaptation of the OHAT risk of bias tool (OHAT, 2019). Existing guidance or review reports such as those found in the grey literature search were appraised using an Assessment Tool developed by NHMRC for water projects. The certainty of the body of evidence was assessed where appropriate. At least one reviewer performed an assessment on each included study. All assessments were checked internally by the NHMRC project team.

Table 5.3 Risk of Bias Summary of Fatality Case Reports for *Naegleria* fowleri

Domain	Fatality from PAM, with water source confirmation of *Naegleria fowleri*						Fatality from PAM, water source testing not conducted or unknown							Fatality, cause not stated
	N1	N2	N3	N4	N5	N41	N6	N7	N8	N9	N10	N11	N12	N13
3. Appropriate comparison groups	+	+	+	+	+	+	+	-	+	+	+	+	+	+
4. Confounding	-	+	+	-	+	+	++	--	+	+	+	+	+	+
7. Missing outcome data	-	+	+	+	-	+	+	-	+	-	+	+	+	-
8. Exposure characteristics	+	+	+	-	-	+	-	--	-	+	+	+	+	-
9. Outcome assessment	+	+	+	+	+	+	-	+	+	+	+	+	+	+
10. Outcome reporting	+	++	++	--	-	+	+	+	+	+	+	+	-	+
11. Other treats	+	+	+			-	-	--	+					-
Overall	Not serious						Not serious							Not serious

Key: Risk of bias rating

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

Study ID for Table 4.3

N1: Booth PJ, Bodager D, Slade TA, Jett S. Primary Amebic Meningoencephalitis Associated with Hot Spring Exposure During International Travel - Seminole County, Florida, July 2014. MMWR Morb Mortal Wkly Rep. 2015 Nov 6;64(43):1226.

N2: Cope JR, Murphy J, Kahler A, Gorbett DG, Ali I, Taylor B, Corbitt L, Roy S, Lee N, Roellig D, Brewer S, Hill VR. Primary Amebic Meningoencephalitis Associated With Rafting on an Artificial Whitewater River: Case Report and Environmental Investigation. Clin Infect Dis. 2018 Feb 1;66(4):548-553. doi: 10.1093/cid/cix810. PMID: 29401275; PMCID: PMC5801760.

N3: Kemble SK, Lynfield R, DeVries AS, Drehner DM, Pomputius WF 3rd, Beach MJ, Visvesvara GS, da Silva AJ, Hill VR, Yoder JS, Xiao L, Smith KE, Danila R. Fatal *Naegleria fowleri* infection acquired in Minnesota: possible expanded range of a deadly thermophilic organism. Clin Infect Dis. 2012 Mar;54(6):805-9.

N4: Nicholls CL, Parsonson F, Gray LE, Heyer A, Donohue S, Wiseman G, Norton R. Primary amoebic meningoencephalitis in North Queensland: the paediatric experience. Med J Aust. 2016 Oct 3;205(7):325-8.

N5: Su MY, Lee MS, Shyu LY, Lin WC, Hsiao PC, Wang CP, Ji DD, Chen KM, Lai SC. A fatal case of *Naegleria fowleri* meningoencephalitis in Taiwan. Korean J Parasitol. 2013 Apr;51(2):203-6.

N41: Abrahams-Sandí E, Retana-Moreira L, Castro-Castillo A, Reyes-Batlle M, Lorenzo-Morales J. Fatal meningoencephalitis in child and isolation of *Naegleria fowleri* from hot springs in Costa Rica. Emerg Infect Dis. 2015 Feb;21(2):382-4. doi: 10.3201/eid2102.141576. PMID: 25625800; PMCID: PMC4313663.

N6: Budge PJ, Lazensky B, Van Zile KW, Elliott KE, Dooyema CA, Visvesvara GS, Beach MJ, Yoder JS. Primary amebic meningoencephalitis in Florida: a case report and epidemiological review of Florida cases. J Environ Health. 2013 Apr;75(8):26-31.

N7: Chen M, Ruan W, Zhang L, Hu B, Yang X. Primary Amebic Meningoencephalitis: A Case Report. Korean J Parasitol. 2019 Jun;57(3):291-294.

N8: Hamaty E Jr, Faiek S, Nandi M, Stidd D, Trivedi M, Kandukuri H. A Fatal Case of Primary Amoebic Meningoencephalitis from Recreational Waters. Case Rep Crit Care. 2020 May 28;2020:9235794.

N9: Lopez C, Budge P, Chen J, Bilyeu S, Mirza A, Custodio H, Irazuzta J, Visvesvara G, Sullivan KJ. Primary amebic meningoencephalitis: a case report and literature review. Pediatr Emerg Care. 2012 Mar;28(3):272-6.

N10: Phu NH, Hoang Mai NT, Nghia HD, Chau TT, Loc PP, Thai le H, Phuong TM, Thai CQ, Man DN, Van Vinh Chau N, Nga TV, Campbell J, Baker S, Whitehorn J. Fatal consequences of freshwater pearl diving. Lancet. 2013 Jan 12;381(9861):176.

N11: Stowe RC, Pehlivan D, Friederich KE, Lopez MA, DiCarlo SM, Boerwinkle VL. Primary Amebic Meningoencephalitis in Children: A Report of Two Fatal Cases and Review of the Literature. Pediatr Neurol. 2017 May;70:75-79.

N12: Vareechon C, Tarro T, Polanco C, Anand V, Pannaraj PS, Dien Bard J. Eight-Year-Old Male With Primary Amebic Meningoencephalitis. Open Forum Infect Dis. 2019 Jul 29;6(8):ofz349.

N13: Matthews, S., D. Ginzl, D. Walsh, K. Sherin, J. Middaugh, R. Hammond, D. Bodager, K. Komatsu, J. Weiss, N. Pascoe, F. Marciano-Cabral, E. Villegas, G. Visvesvara, J. Yoder, B. Eddy, L. Capewell, R. Sriram, K. Bandyopadhyay, Y. Qvarnstrom, A. DaSilva, S. Johnston, L. Xiao, V. Hill, S. Roy and M. J. Beach. Centers for Disease Control and Prevention (CDC). Primary amebic meningoencephalitis--Arizona, Florida, and Texas, 2007. MMWR Morb Mortal Wkly Rep. 2008 May 30;57(21):573-7. PMID: 18509301.

Table 5.4 Risk of Bias Summary of Infection of *Naegleria fowleri*

Q.	Infection N14
3. Appropriate comparison groups	+
4. Confounding	+
7. Missing outcome data	++
8. Exposure characteristics	+
9. Outcome assessment	+
10. Outcome reporting	+
11. Other treats	+
Overall	Not serious

Key: Risk of bias rating

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

Study ID for Table 4.4

N14: Diaz J. Seasonal primary amebic meningoencephalitis (PAM) in the south: summertime is PAM time. J La State Med Soc. 2012 May-Jun;164(3):148-50, 152-5.

Table 5.5 Risk of Bias Summary of Successfully treated case reports of *Naegleria fowleri*

Q.	PAM successfully treated			
	N15	N16	N17	N18
3. Appropriate comparison groups	+	+	+	+
4. Confounding	+	+	+	+
7. Missing outcome data		-	-	+
8. Exposure characteristics	N/A	-	-	+
9. Outcome assessment	+	+	+	+
10. Outcome reporting	+	+	+	-
11. Other treats	+	-	-	+
Overall	Not serious			

Key: Risk of bias rating

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

Study ID for Table 4.5

N15: Dunn AL, Reed T, Stewart C, Levy RA. *Naegleria fowleri* That Induces Primary Amoebic Meningoencephalitis: Rapid Diagnosis and Rare Case of Survival in a 12-Year-Old Caucasian Girl. Lab Med. 2016 May;47(2):149-54.
N16: Heggie TW, Küpper T. Surviving *Naegleria fowleri* infections: A successful case report and novel therapeutic approach. Travel Med Infect Dis. 2017 Mar-Apr;16:49-51.
N17: Linam WM, Ahmed M, Cope JR, Chu C, Visvesvara GS, da Silva AJ, Qvarnstrom Y, Green J. Successful treatment of an adolescent with *Naegleria fowleri* primary amebic meningoencephalitis. Pediatrics. 2015 Mar;135(3):e744-8.
N18: Vargas-Zepeda J, Gómez-Alcalá AV, Vásquez-Morales JA, Licea-Amaya L, De Jonckheere JF, Lares-Villa F. Successful treatment of *Naegleria fowleri* meningoencephalitis by using intravenous amphotericin B, fluconazole and rifampicin. Arch Med Res. 2005 Jan-Feb;36(1):83-6.

Table 5.6 Risk of Bias Summary of Environmental Water Testing for *Naegleria fowleri*

Q.	Testing of recreational water for detection of *Naegleria fowleri*					
	N19	N20	N21	N22	N23	N42
3. Appropriate comparison groups	+	++	+	+	++	++
4. Confounding	+	++	-	+	++	+
7. Missing outcome data	+	-	+	--	+	+
8. Exposure characteristics	+	+	+	++	+	+
9. Outcome assessment	+	+	+	+	+	+
10. Outcome reporting	+	+	+	-	+	+
11. Other treats	-	+	+	+	+	-
Overall	Not serious					

Key: Risk of bias rating

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

Study ID for Table 4.6

N19: Bonilla-Lemus P, Rojas-Hernández S, Ramírez-Flores E, Castillo-Ramírez DA, Monsalvo-Reyes AC, Ramírez-Flores MA, Barrón-Graciano K, Reyes-Batlle M, Lorenzo-Morales J, Carrasco-Yépez MM. Isolation and Identification of *Naegleria* Species in Irrigation Channels for Recreational Use in Mexicali Valley, Mexico. Pathogens. 2020 Oct 7;9(10):820. **N20:** Heggie TW, Küpper T. Surviving *Naegleria fowleri* infections: A successful case report and novel therapeutic approach. Travel Med Infect Dis. 2017 Mar-Apr;16:49-51.

