SAAFE CRC

WHITE PAPER ON

Integrating microbial endpoints, including those related to antimicrobial resistance (AMR), into Australian and New Zealand guideline values for fresh and marine water quality

Australian Government
Department of Climate Change, Energy, the Environment and Water

Australian & New Zealand GUIDELINES FOR FRESH & MARINE WATER QUALITY

CSIRO

SETAC

Water Research AUSTRALIA



Purpose and Guide to the White Paper

This white paper explains why microbial endpoints, including those relevant to antimicrobial resistance, should be incorporated into default guideline values (DVGs) for toxicants within the Australian and New Zealand guidelines for fresh and marine water quality (ANZG).

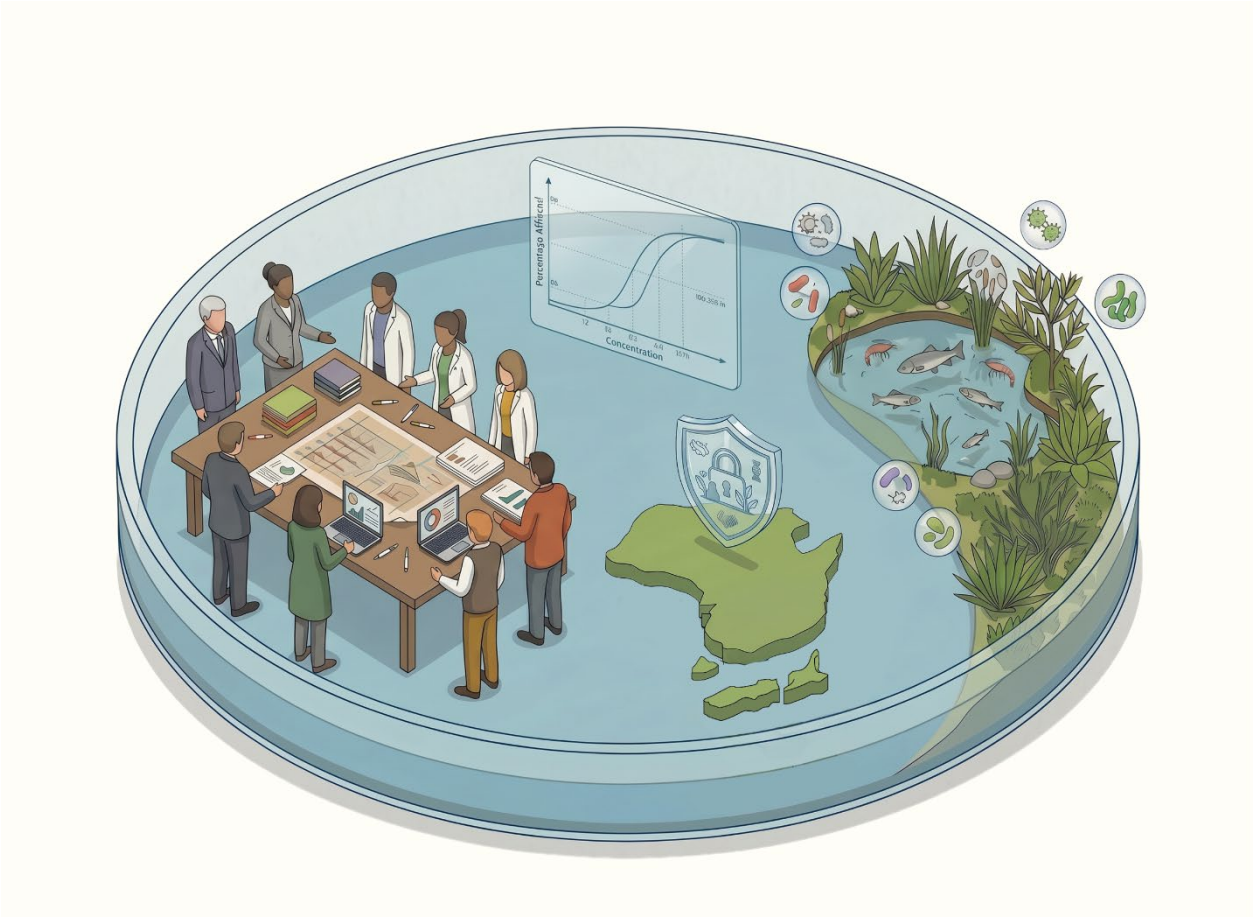
Drawing on syntheses from targeted workshops and conference sessions in Australia and New Zealand, it guides readers through the evidence, gaps, and actions needed to enable routine and defensible use of microbial endpoints when deriving guideline values.

The paper first proposes a co-created supplementary definition for “ecologically relevant microbial endpoints” to address a key gap in current ANZG guidance.

It then critically appraises priority endpoint categories, from single species assays to community function assays, genomics and multi-omics approaches.

Finally, the paper presents a gap analysis and a staged Action Plan and Roadmap (2026–2032) to guide method standardisation, governance, data stewardship, and regulatory readiness.

Together, these recommendations support more ecosystem-representative guideline values for toxicants in waters and sediments to strengthen One Health[1] protection outcomes.



[1] One Health is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals and ecosystems. It recognises the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and inter-dependent. The approach mobilises multiple sectors, disciplines and communities at varying levels of society to work together to foster well-being and tackle threats to health and ecosystems, while addressing the collective need for clean water, energy and air, safe and nutritious food, taking action on climate change, and contributing to sustainable development (One Health High Level Expert Panel, 2023)

Research & Funding Acknowledgements

We would like to formally acknowledge the 36 workshop participants whose contributions were crucial to developing this white paper. Some have chosen not to list their names or affiliations, but we thank all equally.

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The views expressed in this paper are the views of the paper's authors or participants, as individuals, and not the views of the organisations they work for.

Official sponsors of workshops and conference sessions used to inform this white paper included DCCEEW, SAAFE^{CRC} and AIMS. The workshops and conference sessions were co-convened by CSIRO Minimising AMR Mission, SAAFE^{CRC} and the Society for Environmental Toxicology and Chemistry Australasian Chapter (SETAC AU).

This work was supported by SAAFE^{CRC} Kickstart Project K023, which received contributions from DCCEEW and SAAFE^{CRC}. SAAFE^{CRC} receives funding from the Australian Commonwealth government through the Cooperative Research Centre program along with partner organisations. This is SAAFE^{CRC} Approval number 3_K023_PB001.

Water Research Australia (WaterRA) provided administrative support, including publication and production of this white paper. Some member organisations of WaterRA were also represented within the project team and workshop participants.

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Suggested citation

Binet, M.T., Harford, A.J., Donner, E., Hayward, C., Ashbolt, N., Chariton, A., Luter, H., Thomas, M., Negri, A., Stauber, J.L., van Dam, R., Batley, G.E., Weaver, L., Pantos, O., Fisher, R., Adams, M.S., King C.K., Saaristo, M., Lepesteur, M., & Kumar, A. (2026). White paper on integrating microbial endpoints, including those related to antimicrobial resistance (AMR), into Australian and New Zealand guideline values for fresh and marine water quality. Prepared for the Department of Climate Change, Energy, The Environment and Water, SAAFE^{CRC} Publication number 3_K023_PB001, 85 pp.

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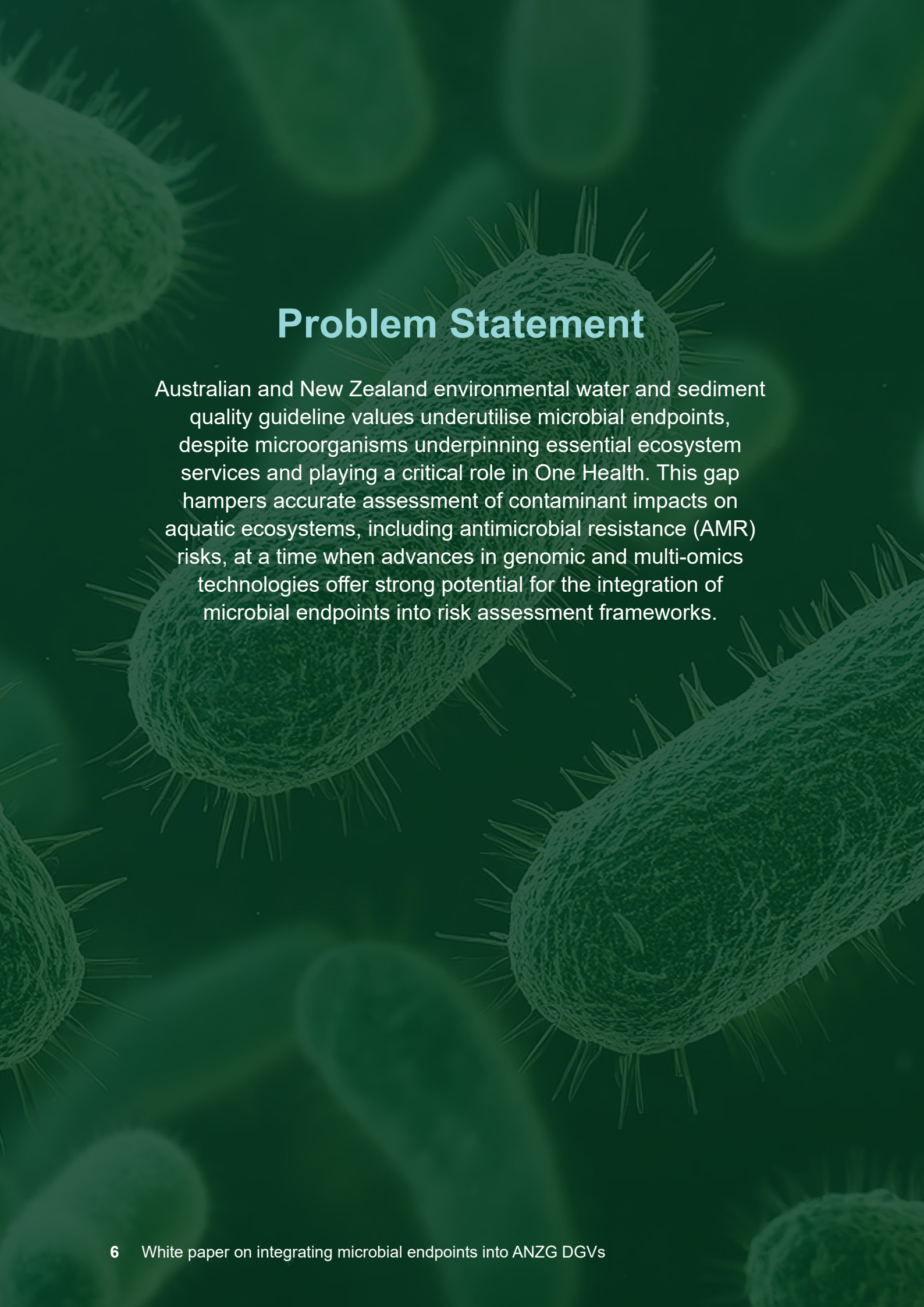
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Problem Statement