N21: Maclean RC, Richardson DJ, LePardo R, Marciano-Cabral F. The identification of *Naegleria fowleri* from water and soil samples by nested PCR. Parasitol Res. 2004 Jun;93(3):211-7.

N22: Miller HC, Morgan MJ, Walsh T, Wylie JT, Kaksonen AH, Puzon GJ. Preferential feeding in *Naegleria fowleri*; intracellular bacteria isolated from amoebae in operational drinking water distribution systems. Water Res. 2018 Sep 15;141:126-134.

N23: Moussa M, De Jonckheere JF, Guerlotté J, Richard V, Bastaraud A, Romana M, Talarmin A. Survey of *Naegleria fowleri* in geothermal recreational waters of Guadeloupe (French West Indies). PLoS One. 2013;8(1):e54414.

N42: Sifuentes LY, Choate BL, Gerba CP, Bright KR. The occurrence of *Naegleria fowleri* in recreational waters in Arizona. J Environ Sci Health A Tox Hazard Subst Environ Eng. 2014 Sep 19;49(11):1322-30.

Table 5.7 Risk of Bias Summary of in-vitro growth conditions for *Naegleria fowleri*

Q.	In-vitro testing of growth conditions	
	N24	N25
3. Appropriate comparison groups	++	++
4. Confounding	+	+
7. Missing outcome data	-	+
8. Exposure characteristics	+	+
9. Outcome assessment	-	+
10. Outcome reporting	+	+
11. Other treats	+	
Overall	Not serious	

Key: Risk of bias rating

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

Study ID for Table 4.7

N24: Goudot S, Herbelin P, Mathieu L, Soreau S, Banas S, Jorand F. Growth dynamic of *Naegleria fowleri* in a microbial freshwater biofilm. Water Res. 2012 Sep 1;46(13):3958-66.
N25: Lam C, He L, Marciano-Cabral F. The Effect of Different Environmental Conditions on the Viability of *Naegleria fowleri* Amoebae. J Eukaryot Microbiol. 2019 Sep;66(5):752-756.

Table 5.8 Risk of Bias Summary of testing of drinking water for *Naegleria fowleri*

Q.	Detection of *Naegleria fowleri* in drinking water		
	N26	N27	N28
3. Appropriate comparison groups	+	-	++
4. Confounding	++	+	++
7. Missing outcome data	+	-	+
8. Exposure characteristics	++	++	++
9. Outcome assessment	+	+	-
10. Outcome reporting	++	-	++
11. Other treats	++	+	++
Overall	Not serious		

Key: Risk of bias rating

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

Study ID for Table 4.8

N26: Morgan MJ, Halstrom S, Wylie JT, Walsh T, Kaksonen AH, Sutton D, Braun K, Puzon GJ. Characterization of a Drinking Water Distribution Pipeline Terminally Colonized by *Naegleria fowleri*. Environ Sci Technol. 2016 Mar 15;50(6):2890-8.

N27: Puzon GJ, Wylie JT, Walsh T, Braun K, Morgan MJ. Comparison of biofilm ecology supporting growth of individual *Naegleria* species in a drinking water distribution system. FEMS Microbiol Ecol. 2017 Apr 1;93(4).

N28: Yu Z, Miller HC, Puzon GJ, Clowers BH. Application of untargeted metabolomics for the detection of pathogenic *Naegleria fowleri* in an operational drinking water distribution system. Water Research. 2018 Nov;145:678-686.

Table 5.9 Risk of Bias Summary of dose response and concentrations for *Naegleria fowleri*

Q.	N19	N29	N23
3. Appropriate comparison groups	+	+	++
4. Confounding	+	+	++
7. Missing outcome data	+	--	+
8. Exposure characteristics	+	+	+
9. Outcome assessment	+	+	+
10. Outcome reporting	+	--	+
11. Other treats	-	-	+
Overall	Not serious	Serious	Not serious

Key: Risk of bias rating

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

Study ID for Table 4.9

N19: Bonilla-Lemus P, Rojas-Hernández S, Ramírez-Flores E, Castillo-Ramírez DA, Monsalvo-Reyes AC, Ramírez-Flores MA, Barrón-Graciano K, Reyes-Batlle M, Lorenzo-Morales J, Carrasco-Yépez MM. Isolation and Identification of *Naegleria* Species in Irrigation Channels for Recreational Use in Mexicali Valley, Mexico. Pathogens. 2020 Oct 7;9(10):820.
N29: Dean K, Weir MH, Mitchell J. Development of a dose-response model for *Naegleria fowleri*. J Water Health. 2019 Feb;17(1):63-71.
N23: Moussa M, De Jonckheere JF, Guerlotté J, Richard V, Bastaraud A, Romana M, Talarmin A. Survey of *Naegleria fowleri* in geothermal recreational waters of Guadeloupe (French West Indies). PLoS One. 2013;8(1):e54414.

Table 5.10 Risk of Bias Summary of Epidemiology studies of *Naegleria fowleri*

Q.	Epidemiology studies of *Naegleria fowleri*	
	N35	N40
3. Appropriate comparison groups	+	+
4. Confounding	+	+
7. Missing outcome data	+	-
8. Exposure characteristics	+	N/A
9. Outcome assessment	+	+
10. Outcome reporting	-	+
11. Other treats	-	-
Overall	Not serious	

Key: Risk of bias rating

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

Study ID for Table 4.10

N35: Gharpure R, Gleason M, Salah Z, Blackstock AJ, Hess-Homeier D, Yoder JS, Ali IKM, Collier SA, Cope JR. Geographic Range of Recreational Water-Associated Primary Amebic Meningoencephalitis, United States, 1978-2018. Emerg Infect Dis. 2021 Jan;27(1):271-274.

N40: Gharpure R, Bliton J, Goodman A, Ali IKM, Yoder J, Cope JR. Epidemiology and Clinical Characteristics of Primary Amebic Meningoencephalitis Caused by *Naegleria fowleri*: A Global Review. Clin Infect Dis. 2021 Jul 1;73(1):e19-e27.

5.2.2 Quality of included studies for *Burkholderia pseudomallei*

The included primary studies were assessed for risk of bias using an adaptation of the OHAT risk of bias tool (OHAT, 2019). Existing guidance or review reports such as those found in the grey literature search were appraised using an Assessment Tool developed by NHMRC for water projects. The certainty of the body of evidence was assessed where appropriate. At least one reviewer performed an assessment on each included study. All assessments were checked internally by the NHMRC project team.

Table 5.11 Risk of Bias Summary of case reports of *Burkholderia pseudomallei*

Q.	B1	B2
3. Appropriate comparison groups	--	-
4. Confounding	-	-
7. Missing outcome data	+	-
8. Exposure characteristics	-	-
9. Outcome assessment	+	-
10. Outcome reporting	+	-
11. Other treats		
Overall	Serious	Serious

Key: Risk of bias rating

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

Study ID for Table 4.11

B1: Alvarez-Hernandez G, Cruz-Loustaunau D, Ibarra JA, Rascon-Alcantar A, Contreras-Soto J, Meza-Radilla G, Torres AG, Estrada-de Los Santos P. Description of two fatal cases of melioidosis in Mexican children with acute pneumonia: case report. BMC Infect Dis. 2021 Feb 23;21(1):204.

B2: Sharif, Saidatulakma. *Ocular Burkholderia Pseudomallei, a Rare Variant in Presentation – A Case Series*. Pediatria i Medycyna Rodzinna. 2020 October 16(3):329-333.

Table 5.12 Risk of Bias Summary of Epidemiological Studies of *Burkholderia pseudomallei*

Q.	B3	B4
3. Appropriate comparison groups	-	+
4. Confounding	-	-
7. Missing outcome data	+	+
8. Exposure characteristics	-	-
9. Outcome assessment	+	+
10. Outcome reporting	+	-
11. Other treats	N/A	N/A
Overall	Serious	Serious

Key: Risk of bias rating

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

Study ID for Table 4.12

B3: Baker A, Tahani D, Gardiner C, Bristow KL, Greenhill AR, Warner J. Groundwater seeps facilitate exposure to *Burkholderia pseudomallei*. Appl Environ Microbiol. 2011 Oct;77(20):7243-6.
B4: Inglis TJ, Foster NF, Gal D, Powell K, Mayo M, Norton R, Currie BJ. Preliminary report on the northern Australian melioidosis environmental surveillance project. Epidemiol Infect. 2004 Oct;132(5):813-20.

Table 5.13 Risk of Bias Summary of Environmental Studies of *Burkholderia pseudomallei*

Q.	B5	B6	B7
3. Appropriate comparison groups	--	-	--
4. Confounding	-	-	-
7. Missing outcome data	+	-	+
8. Exposure characteristics	-	-	-
9. Outcome assessment	-	-	-
10. Outcome reporting	-	-	-
11. Other treats			
Overall	Serious	Serious	Serious

Key: Risk of bias rating

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

Study ID for Table 4.13

B5: Baker AL, Warner JM. *Burkholderia pseudomallei* is frequently detected in groundwater that discharges to major watercourses in northern Australia. Folia Microbiol (Praha). 2016 Jul;61(4):301-5.

B6: Draper AD, Mayo M, Harrington G, Karp D, Yinfoo D, Ward L, Haslem A, Currie BJ, Kaestli M. Association of the melioidosis agent *Burkholderia pseudomallei* with water parameters in rural water supplies in Northern Australia. Appl Environ Microbiol. 2010 Aug;76(15):5305-7.

B7: Kaestli M, O'Donnell M, Rose A, Webb JR, Mayo M, Currie BJ, Gibb K. Opportunistic pathogens and large microbial diversity detected in source-to-distribution drinking water of three remote communities in Northern Australia. PLoS Negl Trop Dis. 2019 Sep 5;13(9):e0007672.

Table 5.14 Risk of Bias Summary of analysis of testing methods for *Burkholderia pseudomallei*

Q.	B8
3. Appropriate comparison groups	-
4. Confounding	+
7. Missing outcome data	+
8. Exposure characteristics	+
9. Outcome assessment	-
10. Outcome reporting	-
11. Other treats	
Overall	Serious

Key: Risk of bias rating

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

Study ID for Table 4.14

B8: Knappik M, Dance DA, Rattanavong S, Pierret A, Ribolzi O, Davong V, Silisouk J, Vongsouvath M, Newton PN, Dittrich S. Evaluation of Molecular Methods To Improve the Detection of *Burkholderia pseudomallei* in Soil and Water Samples from Laos. Appl Environ Microbiol. 2015 Jun;81(11):3722-7.

Table 5.15 Risk of Bias Summary of weather pattern analysis for *Burkholderia pseudomallei*

Q.	B9	B10
3. Appropriate comparison groups	--	--
4. Confounding	--	-
7. Missing outcome data	+	+
8. Exposure characteristics	--	-
9. Outcome assessment	-	-
10. Outcome reporting	-	-
11. Other treats		
Overall	Very Serious	Serious

Key: Risk of bias rating

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

Study ID for Table 4.15

B9: Kaestli M, Grist EPM, Ward L, Hill A, Mayo M, Currie BJ. The association of melioidosis with climatic factors in Darwin, Australia: A 23-year time-series analysis. J Infect. 2016 Jun;72(6):687-697.
B10: Liu X, Pang L, Sim SH, Goh KT, Ravikumar S, Win MS, Tan G, Cook AR, Fisher D, Chai LY. Association of melioidosis incidence with rainfall and humidity, Singapore, 2003-2012. Emerg Infect Dis. 2015 Jan;21(1):159-62.

5.4 Assessment of certainty of primary studies

A certainty of evidence rating of the primary studies was used to support the overall confidence in the evidence to address the research questions. The assessment was conducted as described in the OHAT Handbook. In brief, the primary studies were initially grouped (Tables 4.3-4.15) based on key study design features. The confidence in the results could be downgraded based on multiple factors (Risk of Bias, Unexplained inconsistency, Indirectness, Imprecision, and Publication bias (OHAT Handbook Figure 6)). Conversely the confidence in the results could be upgraded based on multiple factors (Magnitude of effect, does response, residual confounding, and consistency across study designs/populations/animal models or species (OHAT Handbook Figure 6)). After assessment, a final confidence rating was given and summarised in a separate table (Table 4.29 and 4.30).

5.4.1 Assessment of certainty of *Naegleria fowleri*

Table 5.16 Confidence Rating for Fatality Case Reports for *Naegleria fowleri*

Study outcome (number of studies, study type)	Fatalities from PAM, with testing of suspected water source confirming *Naegleria fowleri* (6 case reports)	Fatalities from PAM where testing of water source for *Naegleria fowleri* not conducted or unknown (7 case reports)	Fatalities from *Naegleria fowleri*, cause unknown (1 case report)	**Comment** [(a)]
Initial confidence rating	**LOW**	**LOW**	**LOW**	Based on study design as per OHAT (2019, Table 8).
Risk of Bias	Not serious	Not serious	Not serious	Plausible bias unlikely to seriously alter the results.
Unexplained inconsistency	Not serious	Not serious	Not serious	Case reports appear to be consistent in terms of findings (PAM fatalities caused by *Naegleria fowleri*).
Indirectness	Not serious	Not serious	Not serious	Human studies generally are not downgraded for indirectness.
Imprecision	Serious	Serious	Serious	Small sample sizes inherent of case reports render the results imprecise. Confidence remains low.
Publication bias	Undetected	Undetected	Undetected	No downgrade

Study outcome (number of studies, study type)	Fatalities from PAM, with testing of suspected water source confirming *Naegleria fowleri* (6 case reports)	Fatalities from PAM where testing of water source for *Naegleria fowleri* not conducted or unknown (7 case reports)	Fatalities from *Naegleria fowleri*, cause unknown (1 case report)	**Comment** [(a)]
Magnitude	Not large	Not large	Not large	Case reports with small sample sizes do not fit the classic consideration for magnitude of response. Confidence not upgraded.
Dose response	No	No	No	Case reports with small sample sizes do not lend themselves to a dose response. Confidence not upgraded.
Residual confounding	No	No	No	Not relevant for case reports. Confidence not upgraded.
Consistency across species/population/ study design	Yes Upgraded to **MODERATE**	Yes Upgraded to **MODERATE**	Yes Upgraded to **MODERATE**	All studies were consistent with *Naegleria fowleri* infection causing PAM and resulting in a fatality. Confidence upgraded.
Final confidence rating	**MODERATE**	**MODERATE**	**MODERATE**	
a) Table adapted from guidance provided in OHAT (2019, Table 7)				

Table 5.17 Confidence Rating for Infection of *Naegleria fowleri*

Study outcome (number of studies, study type)	Infection with *Naegleria fowleri* (1 case report)	**Comment** [(a)]
Initial confidence rating	**LOW**	Based on study design as per OHAT (2019, Table 8).
Risk of Bias	Not serious	Plausible bias unlikely to seriously alter the results.
Unexplained inconsistency	Not serious	Confidence not downgraded

Study outcome (number of studies, study type)	Infection with *Naegleria fowleri* (1 case report)	**Comment** [a]
Indirectness	Not serious	The study is relevant to the research questions. Confidence not downgraded.
Imprecision	Serious	Small sample sizes inherent of case reports render the results imprecise. Confidence remains low.
Publication bias	Undetected	No downgrade
Magnitude	Not Large	Report with small sample sizes do not fit the classic consideration for magnitude of response. Confidence not upgraded.
Dose response	No	Report with small sample sizes do not lend themselves to a dose response. Confidence not upgraded.
Residual confounding	No	Not relevant for case reports. Confidence not upgraded.
Consistency across species/population/ study design	Yes Upgraded to **MODERATE**	Cases were consistent with *Naegleria fowleri* infection causing PAM and resulting in a fatality. Confidence upgraded.
Final confidence rating	**MODERATE**	
a) Table adapted from guidance provided in OHAT (2019, Table 7)		

Table 5.18 Confidence Rating for Successfully Treated Case Reports of *Naegleria fowleri*

Study outcome (number of studies, study type)	Successfully treated PAM (4 case reports)	**Comment** [a]
Initial confidence rating	**LOW**	Based on study design as per OHAT (2019, Table 8).
Risk of Bias	Not serious	Plausible bias unlikely to seriously alter the results.
Unexplained inconsistency	Not serious	Confidence not downgraded
Indirectness	Not serious	Human studies generally are not downgraded for indirectness.
Imprecision	Serious	Small sample sizes inherent of case reports render the results imprecise. Confidence remains low.
Publication bias	Undetected	No downgrade
Magnitude	Not Large	Report with small sample sizes do not fit the classic consideration for magnitude of response. Confidence not upgraded.

Study outcome (number of studies, study type)	Successfully treated PAM (4 case reports)	**Comment** [a]
Dose response	No	Report with small sample sizes do not lend themselves to a dose response. Confidence not upgraded.
Residual confounding	No	Not relevant for case reports. Confidence not upgraded.
Consistency across species/population/ study design	No	Some consistency of outcomes across study designs but not considered enough to warrant upgrading. Confidence not upgraded.
Final confidence rating	**LOW**	
a) Table adapted from guidance provided in OHAT (2019, Table 7)		

Table 5.19 Confidence Rating for Environmental Water Testing for *Naegleria fowleri*

Study outcome (number of studies, study type)	Testing of recreational water for detection of *Naegleria fowleri* (6 observational environmental studies)	**Comment** [a]
Initial confidence rating	**LOW**	Based on study design as per OHAT (2019, Table 8).
Risk of Bias	Not serious	Plausible bias unlikely to seriously alter the results.
Unexplained inconsistency	Not serious	Environmental studies seem to be consistent in terms of their findings Confidence not downgraded.
Indirectness	Not serious	The studies are relevant to the research questions. Confidence not downgraded.
Imprecision	Serious	Small sample sizes render the results imprecise. Confidence remains low.
Publication bias	Undetected	Confidence not downgraded.
Magnitude	Not large	Environmental studies with small sample sizes do not fit the classic consideration for magnitude of response. Confidence not upgraded.
Dose response	No	Environmental studies with small sample sizes do not lend themselves to a dose response. Confidence not upgraded.
Residual confounding	No	Confidence not upgraded.