Australian and New Zealand environmental water and sediment quality guideline values underutilise microbial endpoints, despite microorganisms underpinning essential ecosystem services and playing a critical role in One Health. This gap hampers accurate assessment of contaminant impacts on aquatic ecosystems, including antimicrobial resistance (AMR) risks, at a time when advances in genomic and multi-omics technologies offer strong potential for the integration of microbial endpoints into risk assessment frameworks.

Why integrating microbial endpoints now matters

Current Australian and New Zealand environmental water and sediment quality guideline values may not protect key ecosystem processes (nutrient cycling, disease regulation) that sit outside species level endpoints.

Microbial communities are often the most sensitive indicators of chemical stress, providing earlier warning than higher trophic levels.

AMR represents a distinct, escalating environmental, animal and public health risk not captured by existing guideline frameworks.

Advances in genomics and quantitative microbiome methods mean regulatory ready microbial data are now achievable.

Without clear guidance, regulators risk inconsistent or ad hoc use of microbial evidence.



Executive Summary

Overview

Aquatic ecosystem resilience and adaptive capacity are intrinsically linked to the compositional and functional dynamics of their associated microbiomes. However, current environmental water and sediment quality guidelines for toxicants predominantly focus on protecting organisms at higher trophic levels, with limited consideration of microbial communities. This white paper outlines why and how microbial endpoints, including those relevant to antimicrobial resistance (AMR), should be integrated into deriving environmental water and sediment quality default guideline values (DGVs) for toxicants in Australia and New Zealand (ANZ). It synthesises outputs from targeted conference sessions and expert workshops convened in 2025-2026 to identify barriers and enablers for incorporating microbial data into DGV under the Australian and New Zealand Guidelines (ANZG) for Fresh and Marine Water Quality (ANZG, 2018). The focus is on communities of environmental microbes (prokaryotic and eukaryotic microorganisms) as critical drivers of ecosystem services and as a key component of a One Health approach.

This white paper is intended to support regulators, guideline developers, consultants, and researchers in ANZ involved in derivation and/or application of DGVs, site-specific guideline values (SSGVs) for toxicants and direct toxicity assessments in waters and sediments. It consolidates findings into an action plan and roadmap centred around four overarching objectives, with fifteen actions to guide a staged implementation from 2026 to 2032.



In doing so, the white paper delivers four key outcomes that begin to address the problem statement:

1. **A new, co-created supplementary definition** of ‘ecologically relevant microbial endpoints’, tailored specifically for microbes and recognised as a gap in the current ANZG guidance (Warne et al., 2025).
2. **A critical appraisal of five categories of microbial endpoints:** cell-based assays; community functional endpoints; amplicon-based methods; metagenomic/meta transcriptomic approaches; and other omic/multi-omics. Each category was evaluated based on their ecological relevance, regulatory utility, and methodological readiness.
3. **Gap analysis** mapping identified barriers and enablers against current pathways for integrating microbial data into ANZG DGVs.
4. **An Action Plan and Roadmap** structured around four overarching objectives and fifteen key actions to guide staged implementation between 2026 and 2032.

This synthesis draws upon: two curated sessions at the joint conference for the Society for Environmental Toxicology and Chemistry Australasian Chapter (SETAC AU) and Australasian College of Toxicology and Risk Assessment (ACTRA) in Wellington, New Zealand (28 August 2025); an invitation-only workshop in Wellington, New Zealand (29 August 2025); and follow-on online workshops and surveys (October 2025- March 2026). The workshops included 36 participants spanning regulators, scientists, consultants and advisers, who contributed via facilitated discussions, Mentimeter polling, and scribed notes. Outputs were subject to thematic analysis to develop this white paper.

Key findings

What must change to enable better use of microbial endpoints

Ecological relevance must be redefined for microbes within ANZG guidance documents.

The current ANZG definition (Warne et al., 2025) of “ecologically relevant endpoints” for the derivation of DGVs is centred on species-level competitiveness and is only partially applicable to microbes. Participants overwhelmingly supported a supplementary definition tailored to microbiomes that recognises community structure, function and activity as the appropriate units of assessment, including holobiont contexts (host associated microbiomes). A new definition was co-created and is presented in this white paper.

Community and functional endpoints are most relevant, but difficult to operationalise.

Whole-community functional endpoints (e.g., respiration, nutrient cycling) align with ecosystem protection goals [2] but are constrained by context dependence and limited agreement on regulatorily defensible effect thresholds. Under current guidance, they are most readily used as supporting evidence within weight of evidence approaches, particularly for site-specific guideline values (SSGVs), but pathways for their routine contribution to DGV derivation remain unclear.

Sequencing-based endpoints are promising if standardised and quantitative.

Amplicon sequencing can detect community shifts across thousands of taxa. Regulatory application improves substantially when methods incorporate viability assessment and move from relative abundance to quantitative profiling (absolute cell/gene loads), enabling derivation of taxon-specific concentration-response relationships that can be aggregated into microbial sensitivity distributions. Metagenomic and meta-transcriptomic approaches provide mechanistic insights (functional potential, expression, resistomes) but currently lack validated, transferable effect thresholds and consistent quality assurance/quality control (QA/QC) expectations for regulatory decision making. Effective implementation will require agreed data governance, access and stewardship arrangements to enable reuse, transparency and long-term availability of microbial and AMR datasets used in DGV derivation.

[2] Ecosystem protection goals define what ecological components and functions are to be protected and the acceptable level of change, guiding endpoint selection. For microbiomes, which operate as highly dynamic, functionally redundant communities embedded within larger complex biological and environmental systems, impacts are more appropriately assessed at the community and functional level rather than through the loss of individual species.

AMR represents a distinct risk dimension.

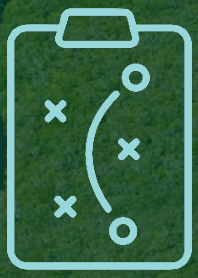
AMR-associated risk cannot be inferred from conventional single species toxicity endpoints or community functional endpoints alone. Metagenomic interrogation of resistance genes and mobile genetic elements is currently the most defensible direct approach for AMR hazard characterisation in environmental systems, but requires agreed endpoints, effect thresholds, and a clear decision context for how AMR information should influence DGV derivation and risk assessment.

Framework misfit is a core barrier.

The dominant DGV estimation method, the species sensitivity distribution (SSD), focuses on effects at the species level. This creates a misalignment between: (i) what is most ecologically relevant for microbes (community/function measures); and (ii) what is most readily incorporated into DGV derivation (single-species endpoints). Current rules limiting the combination of different taxonomic levels (e.g. species, family, community) in a single distribution, enforced to ensure interpretability, risk marginalising microbial evidence to “supporting” status, even when high quality data are available. Alternative metrics and approaches are needed.

Practical constraints matter.

Key implementation barriers include: limited method standardisation and accreditation; disagreement on the “chronic” exposure definition for microbes (the current >24 h convention was challenged); difficulty verifying exposure concentrations in small volume microbial assays at trace levels; and uneven regulatory appetite and awareness, including inconsistent prioritisation of AMR across jurisdictions.



The **Action Plan** and **Roadmap** propose a staged pathway

2026–2027
Establish governance, co-design and expert coordination to oversee and implement the action plan

2027-2031
Targeted research and guidance development

2031-2032
Delivery of endorsed guidance and microbial-inclusive DGV submissions

2030-2032
Capacity building activities for end-users



Key deliverables include

Guidance Briefs (2027 onward)

Microbial guidance addendum (planned 2031)

Formal guidance update (planned 2032)
alongside
Draft default toxicant DGV technical briefs for priority toxicants that incorporate microbial endpoints (planned 2032)

Research publications throughout

An advisory committee should be established early to prioritise the most critical actions within available funding, and to identify collaborations and additional funding sources to support the remaining actions.

Key Actions intended to support a staged transition of microbial endpoints from research approaches to regulatory ready application, with clear decision points and documentation requirements:

1 **Establish governance, oversight and delivery mechanisms.** Form a transdisciplinary expert advisory group to guide method development, interpretation and implementation, and to oversee roadmap delivery. This should include an expression of interest process, clear terms of reference, and quarterly reporting. Develop engagement, funding and communication plans to support implementation.

2 **Define ecosystem protection goals and ecological relevance for microbes.** Define and document ecosystem protection goals that explicitly incorporate microbial community and functional endpoints. Develop a clear and an explicit statement on the ecological relevance of microbial endpoints, and how they support ecosystem protection goals in DGV derivation to support future guidance updates.

3 **Define acceptable microbial endpoints and regulatory-ready methods.** Clarify which whole community and functional endpoints are acceptable for DGV derivation. Develop QA/QC and metadata standards for genomic and omics-based endpoints including access to bioinformatic workflows. Clearly define the regulatory applicability of amplicon sequencing, metagenomic and meta-transcriptomic approaches, including their roles as primary or supporting lines of evidence.

4 **Close key technical gaps for assay defensibility.** Revisit whether the microbial chronic exposure timeframe definition of >24 h remains appropriate or should be extended to better capture longer-term microbial responses. Develop and standardise affordable exposure verification approaches for small volume and trace level assays. Apply conceptual ecological models and adverse outcome pathways (AOP) to inform meaningful thresholds for microbial community-level and functional endpoints.

5 **Clarify and test microbial data integration pathways for DGVs.** Clarify current rules for integrating microbial data and combining evidence across taxonomic levels. Develop practical guidance applying SSD approaches to large, high-resolution community and omics datasets (e.g., weighting and aggregation). Conduct worked case studies on priority toxicants to assess the impact of microbial data inclusion on DGVs considering several integration approaches including separate microbial sensitivity distributions, combined approaches, and weight of evidence or multiple lines of evidence methods.

6 **Advance AMR endpoint development and application.** Define the policy framing and decision context for AMR endpoints in toxicant DGV derivation, with a clear focus on chemical drivers of AMR. This action does not seek to derive guideline values or conduct risk assessments for biological AMR determinants (e.g., antimicrobial resistant pathogens, commensals, and associated resistance genes), although methodological and evidence overlaps will likely inform both areas. Identify priority AMR-relevant microbial endpoints. Establish and validate assays and thresholds (particularly metagenomic approaches), including targeted datasets for priority contaminants including non-antimicrobial co-selectors.

7 **Capacity building for end-users.** Develop targeted capacity building training to enhance regulators' ability to integrate microbial endpoints into risk assessments and apply emerging knowledge in regulatory decision-making. Work with regulators to build awareness and understanding of new analytical technologies and the significance of results obtained.

Scope boundaries and intent

This white paper does not address other water quality guidelines within the National Water Quality Management Strategy (e.g., drinking, recreational, or recycled water). However, as AMR knowledge advances, coordinated engagement across responsible agencies will help align water quality guidelines under a One Health approach for environmental, animal and human protection.

This paper builds on (and does not replace) the ANZG (2018)/Warne et al. (2025) approach for deriving DGVs to protect aquatic ecosystems. Species level ecotoxicology and SSD methods remain essential for consistent guideline derivation across jurisdictions. Accordingly, toxicant DGVs derived using the current approach remain valid and applicable for use in water quality assessments. The paper also notes that endpoints used for higher organisms have recognised limitations in ecological relevance, and that some Action Plan outcomes, particularly for community based endpoints, may be applicable beyond microbial communities. Until more ecologically relevant microbial endpoints and datasets are available, this white paper does not recommend removing microbial endpoints currently used in DGVs.

The paper does not suggest or mandate universal use of metagenomics or multi-omics is not suggested or mandated in this white paper. These are presented as targeted, fit for purpose tools where they add clear ecological or regulatory value.

AMR is recognised as a distinct, evolving risk dimension, but the paper does not attempt to define all pathways, thresholds or regulatory responses at this stage. The focus is on preventing AMR driven by anthropogenic chemicals in the environment, with priorities identified for staged research, co design and guidance development.

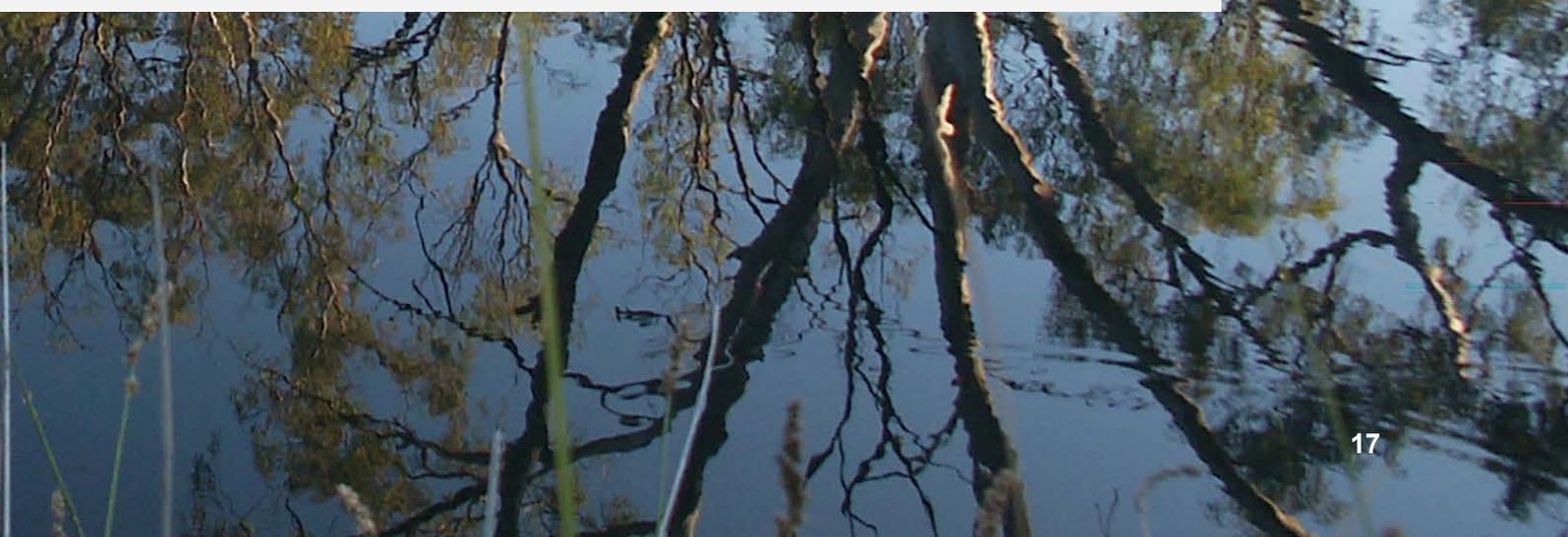
While this white paper primarily focuses on methodology for water quality guideline value derivation, these approaches are also applicable to sediment quality assessment and sediment quality guideline values when sediment-specific considerations such as toxicant bioavailability and exposure pathways are incorporated. Accordingly, the paper also considers the role of microbial endpoints in sediment quality guideline derivation, given the importance of sediment microbial communities in ecosystem function and contaminant cycling. Applying these findings to strengthen microbial endpoints for sediment quality assessments is a key next step, highlighted in the Action Plan. Likewise, many of the concepts discussed are also relevant to groundwater ecosystems, where microbial communities support critical biogeochemical processes and may be vulnerable to toxicant exposure, although groundwater-specific frameworks are not considered in detail. Finally, the overall scope and intent of this paper is consistent with the continual improvement philosophy espoused by ANZG (2018).



Outcome

This white paper establishes a **foundation for integrating microbial endpoints into ANZG DGV derivation**, supporting the development of more robust and comprehensive regulatory guideline values and associated guidance documents. Implementation of the proposed roadmap will enable DGVs to more accurately represent whole ecosystem hazards, including those at the microbial community level, which underpin key ecosystem services. The improved ability to incorporate whole-community endpoints, particularly those at the functional level, extends beyond microbial systems to benefit better use of all available taxonomic-level data in deriving DGVs. Ultimately, implementation of the action plan will enable routine, defensible inclusion of microbial endpoints (including AMR-relevant indicators) in DGVs, SSGVs and direct toxicity assessments (DTAs), strengthening environmental risk assessment, including wider **One Health outcomes and improving protection of aquatic ecosystems**.





Background

Why include microbial endpoints?



The Australian and New Zealand (ANZ) Guidelines for Marine and Fresh Water Quality (ANZG, 2018; referred to herein as ANZG) provide best-practice guidance for assessing, monitoring and managing water and sediment quality and overall aquatic ecosystem health, which can be used for environmental risk assessments (ERAs) and other purposes. They underpin regulatory decision-making at jurisdictional and national scales. ANZG recommends a weight-of-evidence (WoE) approach to water and sediment quality assessment and provides default guideline values (DGVs) for toxicants that can be used as one line of evidence within this approach. ANZG also distinguishes between DGVs and site-specific guideline values (SSGVs), recommending the latter, where possible. However, in practice DGVs are often the only line of evidence available, resulting in a strong reliance on DGVs to inform potential environmental risk or impact. Consequently, there is an incentive to ensure that DGVs are as robust and environmentally representative as possible. This White Paper focuses on methods for deriving DGVs but is also applicable to those for SSGVs (SSGVs are also considered further in “The parking lot”, (page 64).

Although the current method for deriving DGVs for toxicants (Warne et al. 2025) is acknowledged as representing current best practice and is periodically refined (e.g., Batley et al., 2018; Warne et al., 2018; 2025) as scientific understanding advances, a major gap remains: the limited representation of microorganisms, or microbes in the data used to derive DGVs. Despite (i) the ability for species-level microbial data to be included in DGV derivation, and (ii) repeated international calls over more than a decade (Brandt et al., 2015; Hellal et al., 2023; Ford, 2025), microbial taxa, other than microalgae and cyanobacteria, remain significantly underrepresented. As a result, it is unknown if current DGVs adequately protect sensitive environmental microbiomes, that underpin essential ecosystem functions, resilience, and disease regulation in aquatic organisms.