Study outcome (number of studies, study type)	Testing of recreational water for detection of *Naegleria fowleri* (6 observational environmental studies)	**Comment** [a]
Consistency across species/population/study design	Yes Upgraded to **LOW**	Consistency observed for some results across some study designs for considered reasonable for upgrading. Confidence upgraded.
Final confidence rating	**LOW**	
a) Table adapted from guidance provided in OHAT (2019, Table 7)		

Table 5.20 Confidence Rating for in-vitro growth conditions for *Naegleria fowleri*

Study outcome (number of studies)	In-vitro testing of growth conditions for *Naegleria fowleri* (2 observational laboratory studies)	**Comment** [a]
Initial confidence rating	**LOW**	Based on study design as per OHAT (2019, Table 8).
Risk of Bias	Not serious	Plausible bias unlikely to seriously alter the results
Unexplained inconsistency	Not serious	Confidence not downgraded
Indirectness	Not serious	The studies are relevant to the research questions. Confidence not downgraded.
Imprecision	Serious	Small sample sizes render the results imprecise. Confidence remains low.
Publication bias	Undetected	Confidence not downgraded.
Magnitude	Not large	Laboratory studies with small sample sizes do not fit the classic consideration for magnitude of response. Confidence not upgraded.
Dose response	No	Laboratory study with small sample sizes and no health perspective/affects. Confidence not upgraded.
Residual confounding	No	Confidence not upgraded.
Consistency across species/population/ study design	No	Some consistency of outcomes across study designs but not considered enough to warrant upgrading. Confidence not upgraded.
Final confidence rating	**LOW**	
a) Table adapted from guidance provided in OHAT (2019, Table 7)		

Table 5.21 Confidence Rating for testing of drinking water for *Naegleria fowleri*

Study outcome (number of studies)	Testing of drinking water for detection of *Naegleria fowleri* (3 observational studies)	**Comment** [a]
Initial confidence rating	**LOW**	Based on study design as per OHAT (2019, Table 8).
Risk of Bias	Not serious	Plausible bias unlikely to seriously alter the results
Unexplained inconsistency	Not serious	Confidence not downgraded
Indirectness	Not serious.	The studies are relevant to the research questions. Confidence not downgraded.
Imprecision	Serious.	Small sample sizes render the results imprecise. Confidence remains low.
Publication bias	Undetected	Confidence not downgraded.
Magnitude	Not large.	Laboratory studies with small sample sizes do not fit the classic consideration for magnitude of response. Confidence not upgraded.
Dose response	No.	Environmental studies with small sample sizes do not lend themselves to a dose response. Confidence not upgraded.
Residual confounding	No.	Confidence not upgraded.
Consistency across species/population/ study design	Yes. Upgraded to **MODERATE**.	Consistency observed for some results across some study designs for considered reasonable for upgrading. Confidence upgraded.
Final confidence rating	**MODERATE**	

a) Table adapted from guidance provided in OHAT (2019, Table 7)

Table 5.22 Confidence Rating for dose response and concentrations for *Naegleria fowleri*

Study outcome (number of studies)	Pathogenicity testing for *Naegleria fowleri* (1 observational study)	Dose response modelling for *Naegleria fowleri* (1 observational study)	Detection of *Naegleria fowleri* (1 observational study)	**Comment** [a]
Initial confidence rating	**LOW**	**LOW**	**LOW**	Based on study design as per OHAT (2019, Table 8).
Risk of Bias	Not serious	Serious. Downgraded to **VERY LOW**	Not serious	Plausible bias unlikely to seriously alter the results
Unexplained inconsistency	Not serious	Not serious	Not serious	Confidence not downgraded

Study outcome (number of studies)	Pathogenicity testing for *Naegleria fowleri* (1 observational study)	Dose response modelling for *Naegleria fowleri* (1 observational study)	Detection of *Naegleria fowleri* (1 observational study)	**Comment** [(a)]
Indirectness	Not serious	Not serious	Not serious	The studies are relevant to the research questions. Confidence not downgraded.
Imprecision	Serious	Serious	Serious	Small sample sizes render the results imprecise. Confidence remains low.
Publication bias	Undetected	Undetected	Undetected	Confidence not downgraded.
Magnitude	Not large	Not large	Not large	Environmental and Laboratory studies with small sample sizes do not fit the classic consideration for magnitude of response. Confidence not upgraded.
Dose response	No	Yes	No	Laboratory study with small sample size using mice and environmental studies with small sample sizes do not lend themselves to a dose response. Confidence not upgraded.
Residual confounding	No	No	No	Confidence not upgraded.
Consistency across species/population/ study design	No	No	No	Some consistency of outcomes across study designs but not considered enough to warrant upgrading. Confidence not upgraded.
Final confidence rating	**LOW**	**VERY LOW**	**LOW**	

a) Table adapted from guidance provided in OHAT (2019, Table 7)

Table 5.23 Confidence Rating of Epidemiological studies for *Naegleria fowleri*

Study outcome (number of studies)	Epidemiological studies of *Naegleria fowleri* (2 observational studies)	**Comment** [(a)]
Initial confidence rating	**LOW**	Based on study design as per OHAT (2019, Table 8).
Risk of Bias	Not serious	Plausible bias unlikely to seriously alter the results
Unexplained inconsistency	Not serious	Confidence not downgraded
Indirectness	Not serious	The studies are relevant to the research questions. Confidence not downgraded.
Imprecision	Not Serious	No or minimal indications of large standard deviations.
Publication bias	Undetected	Confidence not downgraded.
Magnitude	Not large	Environmental/Epidemiological studies with small sample sizes do not fit the classic consideration for magnitude of response. Confidence not upgraded.
Dose response	No	Reports with small sample sizes do not lend themselves to a dose response. Confidence not upgraded.
Residual confounding	No	Confidence not upgraded.
Consistency across species/population/ study design	Yes Upgraded to **MODERATE**	Consistency observed for some results across some study designs for considered reasonable for upgrading. Confidence upgraded.
Final confidence rating	**MODERATE**	
a) Table adapted from guidance provided in OHAT (2019, Table 7)		

5.4.2 Assessment of certainty of *Burkholderia pseudomallei*

Table 5.24 Confidence Rating for case reports of *Burkholderia pseudomallei*

Study outcome (number of studies, study type)	Confirmed infection (Respiratory Distress Syndrome, septic shock and abscesses) potentially linked to recreational water exposure (1 case report)	Confirmed infection (Ocular infection) potentially linked to recreational water exposure (1 case series)	**Comment** [(a)]
Initial confidence rating	**LOW**	**LOW**	Based on study design as per OHAT (2019, Table 8).
Risk of Bias	Serious. Downgraded to **VERY LOW**	Serious. Downgraded to **VERY LOW**	Confidence downgraded due to consistent potential confounding and inconsistent detection bias across case studies for both health outcomes, as well as selective reporting bias in one study.
Unexplained inconsistency	Not serious	Not serious	Case reports appear to be consistent in terms of their findings (i.e. Respiratory Distress Syndrome with abscess formation or Ocular infection). Confidence not downgraded.
Indirectness	Not serious	Not serious	Human studies generally are not downgraded for indirectness.
Imprecision	Serious Cannot downgrade further	Serious Cannot downgrade further	Small sample sizes inherent of case reports render the results imprecise. Confidence remains very low.
Publication bias	Undetected	Undetected	No downgrade.
Magnitude	Not large	Not large	Case reports with small sample sizes do not fit the classic consideration for magnitude of response. Confidence not upgraded.
Dose response	No	No	Case reports with small sample sizes do not lend themselves to a dose response. Confidence not upgraded.
Residual confounding	No	No	Not relevant for case reports. Confidence not upgraded.

Study outcome (number of studies, study type)	Confirmed infection (Respiratory Distress Syndrome, septic shock and abscesses) potentially linked to recreational water exposure (1 case report)	Confirmed infection (Ocular infection) potentially linked to recreational water exposure (1 case series)	**Comment** [a]
Consistency across species/population/ study design	No	No	Some consistency of outcomes across study designs but not considered enough to warrant upgrading. Confidence not upgraded.
Final confidence rating	**VERY LOW**	**VERY LOW**	
a) Table adapted from guidance provided in OHAT (2019, Table 7)			

Table 5.25 Confidence Rating for Epidemiological Studies of *Burkholderia pseudomallei*

Study outcome (number of studies, study type)	Linkage of water testing and patient cases (1 epidemiological study)	Water and soil testing in communities with confirmed cases of Melioidosis (1 environmental surveillance study)	**Comment** [a]
Initial confidence rating	**LOW**	**LOW**	Based on study design as per OHAT (2019, Table 8).
Risk of Bias	Serious Downgraded to **VERY LOW**	Serious Downgraded to **VERY LOW**	Confidence downgraded due to consistent potential confounding and inconsistent detection bias across case studies for reported associations, as well as selective reporting bias in two studies.
Unexplained inconsistency	Not serious	Not serious	Environmental/Epidemiological studies seem to be consistent in terms of their findings Confidence not downgraded.
Indirectness	Not serious	Not serious	The studies are relevant to the research questions. Confidence not downgraded.
Imprecision	Serious Cannot downgrade further	Serious Cannot downgrade further	Small sample sizes render the results imprecise. Confidence remains very low.
Publication bias	Undetected	Undetected	Confidence not downgraded.

Study outcome (number of studies, study type)	Linkage of water testing and patient cases (1 epidemiological study)	Water and soil testing in communities with confirmed cases of Melioidosis (1 environmental surveillance study)	**Comment** [a]
Magnitude	Not large	Not large	Environmental/Epidemiological studies with small sample sizes do not fit the classic consideration for magnitude of response. Confidence not upgraded.
Dose response	No	No	Environmental/Epidemiological studies with small sample sizes do not lend themselves to a dose response. Confidence not upgraded.
Residual confounding	No	No	Confidence not upgraded.
Consistency across species/population/study design	Yes Upgraded to **LOW**	Yes Upgraded to **LOW**	Consistency of findings across study designs. Confidence upgraded.
Final confidence rating	**LOW**	**LOW**	
a) Table adapted from guidance provided in OHAT (2019, Table 7)			

Table 5.26 Confidence Rating for Environmental Studies of *Burkholderia pseudomallei*

Study outcome (number of studies, study type)	Detection of *Burkholderia pseudomallei* in water seeps and associated contamination of waterways (1 observational environmental study)	Water quality characteristics and the association with the presence of *Burkholderia pseudomallei* in bores (1 observational environmental study)	Water quality parameters to assess microbial levels in ground water and drinking water (1 observational scoping study)	**Comment** [a]
Initial confidence rating	**LOW**	**LOW**	**LOW**	Based on study design as per OHAT (2019, Table 8).
Risk of Bias	Serious Downgraded to **VERY LOW**	Serious Downgraded to **VERY LOW**	Serious Downgraded to **VERY LOW**	Confidence downgraded due to consistent potential confounding, inconsistent detection bias across case studies and selective reporting bias.