Microorganisms, or microbes (herein defined as microscopic prokaryotes (bacteria and archaea) and eukaryotes (fungi and protists, but not animals or plants)) make up the vast majority of biomass in aquatic environments (Bar-On et al., 2018) and drive critical ecosystem services such as primary production, biogeochemical cycling, disease resilience, and contaminant degradation and mitigation (Figure 1). Microorganisms respond rapidly to natural and anthropogenic perturbations, highlighting the utility of microbial community dynamics as valuable indicators of ecosystem stress (Glasl et al., 2017). Anthropogenic contamination of soils, waters and sediments can alter microbial community structure and function resulting in a loss of ecosystem services and potentially promote the emergence and spread of antimicrobial resistance (AMR).

The environmental dimensions of AMR are recognised by the United Nations Environment Program (UNEP) as among the most pressing environmental challenges of our time (UNEP, 2023a). This reflects the dual role that environment plays as both a reservoir and transmission pathway for AMR, as well as the critical influence of environmental management on reducing AMR emergence and spread. Reducing pollution is therefore critical to the prevention of AMR (UNEP, 2023b). Beyond antimicrobials, a wide range of stressors, including metals, pesticides, plastics, biocides and pharmaceuticals can impose selective pressures that stimulate genetic level responses in microbes, which indirectly co-select for AMR (Murray et al., 2024; Gillieatt & Coleman, 2024; Ormsby & Quilliam, 2026). For example, microbes acquiring genetic level resistance mechanisms to copper, will co-select for resistance to antimicrobials. Aquatic environments receiving pollutant inputs, particularly from point sources such as wastewater and industrial discharges, or chemical use in aquatic industries (e.g., aquaculture, mining, shipping/transport) can consequently become localised AMR hotspots (Murray et al., 2024). Resistance genes in hotspots are elevated above background levels, thereby increasing opportunities for horizontal gene transfer. This facilitates the movement of AMR from environmental microbiota into commensal or pathogenic microbes, with the potential to cause untreatable infections in aquatic wildlife (including water birds), aquaculture species, and ultimately humans and other animals, posing particular risks for vulnerable and endangered species (WHA, 2026).

The emergence of AMR and formation of biogeochemically dysfunctional hotspots can thus impact all types of biota, with serious implications for human health and food security, reinforcing the need for a One Health approach within ERA frameworks. Despite this urgency, integrating microbial endpoints into ERAs for toxicants remains limited.

Internationally, comparable guideline valuation derivation frameworks in Europe, Canada and the United States remain largely centred on conventional aquatic toxicity data with microbial endpoints generally absent or treated only as supplementary information. Where microorganisms are formally considered, this is typically in a narrow context, such as European PNECs for sewage treatment plant function (ECHA, 2023), rather than through natural aquatic or sediment microbial community endpoints that capture microbial function or antimicrobial resistance selection. However, progress has been made recently, with the World Health Organisation (WHO) and the AMR Industry Alliance (AMRIA) incorporating bacterial endpoints into the derivation of predicted no effect concentrations (PNECs) to support environmental (toxicity) and human (AMR) wastewater risk assessments (WHO, 2024; AMRIA, 2025). While these approaches are not directly transferable to the ANZ context due to differences in methodology, datasets and species (Binet et al., 2025), they represent an important first step in integrating microbial data into guideline values.

Given the importance of microbial communities in ecosystem health and function, this white paper examines why microbial endpoints remain underutilised DGV derivation in ANZG and identifies practical steps to address current barriers. It synthesises current initiatives, highlights opportunities to leverage existing methods and datasets, and draws on expert input spanning guideline development, environmental microbiology, AMR and ecotoxicology. These insights inform a proposed roadmap for incorporating microbial response data into future DGV derivations.

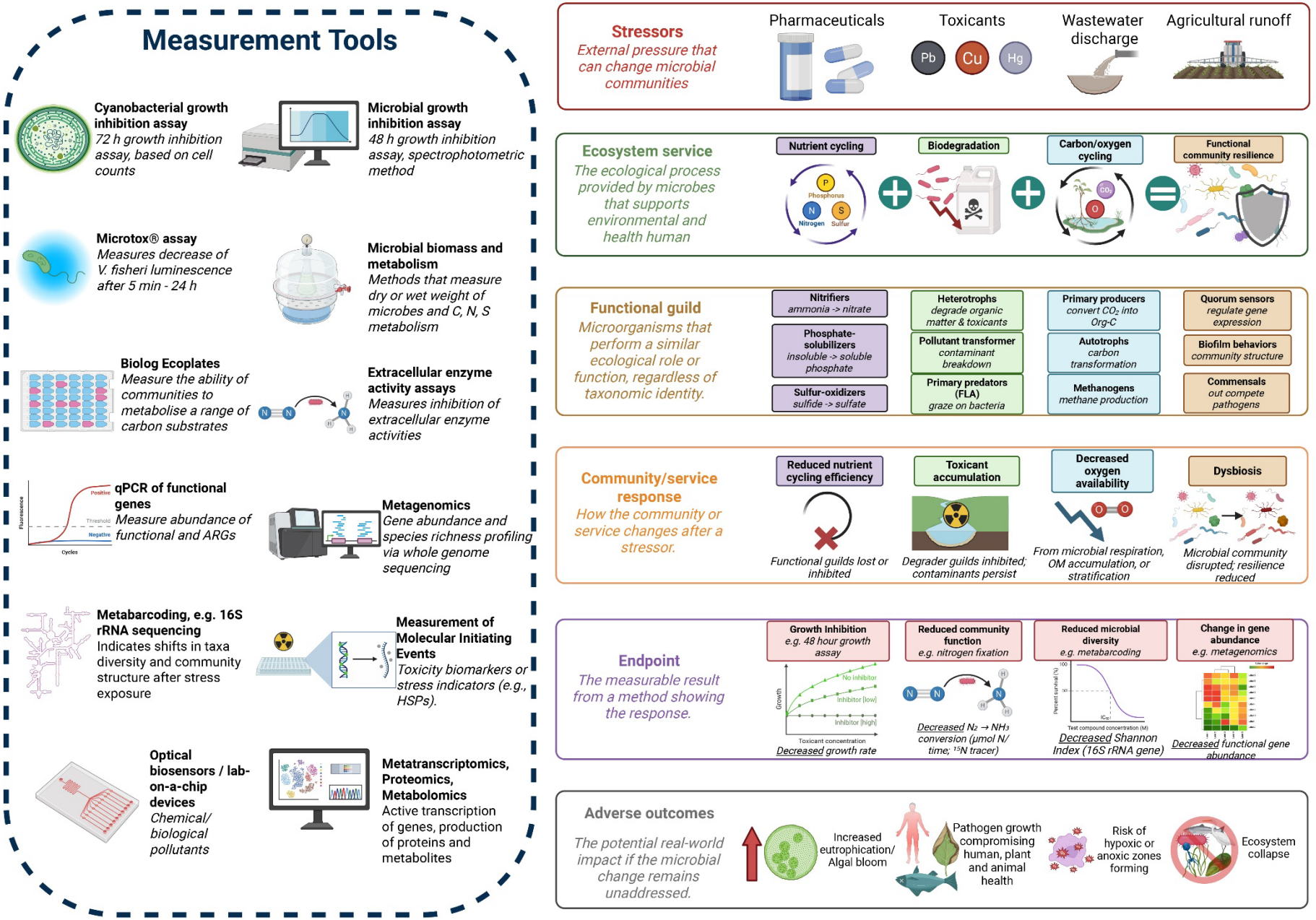


Figure 1. Toxicant (stressor)-microbe interactions in aquatic systems: some example pathways, assays and ecological endpoints



Sessions and Workshops

SETAC AU/ACTRA Joint Conference

As part of the joint SETAC AU and ACTRA conference held in Te Whanganui-a-Tara Wellington, Aotearoa New Zealand, the project team curated a two-part conference session and commenced a series of workshops addressing the topic of 'Identifying 'ecologically relevant endpoints' for microbial processes to inform Environmental Quality Guidelines and One Health approaches'.

Conference sessions

Sessions were held on 28 August 2025 and included ten platform presentations (abstracts provided in Appendix B) and a Q&A panel discussion.

Sessions were chaired by Ms Monique Binet (CSIRO), Prof Erica Donner (SAAFECRC), Dr Andrew Negri (AIMS), and Dr Marie Thomas (AIMS), and were sponsored by SAAFECRC and AIMS. The Q&A panel was facilitated by Monique Binet and Erica Donner, and featured panellists Dr Claire Hayward (SAAFECRC), Assoc Prof Barry Palmer (Massey University), Andrew Negri (AIMS), Dr Rick van Dam (WQadvice), and Marie Thomas (AIMS).

Workshops

An invitation-only workshop was held on 29 August 2025. Co-convened by SETAC AU and SAAFECRC, the workshop was supported through sponsorship from DCCEEW and was facilitated by Monique Binet (CSIRO) and Dr Andrew Harford (DCCEEW).

A follow-on online workshop was subsequently held on 31 October 2025, to enable more detailed exploration of key topics, facilitated by Andrew Harford (DCCEEW) and supported by Dr Heidi Luter (AIMS) and Monique Binet (CSIRO).

These workshops explored how the current ANZG toxicant DGV derivation methodologies (Warne et al., 2025) could be strengthened to better incorporate microbial endpoints into DGV derivation for toxicants.

Participants

The workshops brought together 36 participants comprising researchers, regulators, advisers, consultants and managers across key specific areas of expertise, including ecotoxicology, environmental microbiology, AMR, environmental management, policy, environmental chemistry, marine biology and statistical modelling, with many participants also having a range of additional areas of expertise (Figure 2).

The first workshop was delivered in hybrid format, with 11 online participants. Structured seating for in-person participants ensured a balanced mix of expertise across discussion groups. Each group was supported by an assigned facilitator and scribe. The second workshop was held entirely online as a single discussion group.

Materials and format

Participants received pre reading materials, including a workshop preparation guide and participant information sheet, outlining context, expectations, and information on privacy and consent. A survey was used to elicit expert feedback on the suitability of each endpoint that was identified through the workshops as candidates for use in hazard assessment. Ethical approval for engagement with workshop participants was granted by the CSIRO Human Research Ethics Committee (reference number 082/25).

Workshops combined presentations with facilitated discussions focused on key questions, with insights captured through Mentimeter and scribed notes.

Primary motivations for participants (Figure 2) were:

Learning, networking and contributing. Some participants took part primarily to learn and network and contribute where relevant, whereas others were participating specifically to represent key stakeholder groups.

Continual improvement of DGVs for ecological relevance. Some participants specifically supported the incorporation of microbial response data into the derivation of DGVs, while others wanted to focus specifically on how to incorporate AMR-relevant endpoints and their relevance to DGVs. Others were simply interested in participating and ensuring the ongoing robustness of the DGV approach.

Workshop presentations

The workshop opened with a series of keynote presentations from government, research and guideline experts. These included:

Opening address - DCCEEW - Dr Liz Barnett outlined DCCEEW's role in ANZ water quality management, highlighting the gap in microbial endpoints within current DGVs, and emphasised the importance of this workshop and their partnership with CSIRO and SAAFECRC in progressing that work.

Microbial Endpoints in Ecotoxicology – Ms Monique Binet provided an overview of the research presented at the preceding conference session, covering microbial community responses to mine-derived contaminants, the importance of microbial data in data-poor systems for SSGVs, the use of microbes as pollution bioindicators, a tropical bacterial bioassay, emerging quantitative thresholds for microbial responses, and the combined impacts of heat and pollution on microbial communities.

Microbial Endpoints for AMR – Dr Claire Hayward summarised key themes from the AMR-focused conference session, including AMR as a One Health challenge, the need for more ecologically meaningful microbial endpoints, the limitations of clinical MIC-based approaches, environmental drivers of AMR beyond antimicrobials, and a new framework for integrating AMR and ecotoxicity into water quality DGV development.

The ANZG Guidelines and its default guideline values (DGVs) – Dr Rick van Dam and Ms Yulia Cuthbertson presented an overview of the updated ANZG guidance (Warne et al., 2025) and described the broader governance context within the National Water Quality Management Strategy (NWQMS).

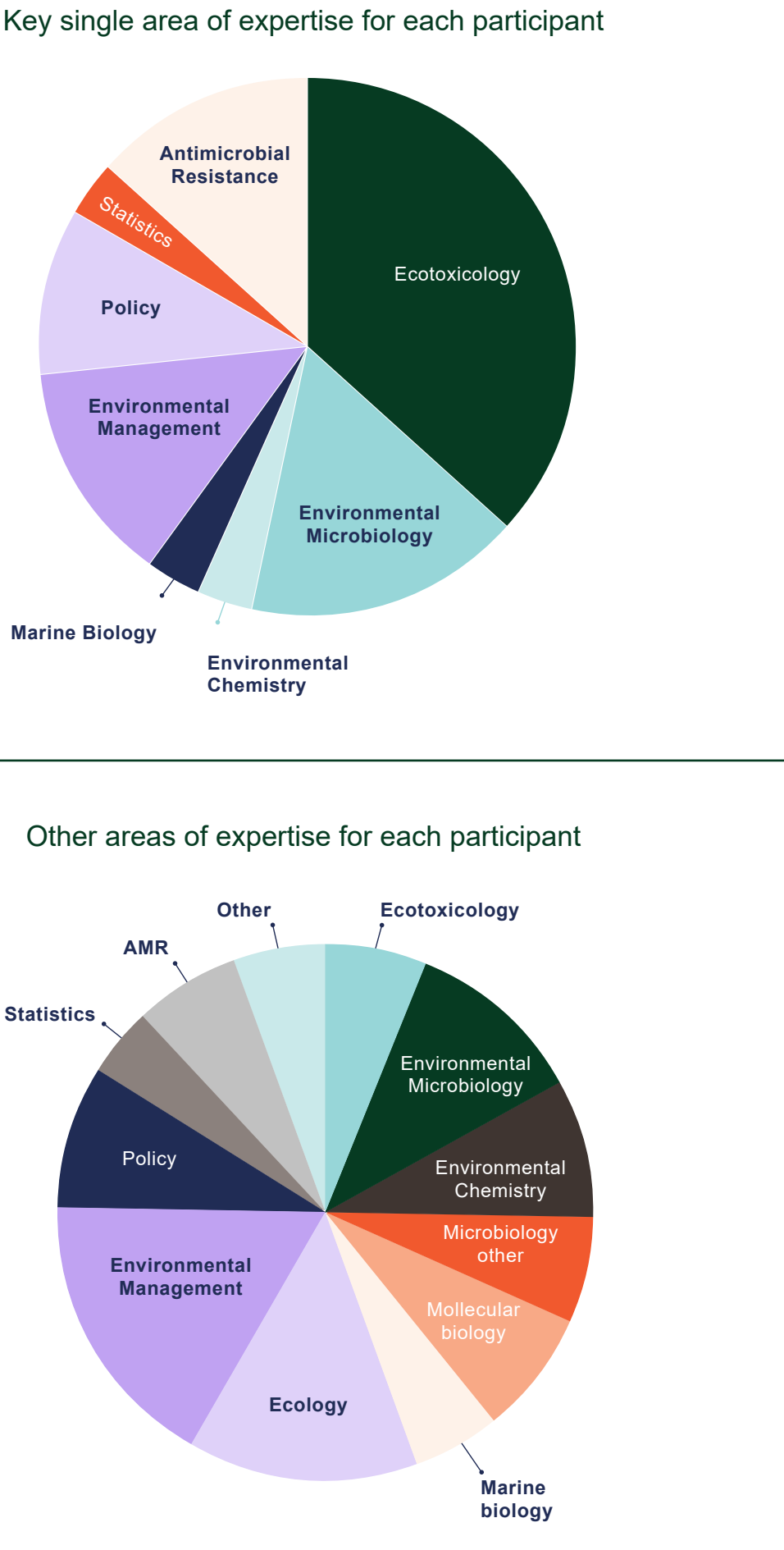
Implementing changes to pathogen monitoring guidelines – Prof Nicholas Ashbolt shared insights into efforts to influence the adoption of rapid qPCR methods for recreational water quality assessments in North America.

Thematic analyses and synthesis

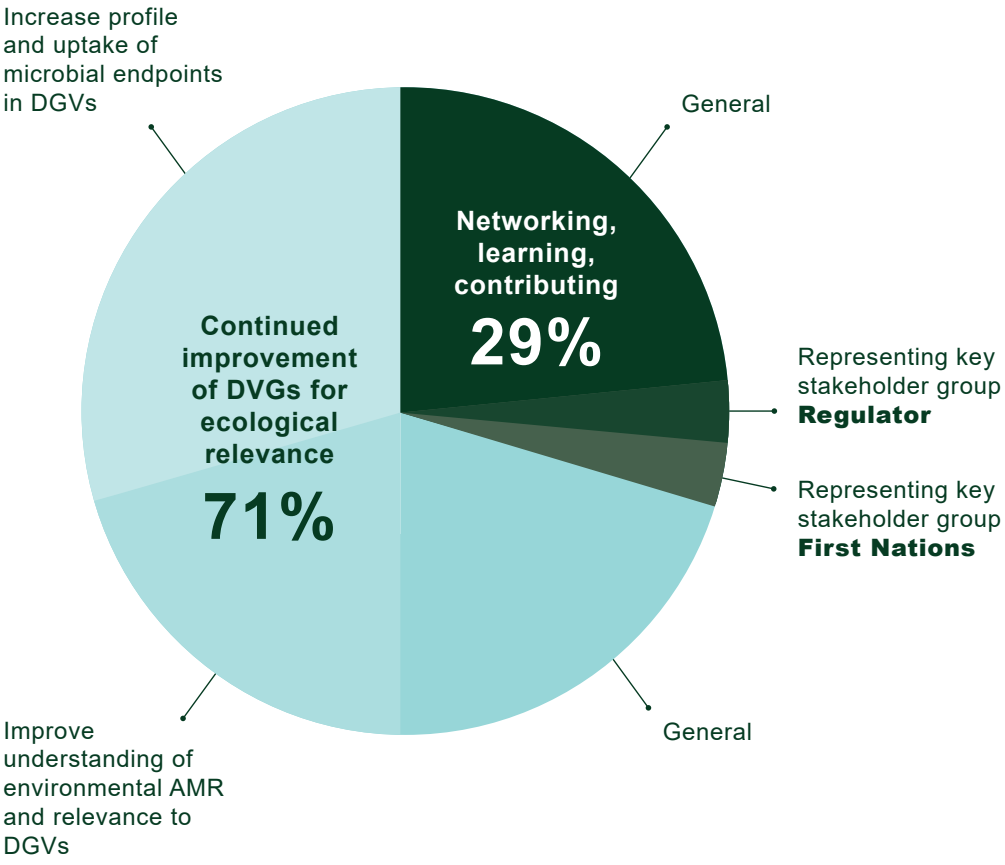
All workshop and conference inputs were collated and analysed thematically to identify key themes, barriers, enablers and knowledge gaps associated with incorporating microbial endpoints into ANZG DGVs. These insights informed a gap analysis of current pathways and underpinned the development of an action plan and roadmap for key research and regulatory priorities.

AI-assisted tools were used to support drafting, consolidation and synthesis of information during preparation of this white paper. However, thematic and gap analyses were done manually by the authors, and all outputs from AI-assisted tools were reviewed and validated by the authors. All data handled by AI were anonymised and deidentified. No personal information was handled by AI-assisted tools at any time.