Study outcome (number of studies, study type)	Detection of *Burkholderia pseudomallei* in water seeps and associated contamination of waterways (1 observational environmental study)	Water quality characteristics and the association with the presence of *Burkholderia pseudomallei* in bores (1 observational environmental study)	Water quality parameters to assess microbial levels in ground water and drinking water (1 observational scoping study)	**Comment** [(a)]
Unexplained inconsistency	Not serious	Not serious	Not serious	Environmental studies seem to be consistent in terms of their findings Confidence not downgraded.
Indirectness	Not serious	Not serious	Not serious	The studies are relevant to the research questions. Confidence not downgraded.
Imprecision	Serious Cannot downgrade further	Serious Cannot downgrade further	Serious Cannot downgrade further	Small sample sizes render the results imprecise. Confidence remains very low.
Publication bias	Undetected	Undetected	Undetected	Confidence not downgraded.
Magnitude	Not large	Not large	Not large	Environmental studies with small sample sizes do not fit the classic consideration for magnitude of response. Confidence not upgraded.
Dose response	No	No	No	Environmental studies with small sample sizes do not lend themselves to a dose response. Confidence not upgraded.
Residual confounding	No	No	No	Confidence not upgraded.
Consistency across species/population/ study design	No	Yes Upgraded to **LOW**	Yes Upgraded to **LOW**	Consistency observed for some results across two study designs for considered reasonable for upgrading. Confidence upgraded.

Study outcome (number of studies, study type)	Detection of *Burkholderia pseudomallei* in water seeps and associated contamination of waterways (1 observational environmental study)	Water quality characteristics and the association with the presence of *Burkholderia pseudomallei* in bores (1 observational environmental study)	Water quality parameters to assess microbial levels in ground water and drinking water (1 observational scoping study)	**Comment** [a]
Final confidence rating	**VERY LOW**	**LOW**	**LOW**	
a) Table adapted from guidance provided in OHAT (2019, Table 7)				

Table 5.27 Confidence Rating of analysis of testing methods for *Burkholderia pseudomallei*

Study outcome (number of studies)	Evaluation of effectiveness of testing methods for the laboratory detection of *Burkholderia pseudomallei* (1 observational methods evaluation study)	**Comment** [a]
Initial confidence rating	**LOW**	Based on study design as per OHAT (2019, Table 8).
Risk of Bias	Serious Downgraded to **VERY LOW**	Confidence downgraded due to inconsistent detection bias across case studies for both health outcomes, as well as selective reporting bias in one study.
Unexplained inconsistency	Not serious	Confidence not downgraded.
Indirectness	Not serious	The study is relevant to the research questions. Confidence not downgraded.
Imprecision	N/A	Single study, unable to assess
Publication bias	Undetected	No downgrade.
Magnitude	Not large	Method validation studies do not fit the classic consideration for magnitude of response. Confidence not upgraded.
Dose response	No	Method validation studies do not lend themselves to a dose response. Confidence not upgraded.
Residual confounding	No	Confidence not upgraded.
Consistency across species/population/ study design	N/A	Not applicable to single study/outcome, unable to assess
Final confidence rating	**VERY LOW**	
a) Table adapted from guidance provided in OHAT (2019, Table 7)		

Table 5.28 Confidence Rating of weather pattern analysis for *Burkholderia pseudomallei*

Study outcome (number of studies, study type)	Association between weather patterns and Melioidosis cases (1 environmental case series)	Association between weather factors and Melioidosis cases (1 epidemiological study)	**Comment** [a]
Initial confidence rating	**LOW**	**LOW**	Based on study design as per OHAT (2019, Table 8).
Risk of Bias	Very Serious Downgraded to **VERY LOW**	Serious Downgraded to **VERY LOW**	Confidence downgraded due to consistent potential confounding and inconsistent detection bias across case studies for both health outcomes, as well as selective reporting bias in one study.
Unexplained inconsistency	Not serious	Not serious	Confidence not downgraded.
Indirectness	Not serious	Not serious	The studies are relevant to the research questions. Confidence not downgraded.
Imprecision	Serious Cannot downgrade further	Serious Cannot downgrade further	Small sample sizes inherent of case reports render the results imprecise. Confidence remains very low.
Publication bias	Undetected	Undetected	No downgrade.
Magnitude	Not large	Not large	Modelling studies do not fit the classic consideration for magnitude of response. Confidence not upgraded.
Dose response	No	No	Modelling studies do not lend themselves to a dose response. Confidence not upgraded.
Residual confounding	No	No	Confidence not upgraded.
Consistency across species/population/ study design	Yes Upgraded to **LOW**	Yes Upgraded to **LOW**	Consistency of some findings across study designs. Confidence upgraded.
Final confidence rating	**LOW**	**LOW**	
a) Table adapted from guidance provided in OHAT (2019, Table 7)			

5.5 Summary of certainty of primary studies

5.5.1 Overall evaluation of *Naegleria fowleri primary studies*

Table 5.29 Conclusions for *Naegleria fowleri*

Measured outcomes (number of studies, study type)	Certainty rating	Conclusion
Case studies/series of fatalities due to *Naegleria fowleri*		
Fatalities from PAM, with testing of suspected water source confirming *Naegleria fowleri* (6)	**MODERATE**	There is moderate certainty linking exposure to *Naegleria fowleri* in recreational waters to potential infection and adverse health effects, i.e. fatality. Cases all linked to recreational water activity and *Naegleria fowleri* confirmed in water sources.
Fatalities from PAM where testing of testing of water source for *Naegleria fowleri* not conducted or unknown (7)	**MODERATE**	There is moderate certainty linking exposure to *Naegleria fowleri* in recreational waters to potential infection and adverse health effects, i.e. fatality. Cases all linked to recreational water activity but *Naegleria fowleri* not confirmed in water sources.
Fatalities from *Naegleria fowleri,* cause unknown (1)	**MODERATE**	There is moderate certainty linking exposure to *Naegleria fowleri* to potential adverse health effects, i.e. fatality. Cases were linked to potential recreational water activity and *Naegleria fowleri* not confirmed in water sources.
Case studies/series of infections due to *Naegleria fowleri*		
Infection with *Naegleria fowleri* (1)	**MODERATE**	There is moderate certainty linking exposure to *Naegleria fowleri* to potential infection and adverse health effects, i.e. fatality. Some cases were linked to recreational water activity.
Case studies/series of successfully treated case reports of *Naegleria fowleri*		
Successfully treated PAM (4)	**LOW**	There is low certainty linking the different types of treatments methods with successful prevention of PAM following *Naegleria fowleri* infection.
Environmental Water Testing for *Naegleria fowleri*		
Testing of recreational water for detection of *Naegleria fowleri* (5)	**LOW**	There is low certainty of detection of *Naegleria fowleri* in recreational waters and a direct adverse health outcome. One study found *Naegleria fowleri* in recreational waters in winter while another study did confirm the presence of *Naegleria fowleri* in waters where a PAM fatality previously occurred.
In-vitro growth conditions for *Naegleria fowleri*		
In-vitro testing of growth conditions for *Naegleria fowleri* (2)	**LOW**	There is low certainty from a single study which identified the concentration of bacteria to support *Naegleria fowleri* growth and a single study testing *Naegleria fowleri* growth conditions. Neither study links to adverse health outcomes but tests secondary questions.
Testing of drinking water for *Naegleria fowleri*		
Testing of drinking water for *Naegleria fowleri* (3)	**MODERATE**	There is moderate certainty from three studies which identify biotic and abiotic factors which may support *Naegleria fowleri* growth. No study links to adverse health outcomes but tests secondary questions.
Dose response and concentrations for *Naegleria fowleri*		
Pathogenicity testing for *Naegleria fowleri* (1)	**LOW**	There is low certainty of detection of *Naegleria fowleri* in recreational waters leading to a direct adverse health outcome.
Dose response modelling for *Naegleria fowleri* (1)	**VERY LOW**	There is very low certainty from the study which shows *Naegleria fowleri* is fatal to mice at given concentrations and activities but cannot be directly related to humans (other than the knowledge that *Naegleria fowleri* infections are fatal).
Detection of *Naegleria fowleri* (1)	**LOW**	There is low certainty of detection of *Naegleria fowleri* at a given concentration in recreational waters and a direct adverse health outcome. Study did confirm the presence of *Naegleria fowleri* in waters where a PAM fatality previously occurred.
Epidemiological studies for *Naegleria fowleri*		
Epidemiological studies for *Naegleria fowleri* (2)	**MODERATE**	There is moderate certainty linking exposure to *Naegleria fowleri* in recreational waters which can result in infection and adverse health effects, i.e. fatality. Some cases were linked to recreational water activity.