Figure 2. Workshop participants expertise and motivation



Motivation for participating





Synthesis: SETAC AU/ACTRA Joint Conference

Key Insights from the Q&A Panel

Although the updated ANZG guidance provides greater flexibility, the current ANZG definition of ecological relevance is not fit for purpose for microorganisms, highlighting the need for revised criteria.

The updated ANZG method for deriving DGVs for toxicants (Warne et al., 2025) now provides greater flexibility, allowing modifications for unusual chemicals or mechanisms, which is more adaptable than those used by many international jurisdictions. The definition of an ecologically relevant endpoint within the updated ANZG guidance remains unchanged from previous versions of the method. Microbial data are considered acceptable for deriving DGVs, provided their ecological relevance can be demonstrated. However, the current ANZG definition of an ecologically relevant endpoint is not fit for purpose when considering microorganisms.

Societal perception and technical barriers that continue to hinder the effective use of microbial response data in deriving ANZG DGVs, include limited public and regulatory awareness of the ecological importance of microorganisms and the complexity of available microbial response data. Although single-species microbial data are widely considered insufficient for application in DGV derivations, clarity over which endpoints should be used, and how best to integrate them into DGVs are lacking.

A persistent lack of public and regulatory awareness stems in part from their inherent invisibility and their association with risk of disease. While microbial metrics can align with traditional single species ecotoxicology assays, these are not ecologically relevant, as microbes don’t exist as single species, rather as complex microbiomes, and more ecologically relevant information will come from function and community composition metrics. It should be recognised that similar arguments could be made for all organisms, which exist within complex, highly interactive ecosystems. However, in deriving effects-based DGVs, this complexity has been simplified to the highest level of biological organisation for which robust cause-effect data can be readily obtained (i.e.

the species or population level). While this simplification is not without limitations, it has, until now, been considered acceptable. This simplified model is not, however, acceptable for microorganisms. The loss of a single microbial species cannot be directly equated with the loss of a macro-scale species (e.g., an invertebrate), as microbiomes operate as highly dynamic, functionally redundant communities, with fine-scale spatial heterogeneity, embedded within larger complex biological and environmental systems. Consequently, ecological impact at the microbial scale is better understood in terms of shifts in community structure and function, rather than discrete species loss. However, such data are currently only acceptable for use as supporting lines of evidence when deriving ANZG DGVs, and are not widely used, due to the complexity of data, variability in methods, and lack of guidance on which endpoints might be considered most ecologically relevant. The complexity of microbiomes is also hard to translate into simple, regulator-friendly outputs. Whilst ANZG has a relatively flexible DGV derivation approach that allows for use of multiple lines of evidence (MLoE), and can incorporate data from many species', the most effective, relevant and efficient approach to use microbial data is still under debate.

Establishing appropriate microbial assay methodology within the ANZG context will need to balance environmental realism, practicality, revisiting 'chronic' while addressing challenges in quantitatively resolving microbial responses.

Direct Toxicity Assessment (DTA) is a tool to determine the impact of complex mixtures to receiving environments. The development of defensible and relevant DTA methodologies for microbes must balance the needs for sensitive and practical endpoints, ensuring minimal nutrients for growth-based measures to ensure environmental relevance, and resolving uncertainties around exposure conditions and duration. The ANZG definition of exposures greater than 24 hours as 'chronic' for microbes was challenged by some as being too short, while others disagreed, claiming it was sufficient, based on the rapid microbial generation times. There are also challenges in distinguishing viable from non-viable microbes. Ultimately, the environmental processes and services that we are aiming to protect in the environment must first be identified, and exposure conditions must be aligned with a realistic environmental context.

Although gene-abundance data show promise for future DGV development, key uncertainties around natural environmental baselines, gene activity, and ecosystem protection goals mean substantially more evidence and guidance are required.

This discussion point raised more questions than answers. Key uncertainties include how to establish baselines for microbial communities, whether detected genes are functionally active, and how AMR and toxicity information might be combined to identify sensitive endpoints. Substantially more data are required to determine whether gene-level data are sufficiently quantitative and protective for regulatory application. However, emerging developments, such as CSIRO's work on rapid gene detection, may improve the accessibility and applicability of gene-based indicators. From an ecological perspective, a fundamental question also remains regarding what should be protected (e.g., species, functions or ecosystem processes), with research from AIMS indicating that important functions can be readily lost.

Key panel recommendations to support inclusion of microbial data

1. **Review and update the ANZG definition of ecologically relevant endpoints** to ensure it reflects microbial community structure, function, and ecosystem processes. (Workshop Topic 1)

2. **Implement targeted awareness and engagement initiatives** to improve understanding of the ecological importance of environmental microbes among regulators, policymakers, and the broader community. (Roadmap)

3. **Convene expert panels to elicit consensus and develop guidance** on which microbial endpoints are most ecologically relevant and should be prioritised for use in DGV development. (Workshop Topic 2)

4. **Develop and adopt standardised methodologies** for generating microbial data to support derivation of DGVs, including recommended approaches for managing, refining, and interpreting complex microbial datasets. (Workshop Topic 2, Roadmap)

5. **Retain single-species microbial toxicity data**, while supporting a staged transition toward more ecologically relevant, community-based approaches as methods become standardised and accepted. (Workshop Topics 1 and 2)

6. **Reassess and, if necessary, revise the ANZG definition of 'chronic' exposure for microbial endpoints**, recognising that while the current >24-hour threshold allows for longer studies, its low minimum may encourage short-term testing that fails to capture genuine long-term microbial responses. (Roadmap)

7. **Prioritise microbial functions and ecosystem processes over individual** species when considering ecologically relevant endpoints for DGVs. (Workshop Topics 1, 2 and 3).



Synthesis: Workshops

Topic 1: Redefining 'ecologically relevant endpoints' for environmental microbes

For this topic, participants focused on redefining "ecologically relevant" for microbial endpoints in a DGV-derivation context. As previously stated, for the purposes of this white paper, the term 'environmental microbes' includes any microscopic eukaryote (protists and fungi, but not animals or plants) and prokaryotes (archaea and bacteria) inhabiting natural environments.

The current definition in Warne et al., (2025) for an ecologically relevant endpoint is:

"An endpoint is considered to have ecological relevance when it negatively affects a species' ecological competitiveness (i.e., its ability to increase the frequency of its genes in subsequent generations). What is considered ecologically relevant will be both species- and toxicant-specific. An effect on a species' competitiveness can be direct or indirect in the case of a symbiotic organism such as zooxanthellae in corals."

Overwhelmingly, the majority (>90%) of participants felt this definition, which focuses heavily on species-level outcomes (e.g., survival, reproduction, growth, development) was, at best, only partially relevant for microbial systems because:

- The concept of "species" is problematic for microbes; communities, consortia, and functional groups are more appropriate units of assessment.
- The "ability to increase the frequency of genes in subsequent generations" is not always a suitable indicator for microbes, particularly where increases may undermine ecosystem health, e.g., pathogens, AMR and toxin-producing genes.
- Microbial responses may be adverse, neutral, or beneficial to the microbial community, and to the whole ecosystem, depending on context, and can include enrichment, competitive displacement, functional activation, or stasis.

Focusing narrowly on gene or taxon abundance risks overlooking the microbial functions that underpin ecosystem processes, such as nutrient cycling, quorum sensing, or holobiont interactions.

Participants identified the need to either broaden the current definition or create an alternative supplementary definition specifically related to microbes, to include:

- **Shift in focus from species to microbiomes:** Emphasising microbial functional groups, community dynamics, and ecosystem-level processes relevant to ecosystem resilience and health.
- **Function and activity, not just presence:** Microbes may exist without performing critical functions; endpoints should aim to capture changes in functional activity.
- **Change, not only "negative" effects:** Any kind of significant shift in a microbial community can have ecological consequences. Wording should reflect "change" rather than only "negative", noting that ecological consequences will need to be proven for community change endpoints to be considered ecologically relevant, i.e., not every microbial community change will result in ecologically relevant consequences.

- **Natural variability and baselines:** Participants raised the point that there was a need to differentiate between natural seasonal or environmental shifts from stressor-impacted shifts. While this is essential for field-based impact or risk assessment, this challenge can be addressed in DGV data generation through appropriate experimental design, consistent with existing approaches for managing known variability in field-collected test organisms. For example, ensuring the field-collected consortia are homogenised before inoculating test vessels, helping to ensure that all controls and positive controls aim to have the same starting community structure as those in the toxicant treatments. Therefore, we suggest there is no need to specifically identify natural variability and baselines in the definition of ecological relevance for microbial endpoints used for deriving DGVs.
- **Holobiome and consortia:** Endpoints must consider microbes in symbiosis (e.g., corals, fish, aquatic plants) where dysbiosis can impair host and ecosystem health. The original definition already considers this ("An effect on a species' competitiveness can be direct or indirect in the case of a symbiotic organism such as zooxanthellae in corals") however, modification of this statement to better clarify other holobiome-consortia examples will be useful for enhancing applicability for microbes.
- **Mode of action specificity:** The new definition should retain a statement that aligns with original wording around effects possibly being both toxicant-specific and/or species-specific (e.g., herbicide effects on photosynthetic microbes).
- **Evolutionary ecotoxicology:** Recognition of keystone microbial groups and traits linked to resilience, redundancy, ecological drift, and long-term stability.
- **One Health and AMR:** Ecologically relevant microbial endpoints must consider cross-domain consequences for environmental/microbiome, plant, animal, and human health, particularly where microbial responses influence the emergence, maintenance, or spread of AMR.

Conclusion

A supplementary statement is proposed for future guidance documents to specifically address environmental microbial endpoints for DGV derivation for toxicants in waters and sediments, to augment the existing definition, where 'environmental microbes' include any microscopic eukaryotes (protists and fungi, but not animals or plants) and prokaryotes (archaea and bacteria) inhabiting natural environments:

"An ecologically relevant microbial endpoint is a measurable attribute of environmental microbial community structure, function or activity whose perturbation is associated with adverse changes in ecosystem processes or services. Consistent with ecological endpoints used for higher organisms, such endpoints should capture both direct and indirect stressor-mediated effects, e.g., impacts on microbial symbionts.