5.5.2 Overall evaluation of *Burkholderia pseudomallei primary studies*

Table 5.30 Conclusions for *Burkholderia pseudomallei*

Measured outcomes (number of studies, study type)	Certainty rating	Conclusion
Case studies/series linking confirmed cases of infection to potential recreational water exposure		
Respiratory distress syndrome, septic shock and abscesses (1)	**VERY LOW**	There is very low certainty from one study of an association between infection with *Burkholderia pseudomallei* and the development of respiratory distress syndrome, septic shock and abscesses with recreational water exposure.
Ocular infection (1)	**VERY LOW**	There is very low certainty from one study of an association between infection with *Burkholderia pseudomallei* and the development of ocular infection from recreational water exposure.
Epidemiological Studies linking clinical cases to potential sources of exposure		
Linkage of water testing and patient cases (1)	**LOW**	There is low certainty from one study of an association between seep water containing *Burkholderia pseudomallei* and human infection.
Water and soil testing in communities with confirmed cases of Melioidosis (1)	**LOW**	There is low certainty from one study of an association between the presence of *Burkholderia pseudomallei* in water and soil and human infection.
Observational Environmental Studies confirming sources of exposure and linking to associated environmental conditions		
Detection of *Burkholderia pseudomallei* in water seeps and associated contamination of waterways (1)	**VERY LOW**	There is very low certainty from one study of an association between the detection of *Burkholderia pseudomallei* in groundwater and contamination of local waterways with the bacteria after heavy rainfall.
Water quality characteristics and the association with the presence of *Burkholderia pseudomallei* in bores (1)	**LOW**	There is low certainty from one study of an association between water characteristics such as chemical and microbial measurements and the presence of *Burkholderia pseudomallei* in bore water.
Water quality parameters to assess microbial levels in ground water and drinking water (1)	**LOW**	There is low certainty from one study of an association between water quality parameters such as chemical and microbial measurements in ground and drinking water and detection of microbes in water samples and biofilms.
Observational methods evaluation assessing effectiveness of methods for sample analysis		
Evaluation of testing methods for the laboratory detection of *Burkholderia pseudomallei* (1)	**VERY LOW**	There is very low certainty from one study of that the use of enrichment and DNA detection is the most effective laboratory method for the detection of *Burkholderia pseudomallei.*
Observational studies assessing weather pattern and incidence of infections		
Association between weather patterns and Melioidosis cases (1)	**LOW**	There is low certainty from one study of an association between weather conditions including dew point, cloud cover, rainfall and temperature and groundwater fluctuations and increased cases of human infections with *Burkholderia pseudomallei.*
Association between weather factors and Melioidosis cases (1)	**LOW**	There is low certainty from one study of an association between increased rainfall and humidity and increased cases of human infection with *Burkholderia pseudomallei.*

6 Results for *Naegleria fowleri* in recreational waters

6.1 Review of existing guidelines

6.1.1 Primary research question

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

6.1.2 Secondary research questions

1. What are the indicators/surrogates of this/these risk/s?
2. What is the frequency of occurrence of identified health outcomes in Australia? Is there an association with exposure to recreational waters?
3. What is known about the occurrence of these organisms in natural waters in Australia?
4. What are the conditions associated with increased occurrence? What are the conditions associated with absence of these microorganisms?
5. What is known about the exposure pathway for each organism?
6. What is known about the dose-response for each organism?
7. What are the current practices to minimise or manage this/these risk/s?

Table 6.1 Summary of evidence from Guidelines for *Naegleria fowleri*

Guidelines	Guideline type	Exposure	Outcomes	Other	Questions addressed
Naegleria response protocol for drinking water supply systems					
Western Australian Government (Study ID N42)	Guideline for water utilities	Overview of *Naegleria fowleri's* presence and management in drinking water supply systems	Detection and management of *Naegleria fowleri*	*Naegleria fowleri* not connected with the presence of faecal coliforms and *E. coli*.	Secondary question 1

6.2 Review of Primary studies

6.2.1 Primary research question

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

6.2.2 Secondary research questions

1. What are the indicators/surrogates of this/these risk/s?
2. What is the frequency of occurrence of identified health outcomes in Australia? Is there an association with exposure to recreational waters?
3. What is known about the occurrence of these organisms in natural waters in Australia?
4. What are the conditions associated with increased occurrence? What are the conditions associated with absence of these microorganisms?
5. What is known about the exposure pathway for each organism?
6. What is known about the dose-response for each organism?
7. What are the current practices to minimise or manage this/these risk/s?

Table 6.2 Summary of evidence from Primary studies for *Naegleria fowleri*

Paper	Study type	Exposure	Outcomes	Other	Questions addressed
Case studies					
Fatalities from PAM, with testing of suspected water source confirming *Naegleria fowleri*					
Booth et al. 2015 (Study ID N1)	Case study	Swimming and water slide	Fatality from primary amoebic meningoencephalitis (PAM)	Hot springs confirmed to have *Naegleria fowleri*	Primary question and Secondary question 4 & 6
Cope et al. 2018 (Study ID N2)	Case report with epidemiological and environmental investigation	Artificial whitewater river - rafting	Primary amoebic meningoencephalitis (PAM) and Cardiac death	Water, sediment and surface swabs tested	Primary question and Secondary question 1, (multiple), 5
Kemble et al. 2012 (Study ID N3)	Case study with epidemiological and environmental investigation	Recreational freshwater – lake	Fatality from primary amoebic meningoencephalitis (PAM)	*Naegleria fowleri* water and sediment testing from lake	Primary question and Secondary question 1, (multiple), 4, & 5
Nicholls et al. 2016 (Study ID N4)	Case reports	Geothermal bore water, cooled in open surface dams and used domestically	Fatalities from primary amoebic meningoencephalitis (PAM)	Water testing conducted	Primary question and Secondary question (potentially play), 2, 4

Paper	Study type	Exposure	Outcomes	Other	Questions addressed
Su et al. 2013 (Study ID N5)	Case study	Presumed hot spring- bathing	Fatality from primary amoebic meningoencephalitis (PAM)	*Naegleria fowleri* detected in hot spring	Primary question
Fatalities from PAM, water source testing not conducted or unknown					
Budge et al. 2013 (Study ID N6)	Case study and review (cases between 1962-2010)	Freshwater swimming (lake), waterslide	Fatality from primary amoebic meningoencephalitis (PAM)	Testing of lake not conducted	Primary question and Secondary question 1 (Temp), 5 (rough play)
Chen et al. 2019 (Study ID N7)	Case report	Warm freshwater at recreational water park	Fatality from primary amoebic meningoencephalitis (PAM)	NA	Primary question
Hamaty et al. 2020 (Study ID N8)	Case study	Suspected recreational surf park	Fatality from primary amoebic meningoencephalitis (PAM)	Testing of surf park not conducted	Primary question Secondary question 5
Lopez et al. 2012 (Study ID N9)	Case study	Suspected lake - swimming	Fatality from primary amoebic meningoencephalitis (PAM)	NA	Primary question Secondary question 5. (Note case is same as Budge 2013)
Phu et al. 2013 (Study ID N10)	Case study	Pearl diving	Fatality from primary amoebic meningoencephalitis (PAM)	NA	Primary question, Secondary question 5
Stowe et al. 2017 (Study ID N11)	Case study and case review	Recreational lake – swimming	Fatalities from primary amoebic meningoencephalitis (PAM)	Review of current and previous patients with PAM comparing treatments	Primary question, Secondary question 5
Vareechon 2019 (Study ID N12)	Case study	Hot spring - swimming	Fatality from primary amoebic meningoencephalitis (PAM)	NA	Primary question, Secondary question 5
Fatality, cause not stated					
Matthews et al. 2008 (Study ID N13)	Case reports	Recreational lakes – swimming and wakeboarding	Death	NA	Primary question, Secondary question 1 (water and air temp) 5 (multiple recreational activities).
Infection					

Paper	Study type	Exposure	Outcomes	Other	Questions addressed
Diaz 2012 (Study ID N14)	Case series (Review)	Recreational freshwater (3 cases – wakeboarding)	Infection with *Naegleria fowleri*	Statistical analysis of risk factors (location, sex, time-frame of exposure)	Primary question, Secondary question 5 (wakeboarding)
PAM successfully treated					
Dunn et al. 2016 (Study ID N15)	Case study	Freshwater park	Primary amoebic meningoencephalitis (PAM) – successfully treated	Methodology for rapid detection included in the paper	Primary question, Secondary question 5 (suspected swimming), 7 (treatment with survival)
Heggie 2017 (Study ID N16)	Case study	Recreational water park – swimming in lake	Primary amoebic meningoencephalitis (PAM) – successfully treated	NA	Primary question (potential Cl use), Secondary question 5
Linam 2015 (Study ID N17)	Case study	Outdoor water park - swimming	Primary amoebic meningoencephalitis (PAM) – successfully treated	Detection in both patient was source water.	Primary question, Secondary question 5 (Note same case as Heggie 2017)
Vargas-Zepeda et al. 2005 (Study ID N18)	Case study	Irrigation canal – swimming	Primary amoebic meningoencephalitis (PAM) – successfully treated	NA	Primary question, Secondary question 5, 7 (successful treatment)
Observational Studies					
Water testing of recreational water for detection of *Naegleria fowleri*					
Bonnilla-Lemus 2020 (Study ID N19)	Observational study	Testing of water from irrigation canals that are used for swimming	Detection of *Naegleria fowleri* in water samples	Includes mouse pathogenicity testing	Secondary question 1 & 4
Jamerson et al. 2009 (Study ID N20)	Observational study	Testing of water and sediment from recreational freshwater lake thermally impacted by industry	Detection of *Naegleria fowleri* in water samples	NA	Secondary question 1 & 4
Maclean 2004 (Study ID N21)	Observational study	Testing of water and sediment samples	Detection of *Naegleria fowleri* in water and sediment samples	NA	Secondary question 1

Paper	Study type	Exposure	Outcomes	Other	Questions addressed
Miller 2018 (Study ID N22)	Quantitative ecological correlational study	Testing of water samples	Detection of *Naegleria fowleri* and correlation with environmental conditions	NA	Secondary question 1 & 4
Moussa et al. 2013 (Study ID N23)	Observational study	Testing of water, sediment and swab samples from geothermal recreational waters	Detection of *Naegleria fowleri* in water and sediment samples	Geothermally fed lakes previously had *Naegleria fowleri* death connected. *Naegleria fowleri* detected below the French standard of 100 amoebae/litre	Primary question (Previous PAM death), Secondary question 1, 5 (Previous PAM death), and 6 (*Naegleria fowleri* concentration)
In-vitro testing of growth conditions					
Goudot et al. 2012 (Study ID N24)	Diagnostic/quantitative observational study	Laboratory study of *Naegleria fowleri* growth conditions	Measurement of total *Naegleria fowleri* in biofilm	NA	Secondary question 1 (potentially) & 4
Lam 2019 (Study ID N25)	Diagnostic/quantitative observational study	Laboratory analysis of the effect of environmental conditions on *Naegleria fowleri* viability	Detection of viable *Naegleria fowleri* at a range of environmental conditions	NA	Secondary question 1 & 5 (absence factors)
Naegleria fowleri in drinking water					
Morgan 2016 (Study ID N26)	Quantitative observational/ correlational study	Testing of drinking water distribution system	Water quality results and presence/absence of *Naegleria fowleri*	NA	Secondary question 1 & 4 (Note chlorinated system, but lacking chlorine).
Puzon 2017 (Study ID N27)	Quantitative observational/ correlational study	Testing to identify and compare biofilm ecology	Quantification of colonising amoeba and ecology	NA	Secondary question 1 (Note chlorinated system, but lacking chlorine).
Yu 2018 (Study ID N28)	Diagnostic or quantitative observational study	Study of metabolomics for detection of *Naegleria fowleri* in drinking water distribution systems	Detection of *Naegleria fowleri* in drinking water	NA	Secondary question 1 (Note chlorinated system, but lacking chlorine).
Dose response/concentrations					