Microbial endpoints should reflect underlying biological processes, including resistance, resilience, and functional redundancy, within the broader microbiome and holobiont context. Endpoints derived from single-species exposure assays generally have less ecological relevance compared with multi-species and community-based approaches."

Topic 2: Critical appraisal of microbial endpoints for DGV derivation

Microbial endpoints were evaluated for ecological relevance, regulatory utility, and methodological robustness within the Warne et al. (2025) framework for deriving national default water and sediment quality DGVs. Five endpoint categories were assessed: (1) cell-based assays, (2) community functional endpoints, (3) amplicon-based methods, (4) metagenomic and metatranscriptomic approaches, and (5) other omics and multi-omics techniques. Endpoints were examined across field, laboratory, and semi-field (microcosm and mesocosm) approaches, each contributing complementary lines of evidence. While the scope was intended to encompass diverse microbial groups, discussions ultimately centred on bacteria, reflecting where the majority of available research and participant expertise currently lies. Fungi, protists and archaea remain important but underexplored gaps. Following workshop discussions, survey responses (14 of 36 participants) was used to inform trends for alignment on the use of each endpoint for deriving DGVs (Table 1, Table C1).

Cell-based endpoints

Cell-based assays, using single or multiple cultured microbial species, align conceptually with the SSD framework but have limited ecological relevance at the ecosystem level. Laboratory-adapted strains and artificial culture conditions (enriched media, simplified matrices) poorly represent in situ communities and can substantially alter contaminant bioavailability. Multi-species assays (e.g., Stone et al., 2024) improve ecological complexity by capturing differential sensitivity across taxa simultaneously, and single-species toxicity estimates can be extracted from community-based assays for use in SSDs. When combined with environmentally relevant exposure conditions, these estimates offer a more realistic alternative to standard OECD/USEPA designs. With the exception of microalgae and cyanobacteria, cell-based tests have rarely been incorporated into SSDs for DGV derivation. They are particularly unsuitable for AMR risk assessment, as they cannot capture horizontal gene transfer, resistome evolution, or co-selective gene enrichment.

Community functional endpoints

Whole-community functional assays, measuring respiration, nutrient cycling, and enzymatic activity, provide a direct link to ecosystem function and protection goals, capturing interactions and redundancy absent from single-species tests. Regulatory precedent exists in soil guideline derivation (standardised OECD/ISO carbon and nitrogen transformation tests), where environmental stability and agreed effect thresholds support their use. In surface water and sediment systems, these assays are constrained by strong context dependence, limited cross-system transferability, and the absence of agreed effect thresholds. They are therefore best applied as supporting lines of evidence within WoE frameworks, particularly for SSGV refinement, and offer only indirect insight into AMR dynamics, unless they are modified or combined with other approaches.



Amplicon-based endpoints

Amplicon sequencing (e.g., 16S rRNA gene, ITS regions, aka metabarcoding) enables detailed characterisation of community structure across hundreds of thousands of taxa (Thomas et al., 2023), overcoming key limitations of single-species assays. Early applications were constrained by reliance on relative abundance data and an inability to distinguish viable from non-viable cells. Integration with viability PCR and quantitative microbiome profiling (QMP), normalising sequencing data to absolute cell counts, addresses both limitations (Thomas et al., 2025a), enabling derivation of taxon-specific effect thresholds that can be aggregated into prokaryotic sensitivity distributions (PSDs) aligned with SSD frameworks (Thomas et al., 2025b).

RNA-based metabarcoding, typically targeting rRNA or reverse-transcribed marker transcripts, provides a complementary approach by targeting the metabolically-active fraction of the community. In this context, RNA-based metabarcoding may provide a more responsive endpoint for detecting toxicant-induced shifts in active microbial assemblages, particularly where changes in community function are expected to occur before major changes in total community composition are apparent. However, RNA-based approaches also introduce additional technical complexity, including rapid RNA degradation, sensitivity to sampling and preservation methods, variability in ribosomal RNA content among taxa and physiological states, and challenges in standardisation across studies.

Overall, amplicon-based endpoints provide valuable information on microbial community composition and, when paired with viability or quantitative approaches, can support more ecologically interpretable measures of taxon-specific sensitivity. However, both DNA- and RNA-based amplicon sequencing can only infer function and resistance through taxonomic composition, rather than directly detecting functional genes or mobile genetic elements. Their application is further limited by amplification bias, restricted taxonomic resolution and an inability to identify novel resistance genes, providing a limited functional picture compared to metagenomics.

Metagenomic and metatranscriptomic endpoints

Compared with metabarcoding, metagenomics and metatranscriptomics provide complementary DNA- and RNA-based information on microbial structural and functional potential and active gene expression, respectively. Metagenomics, based on community DNA, characterises the taxonomic composition, genes and pathways present within a microbial community, including resistance genes, mobile genetic elements, and broader metabolic capacity. This makes it particularly useful for assessing functional potential and for AMR surveillance, although the presence of a gene does not necessarily indicate that the function is active under the environmental conditions being assessed.

Metatranscriptomics, based on community RNA, provides a closer indication of realised biological activity by identifying genes that are actively expressed at the time of sampling. This can strengthen interpretation of toxicant effects on microbial processes and help distinguish latent functional potential from active functional response. However, RNA-based profiles are more sensitive to short-term environmental variation, temporal dynamics, sample handling, and preservation, making study design and interpretation more challenging.

Both approaches can provide mechanistic insight beyond taxonomic composition, but their regulatory application remains constrained by high natural variability, analytical complexity, limited standardisation and the absence of transferable effect thresholds. Metagenomics is currently the more defensible approach for AMR surveillance because it can characterise resistome composition, resistance genes, and mobile genetic elements, although it cannot alone quantify resistance risk, gene transfer dynamics, or realised functional activity. In addition, commercial laboratories now offer

specialised services for ARG detection and quantification, making these data more accessible to those without the required in-house capability. Metatranscriptomics can provide stronger evidence of active functional disruption, but remains more complex and sensitive to transient environmental conditions.

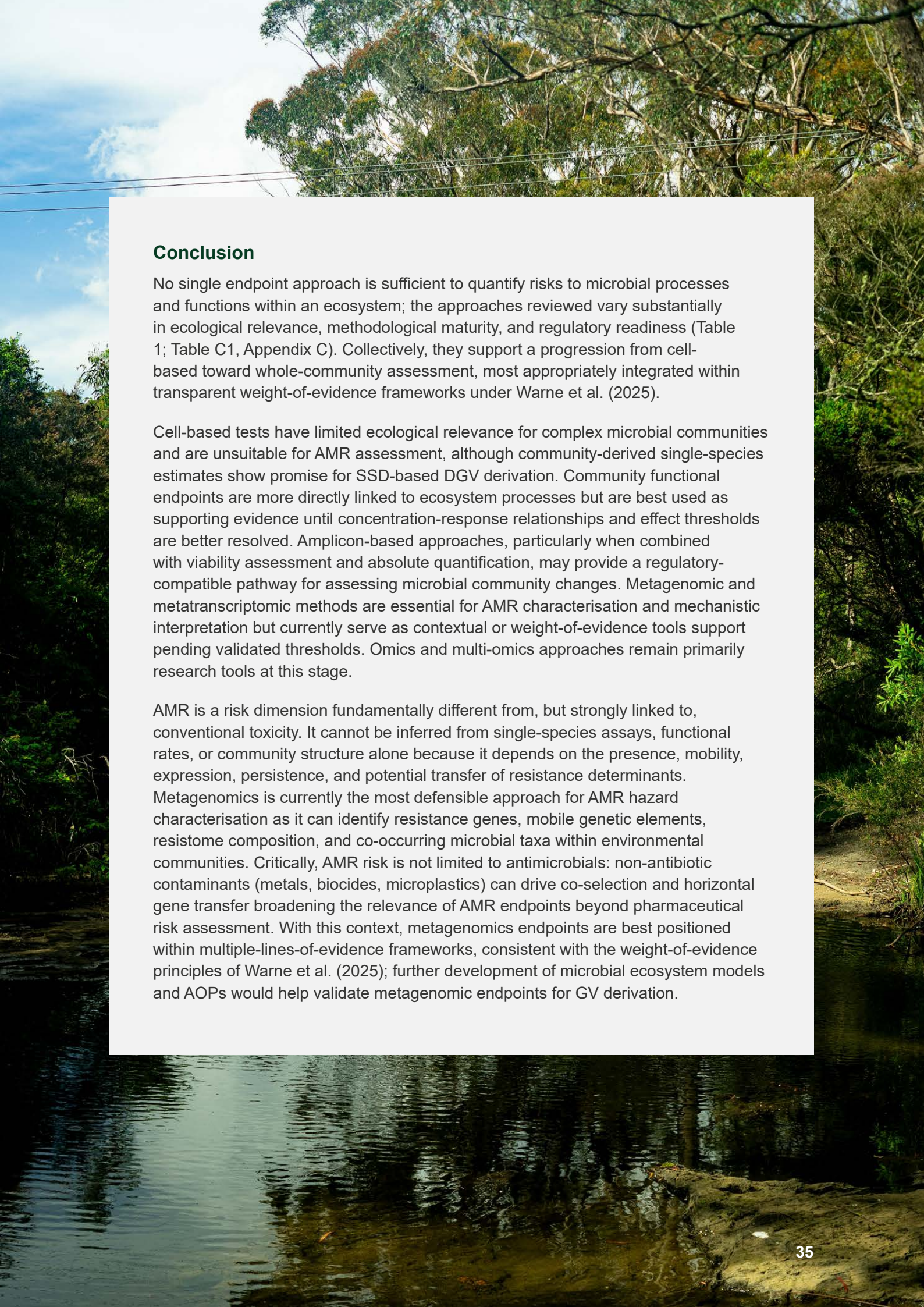
At present, metagenomic and metatranscriptomic endpoints are therefore best used as mechanistic or contextual lines of evidence rather than primary DGV drivers. However, emerging concentration–response modelling from metagenomic data (Webster et al., 2018) provides a possible pathway toward SSD alignment. As sequencing costs decline and workflows become more mainstream, cost is likely to become a lesser barrier. However, interpretation, reproducibility, standardisation, and linkage to ecologically meaningful adverse effects are likely to remain the major constraints on regulatory uptake.