Paper	Study type	Exposure	Outcomes	Other	Questions addressed
Bonnilla-Lemus 2020 (Study ID N19)	Observational study	Testing of water from irrigation canals that are used for swimming	Detection of *Naegleria fowleri* in water samples	Includes mouse pathogenicity testing	Secondary question 1 5 (Potential recreational use-linked to Vargas-Zepeda et al. 2005)
Dean et al. 2019 (Study ID N29)	Statistical modelling – dose response model	Surface water/drinking water	Death	Study conducted in mice	Secondary question 6
Moussa et al. 2013 (Study ID N23)	Observational study	Testing of water, sediment and swab samples from geothermal recreational waters	Detection of *Naegleria fowleri* in water and sediment samples	Geothermally fed lakes previously had *Naegleria fowleri* death connected. *Naegleria fowleri* detected below the French standard of 100 amoebae/litre	Primary question (Previous PAM death), Secondary question 1, 5 (Previous PAM death), 6 (*Naegleria fowleri* concentration)

7 Results for *Burkholderia pseudomallei* in recreational waters

7.1 Review of existing guidelines

7.1.1 Primary research question

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

No Guidelines for *Burkholderia pseudomallei* were included in the review.

7.1.2 Secondary research question

1. What are the indicators/surrogates of this/these risk/s?
2. What is the frequency of occurrence of identified health outcomes in Australia? Is there an association with exposure to recreational waters?
3. What is known about the occurrence of these organisms in natural waters in Australia?
4. What are the conditions associated with increased occurrence? What are the conditions associated with absence of these microorganisms?
5. What is known about the exposure pathway for each organism?
6. What is known about the dose-response for each organism?
7. What are the current practices to minimise or manage this/these risk/s?

7.2 Review of Primary studies

7.2.1 Primary research question

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

7.2.2 Secondary research question

1. What are the indicators/surrogates of this/these risk/s?
2. What is the frequency of occurrence of identified health outcomes in Australia? Is there an association with exposure to recreational waters?
3. What is known about the occurrence of these organisms in natural waters in Australia?

4. What are the conditions associated with increased occurrence? What are the conditions associated with absence of these microorganisms?
5. What is known about the exposure pathway for each organism?
6. What is known about the dose-response for each organism?
7. What are the current practices to minimise or manage this/these risk/s?

Table 7.1 Conclusions for *Burkholderia pseudomallei*

Paper	Study type	Exposure	Outcomes	Other	Questions addressed
Case studies linking potential recreational exposure to *Burkholderia pseudomallei* infection					
Alvarez-Hernand 2021 (Study ID B1)	Case study – 2 patients	Swimming in rainwater pool	Confirmed infection. Presented with respiratory distress syndrome and septic shock, abscess found on autopsy	Environmental samples taken detected *Burkholderia pseudomallei*	Primary question, Secondary question 5
Shariff 2020 (Study ID B2)	Case series	Possible link to swimming in river water (1 case)	Confirmed infection. Ocular infection	Case history and diagnosis only, laboratory identification of *Burkholderia pseudomallei* in patient samples or water	Primary question, Secondary question 5, 7
Epidemiological studies					
Baker 2011 (Study ID B3)	Epidemiological and Environmental study (observational)	Testing of ground water seeps and soil samples Comparisons with samples isolated from patients at the local hospital	Detection of *Burkholderia pseudomallei* in samples and linking with hospital cases	Statistical comparison of prevalence Comparison of dry vs wet season	Secondary question 3, 4
Inglis 2004 (Study ID B4)	Environmental surveillance (observational)	Testing of patient isolates Testing of water and soil samples, locations based	Detection of *Burkholderia pseudomallei* in samples and analysis of water parameters	Range of water sources tested including natural waterways	Primary question, Secondary question 3

Paper	Study type	Exposure	Outcomes	Other	Questions addressed
		on positive cases identified			
Environmental (water and soil) testing					
Baker and Warner 2016 (Study ID B5)	Environmental Study (observational)	Testing of ground water seeps	Detection of *Burkholderia pseudomallei* in samples	Detection in natural waters post rain event	Secondary question 3
Draper 2010 (Study ID B6)	Environmental Study (observational)	Testing of water bores	Detection of *Burkholderia pseudomallei* in samples and analysis of water parameters	Comparison of dry vs wet season (repeat testing)	Secondary question 3, 4
Kaestli 2019 (Study ID B7)	Scoping study (observational)	Testing of ground water and drinking water	Detection of *Burkholderia pseudomallei* in samples and analysis of water parameters	Info on factors associated with the presence of *Burkholderia pseudomallei*	Secondary question 3, 4
Analysis of testing methods					
Knappik 2015 (Study ID B8)	Methods evaluation (observational)	Comparison of culture and molecular methods to detect *Burkholderia pseudomallei* in water and soil samples	Comparison of detection methods	Focused just on detection methods.	Secondary question 7 (improved detection methods for management in natural waters)
Analysis of weather patterns					
Kaestli 2016 (Study ID B9)	Environmental case series	Analysis of weather and climate factors preceding identified positive cases	Association between weather patterns and positive cases	Data includes cloud cover, dew point and rainfall	Secondary question 2, 4, 5 (positive association due to groundwater fluctuations)
Liu 2015 (Study ID B10)	Epidemiological study (observational)	Analysis of case numbers and weather data	Association of melioidosis incidence with rainfall and climate	Data looks at rainfall and humidity	Secondary question 4

8 Discussion

8.1 Primary research question

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

Naegleria fowleri

Naegleria fowleri is a global freshwater parasite that can enter the brain through the nose and cause the deadly disease called primary amoebic meningoencephalitis (PAM). Most cases of PAM are associated with recreational water activities, especially swimming and diving, in warm water bodies. The infection is very rare but almost always fatal, and there is no known safe level of exposure (Booth et al., 2015; Cope et al., 2018; Kemble et al., 2012; Nicholls et al., 2016; Su et al., 2013; Budge et al., 2013; Chen et al., 2019; Hamaty et al., 2020; Lopez et al., 2012; Phu et al., 2013; Stowe et al., 2017; Vareechon, 2019; Matthews et al., 2008; Diaz, 2012; Dunn et al., 2016; Heggie, 2017; Linam, 2015; Vargas-Zepeda et al., 2005).

Swimming is the most common recreational activity linked to *Naegleria fowleri* infections (Gharepure et al., 2021a); however, multiple recreational activities, including swimming/diving (58%), water sports (e. g. waterskiing, wakeboarding and jet skiing) (10%), and multiple recreational water sources such as lakes, ponds, reservoirs, rivers/streams, and geothermal waters, have all been linked to confirmed *Naegleria fowleri* infections with fatal outcomes (Gharepure et al., 2021a). *Naegleria fowleri's* presence and fatalities have also been connected to other lower impact activities in recreational waters, such as bathing in geothermal waters (Booth et al., 2015; Su et al., 2013; Moussa et al., 2013). The median age for *Naegleria fowleri* infections is 14 years old (ranging from 1-month old to 85 years old) with 75% of cases being male and 25% female (Gharepure et al., 2021a).

In 2008, a fatality from *Naegleria fowleri* infection occurred in recreational waters in Guadeloupe (French West Indies) where the *Naegleria fowleri* concentration was noted to be "rather low" at 0 to 22 cells/L, which is below the 100 amoebae/L standard set by the public health ministry of France (Moussa et al., 2013).

Burkholderia pseudomallei

Burkholderia pseudomallei is a soil and water bacterium that is mainly found in tropical regions, especially northern Australia (Kaestli et al., 2016). Weather conditions are linked to an increased in presence of *Burkholderia pseudomallei* in the broader environment i.e. soils, air, and groundwater seeps (Kaestli et al., 2016; Kaestli et al., 2019; Hsueh et at., 2018).

Burkholderia pseudomallei can infect humans through skin wounds, inhalation, or eye contact and can cause a chronic and potentially fatal infection called melioidosis. Infection with *Burkholderia pseudomallei* is more common in people with underlying medical conditions, such as diabetes, alcoholism, or chronic renal disease (Inglis and Sousa, 2009). Few cases of *Burkholderia pseudomallei* infections have been linked to recreational water exposure, with most infections occurring during non-recreational water activity. In Australia, the northern indigenous population is noted to account for 30% of *Burkholderia pseudomallei* infections but are 67% of the cases presenting at the ICU (Stephens et al., 2016). The mortality rate due to *Burkholderia pseudomallei* infections is about 14% in northern Australia, with a rising number of cases globally (Kaestli et al., 2016). Two fatal cases of *Burkholderia pseudomallei* infections associated with

recreational water activity (i.e. swimming) were reported in Mexico (Alvarez-Hernandez et al., 2021) and a third case linked to swimming resulted in ocular melioidosis in Malaysia (Shariff et al., 2020).

8.2 Secondary research questions

- What are the indicators/surrogates of this/these risk/s?

Indicators/surrogates of *Naegleria fowleri*

The abiotic indicators/surrogates of *Naegleria fowleri* risks are elevated water temperatures during summer/autumn months when 85% of cases occurred, which corresponds to *Naegleria fowleri* being a thermophilic organism and also coincides with increased recreational activity (Gharepure et al., 2021a). However, *Naegleria fowleri* has been detected in recreational water bodies with a wide water temperature range (16-47 °C) (Stahl and Olson, 2021) and during winter months (Sifuentes et al., 2014). The increase in summer/autumn PAM cases likely has something to do with increased human activity in recreational waters. *Naegleria fowleri* is noted to remain viable in moderately saline conditions (0-1.4% NaCl for 48 h), across a broad pH range (3-11 for 48 h) and temperatures up to 48 °C for 48 h (Lam et al., 2019). The salinity for brackish water is listed as 0.05-3% and seawater is 3.5%. Biotic indicators/surrogates noted to support *Naegleria fowleri* growth include concentrations of bacterial food (> 10^4 bacteria per amoeba) (Goudot et al 2012), microbial community composition (Morgan et al., 2016; Puzon et al., 2017) and preferential microbial food sources (Miller et al., 2018). The Goudot et al (2012) study was conducted in a laboratory, while Morgan et al (2016), Puzon et al (2017) and Miller et al (2018) were all conducted in drinking water distribution systems which lacked a detectable chlorine residual. It is noted that measurements of most of the abiotic and biotic indicators/surrogates are lacking from confirmed *Naegleria fowleri* cases linked to recreational waters/activities and no studies of the microbial community composition, bacterial food source or concentrations in relation to *Naegleria fowleri*'s presence in recreation waters have been reported.

Indicators/surrogates of *Burkholderia pseudomallei*

The potential abiotic indicators or surrogates of *Burkholderia pseudomallei* increased risk are weather-related (increased dew point, cloud cover, rainfall, and maximum temperature) and groundwater (Kaestli et al., 2016), as well as potential increases due to climate change-driven extreme weather events (Inglis and Sousa, 2009). A positive association between *Burkholderia pseudomallei*, low organic carbon and elevated total iron levels has been found in bore water (Kaestli et al., 2019). No biotic indicators/surrogates of *Burkholderia pseudomallei* increased risk have been reported.

- What is the frequency of occurrence of identified health outcomes in Australia? Is there an association with exposure to recreational waters?

Frequency of health outcomes associated with *Naegleria fowleri* in Australia

The frequency of occurrence of identified health outcomes of *Naegleria fowleri* infections in Australia is rare with three fatalities linked to bore water on rural properties in Queensland since 2002 (Nicholls et al., 2016). The historical Australian cases are linked to water piped above ground over long distances and with the most recent cases being associated with rural properties where children had the opportunity to play with the water via hoses and bathing (Nicholls et al., 2016). While there has been no association with exposure to recreational waters in Australia during the time review timeframes, there has been multiple

infections and deaths globally (Gharpure et al., 2021a) with an indication that climate change maybe be expanding the geographical range of *Naegleria fowleri* infections (Gharpure et al., 2021a; Gharpure et al., 2021b).

Frequency of health outcomes associated with *Burkholderia pseudomallei* in Australia

Although multiple identified health outcomes ranging from minor infections to fatalities are known to be associated with *Burkholderia pseudomallei*, there have been no *Burkholderia pseudomallei* infections associated with recreational water exposure recorded in Australia. *Burkholderia pseudomallei* is known to be present throughout Northern Australia (Inglis et al., 2009, Kaestli et al., 2016; Kaestli et al., 2019), but only two fatal cases related to swimming in Mexico (Alvarez-Hernandez et al., 2021) and a third ocular infection case related to swimming in Malaysia (Shariff et al., 2020) have been reported.

- What is known about the occurrence of these organisms in natural waters in Australia?

Occurrence of *Naegleria fowleri* in natural waters in Australia

There is no information published within the review time period on the understanding of the presence/occurrence of *Naegleria fowleri* in the natural waters (e.g. lakes, rivers, ponds, and ski parks) in Australia used for recreational purposes. The majority of historical detections and fatalities have been associated with water piped overland (Morgan et al., 2016; Nicholls et al., 2016; Puzon et al., 2017). *Naegleria fowleri* is not found in saltwater.

Occurrence of *Burkholderia pseudomallei* in natural waters in Australia

In Australia, *Burkholderia pseudomallei* is known to part of the natural environment and has been detected in soils, rural water supplies, groundwater and groundwater seeps (Baker and Warner, 2016; Baker et al., 2011; Draper et al., 2010; Foong et al., 2014; Inglis et al., 2004; Inglis and Sousa, 2009; Kaestli et al., 2016; Kaestli et al., 2019).

- What are the conditions associated with increased occurrence? What are the conditions associated with absence of these microorganisms?

What conditions increase or decrease *Naegleria fowleri* occurrence?

Naegleria fowleri can be found in the environment year-round (Sifuentes et al., 2014; Stahl and Olson, 2021) but are more abundant on a seasonal basis and increase with warmer temperatures (Puzon et al., 2017; Stahl and Olson, 2021; Yoder et al 2010). Fatal *Naegleria fowleri* cases have occurred in recreational waters with reported water temperatures between 22 °C (Kemble et al., 2012) and >30°C (Moussa et al., 2013). The corresponding need for ample concentrations of bacterial food (Goudot et al., 2012) along with microbial abundance/composition (Morgan et al., 2016) and food sources (Miller et al., 2018) are also linked with the increased presence/abundance of *Naegleria fowleri*. Climate change is indicated as expanding the range of *Naegleria fowleri* infections (Gharpure et al., 2021a). The majority of *Naegleria fowleri* fatalities globally are linked to warmer temperatures and increased recreational water activity (Gharpure et al., 2021a). High salt concentrations, such as those in sea water, prevent *Naegleria fowleri* growth (Lam et al., 2019).

What conditions increase or decrease *Burkholderia pseudomallei* occurrence?

Burkholderia pseudomallei is known to increase in presence and abundance with weather conditions (increased dew point, cloud cover, rainfall, and maximum temperature) (Kaestli et al., 2016) with a potential increase due to climate change (Inglis and Sousa, 2009).

- What is known about the exposure pathway for each organism?

Exposure pathway for *Naegleria fowleri*

The exposure pathway for *Naegleria fowleri* is through inhalation of the organism into the nasal passages (Gharpure et al., 2021a; Gharpure et al., 2021b). Ruptured eardrums have also been listed as a potential pathway.

Exposure pathway for *Burkholderia pseudomallei*

The exposure pathway for *Burkholderia pseudomallei* is skin cuts and abrasions, inhalation, through the eyes (Inglis et al., 2009; Kaestli et al., 2016; Kaestli et al., 2019; Shariff et al., 2020). Adverse outcomes are noted to be connected to poor overall health of individuals, such as excess alcohol dependency, diabetes, or chronic renal disease (Inglis and Sousa, 2009).

- What is known about the dose-response for each organism?

Dose response level for *Naegleria fowleri*

For swimming mice, 13,257 *Naegleria fowleri* cells were predicted to have a lethal dose in 50% of the mice, whereas intranasal inoculation of 1000 *Naegleria fowleri* cells resulted in the death of 70% of the male mice (Dean et al., 2019). For humans little is known about the dose-response level for *Naegleria fowleri*. The public health ministry of France has published a safe recreation level of 100 organisms per litre, however fatalities have occurred in geothermal waters where *Naegleria fowleri* concentrations were significantly below this (0-22 organisms/L) (Moussa et al., 2013). The Australian Drinking water guidelines list 2 *Naegleria fowleri* organisms per litre as a risk (NHMRC, 2011).

Dose response level for *Burkholderia pseudomallei*

No dose-response levels are currently listed for *Burkholderia pseudomallei.*

- What are the current practices to minimise or manage this/these risk/s?

Risk minimisation of *Naegleria fowleri* infections

Current practices for *Naegleria fowleri* infections in recreational waters include:

(1) avoiding water-related activities in bodies of warm freshwater such as hot springs and thermally polluted waters;

(2) avoiding water-related activities in bodies of warm freshwater during periods of high water temperature and low water volume;

(3) using nose clips or holding nose closed while taking part in water-related activities; and

(4) avoiding digging up or disturbing sediment while taking part in water related activities (Yoder et al., 2010).

Routine testing of water bodies used for recreational purposes and signage of associated risks occurs in some areas of Western Australia but not nationally.

Risk minimisation of *Burkholderia pseudomallei* infections

For *Burkholderia pseudomallei* no risk minimisation practices are currently listed but knowledge of endemic areas and conditions which enhance risk of infection are known (Inglis et al., 2004; Kaestli et al., 2016).

8.3 Deviations from protocol

The term “water” was added as an Exposure Term in the literature search keywords (Technical report Table 2.3).

8.4 Research needs

A national assessment of the presence and abundance of *Naegleria fowleri* and *Burkholderia pseudomallei* in recreational waters should be conducted. Assessment of the biotic and abiotic factors which are associated with the presence of *Naegleria fowleri* and *Burkholderia pseudomallei* in recreational waters should be conducted. In addition, research to develop updated dose response and quantitative microbial risk assessments (QMRA) for humans for both *Naegleria fowleri* and *Burkholderia pseudomallei* are needed to enable better management to prevent or reduce infection.

8.5 Conclusions

Risk from *Naegleria fowleri* is present in Australia but the extent is not well understood. The seriousness of this risk derives from the fact that *Naegleria fowleri* infection almost always results in death. Risk of *Burkholderia pseudomallei* is present in Northern Australia. Links between *Burkholderia pseudomallei* and recreational water, are not well documented, but the potential for outdoor recreational interactions cannot be ruled out.

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