Other emerging technologies including omics and multi-omics endpoints

Proteomics and metabolomics offer more direct measures of functional activity than DNA-based approaches, by characterising enzyme expression and metabolic pathway responses, with some application to ecological surveillance (Beale et al., 2022). These endpoints can help identify whether changes in microbial community composition or gene abundance translate into altered biological activity, including shifts in nutrient cycling, contaminant transformation, stress responses or metabolic disruption. Metabolomics may be particularly useful for detecting changes in microbial products, intermediates and biochemical pathways, while proteomics can provide stronger evidence that specific functional proteins or enzymes are being expressed. However, both approaches remain sensitive to environmental variability, sample handling and analytical workflow, and their interpretation is often constrained by incomplete databases, complex data processing and limited linkage to transferable ecological effect thresholds.

Multi-omics approaches, which integrate taxonomic, genomic, transcriptomic, proteomic and metabolomic information, offer a pathway to linking microbial structure and function across biological scales. When combined with systems biology and ecological modelling, these approaches integrate microbial responses with broader biogeochemical processes, including nutrient cycling, ecosystem function, and contaminant fate. In parallel, causal inference methods such as Bayesian networks and structural equation modelling are emerging as useful tools to move beyond correlation and strengthening mechanistic links between environmental exposure, microbial response and ecological outcome.

Overall, these emerging technologies are valuable for mechanistic interpretation, surveillance and hypothesis generation, but their regulatory readiness for guideline value derivation remains low. For AMR, they are currently best positioned as complementary research and monitoring tools that can characterise resistance genes, expression, protein activity, metabolites and environmental context, rather than as standalone endpoints for deriving DGVs. Their future regulatory value will depend on improved standardisation, reproducibility, concentration–response interpretation and stronger linkage to adverse ecological outcomes.



Conclusion

No single endpoint approach is sufficient to quantify risks to microbial processes and functions within an ecosystem; the approaches reviewed vary substantially in ecological relevance, methodological maturity, and regulatory readiness (Table 1; Table C1, Appendix C). Collectively, they support a progression from cell-based toward whole-community assessment, most appropriately integrated within transparent weight-of-evidence frameworks under Warne et al. (2025).

Cell-based tests have limited ecological relevance for complex microbial communities and are unsuitable for AMR assessment, although community-derived single-species estimates show promise for SSD-based DGV derivation. Community functional endpoints are more directly linked to ecosystem processes but are best used as supporting evidence until concentration-response relationships and effect thresholds are better resolved. Amplicon-based approaches, particularly when combined with viability assessment and absolute quantification, may provide a regulatory-compatible pathway for assessing microbial community changes. Metagenomic and metatranscriptomic methods are essential for AMR characterisation and mechanistic interpretation but currently serve as contextual or weight-of-evidence tools support pending validated thresholds. Omics and multi-omics approaches remain primarily research tools at this stage.

AMR is a risk dimension fundamentally different from, but strongly linked to, conventional toxicity. It cannot be inferred from single-species assays, functional rates, or community structure alone because it depends on the presence, mobility, expression, persistence, and potential transfer of resistance determinants. Metagenomics is currently the most defensible approach for AMR hazard characterisation as it can identify resistance genes, mobile genetic elements, resistome composition, and co-occurring microbial taxa within environmental communities. Critically, AMR risk is not limited to antimicrobials: non-antibiotic contaminants (metals, biocides, microplastics) can drive co-selection and horizontal gene transfer broadening the relevance of AMR endpoints beyond pharmaceutical risk assessment. With this context, metagenomics endpoints are best positioned within multiple-lines-of-evidence frameworks, consistent with the weight-of-evidence principles of Warne et al. (2025); further development of microbial ecosystem models and AOPs would help validate metagenomic endpoints for GV derivation.

Summary of key findings



Microbial endpoints vary widely in ecological relevance, use in regulatory context, and methodological robustness and maturity.



Whilst there was a focus on bacterial endpoints in the assessment, microbial endpoints using other prokaryotes (archaea) and micro-eukaryotes (e.g., protists, fungi, and protozoa) were also considered important.



Microbial single-species, cell-based endpoints have limited ecological relevance at the ecosystem level.



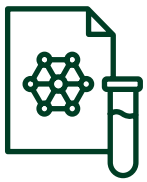
Community functional endpoints align closely with ecosystem protection goals but face practical challenges.



Amplicon sequencing captures the concurrent responses of hundreds to thousands of taxa within the entire community but requires the combination with cell viability assessment and absolute cell load quantification for use in deriving DGVs. Functional profiles should not be inferred from this data using predictive tools unless empirically validated.



Metagenomic and metatranscriptomic approaches offer mechanistic insight into contaminant impacts. Ecosystem function may be more directly inferred from the presence and abundance of metabolic pathways.



Proteomics and metabolomics provide more direct measures of functional activity but these need to be linked to broader ecosystem function and impacts. Emerging sensor technologies and modelling approaches will support interpretation across environmental gradients and exposure scenarios. Together, these will help establish genomic endpoints that reflect ecological functions.



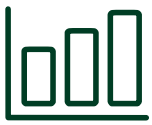
AMR emerged as a risk dimension, fundamentally different from, but crucially linked to conventional toxicity. Integrated community-level and molecular approaches are essential for AMR hazard assessment.



Combined approaches that integrate metagenomics-based AMR endpoints with community functional endpoints for natural environmental consortia have significant potential for use as AMR endpoints for DGVs.



Several ‘omics’-based analyses have been standardised in commercial laboratories, including those that detect antimicrobial resistance genes (ARGs). These may offer a cost-effective approach to enable use of some of the microbial endpoints discussed, particularly where in-house access to the necessary equipment and technical resources is limited.



Survey responses from 14 of 36 participants was used to assess endpoint suitability for deriving DGVs (Table 1), but uncertainty was often high, reflecting variable familiarity with endpoint types, possible differences in how respondents interpreted the task, and limited clarity around how endpoints could be incorporated into guideline value derivation. Further targeted consultation with topic-area experts, using refined survey methods and clearer endpoint-use scenarios, is required to reach formal consensus.

Table 1. Summary of survey responses regarding the relevance and potential use of microbial endpoints in Topic 2, under the newly proposed definition for ecologically relevant microbial endpoints, presented in Topic 1. Full details of each method, strengths and weaknesses and references provided in the Appendix in Table C1a

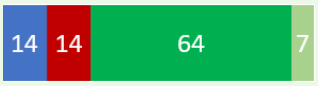
Endpoint Category

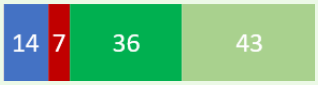
Cell-based endpoints (single-species endpoints from single- and/or multiple-species exposures)

Recommendations

Continue to use traditional growth endpoints in DGV datasets.

However, the use of more ecologically relevant test and exposure scenarios is strongly favoured (e.g., low nutrients, appropriate light/temperature conditions, community-based exposures).

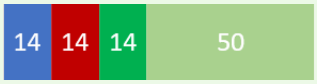
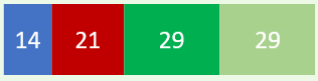
Endpoint	Mean score for ecological relevance[b]	Support for use in DGVs (% of participants)[c]	Summary
Bacterial luminescence inhibition	3.5		Respondents generally did not consider this ecologically relevant, noting that it is a rapid single-species assay with weak linkage to real microbial community structure or ecosystem processes. It may remain useful as a screening tool but is not suitable as a primary endpoint for DGV derivation.
Microalgal growth inhibition	8.0		This was one of the most strongly supported endpoints, reflecting the ecological importance of microalgae in primary production and food-web support, and historical use for DGV derivations. Respondents considered it directly relevant, though the need for environmentally realistic exposure conditions and species selection was noted.
Cyanobacterial growth inhibition	6.8		Respondents saw this as relevant in systems where cyanobacteria play key ecological roles, particularly in nutrient cycling and bloom dynamics. However, moderate uncertainty reflects concerns about general applicability and the need for improved ecological realism in test design.

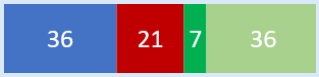
Endpoint	Mean score for ecological relevance[b]	Support for use in DGVs (% of participants)[c]	Summary
Microbial growth inhibition (bacteria, protozoa)	6.5		Respondents saw growth inhibition as relevant because it reflects impaired survival and productivity but questioned how well simple culture-based tests represent natural microbial communities. Improvements are needed to better link responses to ecosystem-level outcomes and better reflect environmentally relevant exposure conditions.
Fungal growth inhibition	6.4		This endpoint was considered relevant where fungi play important roles in decomposition and nutrient cycling, but respondents noted that its importance varies across ecosystems. Uncertainty reflected variability in ecological function and the need for context-specific application.
Yeast growth inhibition	6.0		Respondents showed high uncertainty regarding this endpoint, reflecting unclear ecological relevance in many environmental contexts. While yeast can be important in some systems, its role is often not representative of broader microbial community responses.
Change in viable microbial abundance	7.6		Respondents viewed this as a more ecologically meaningful measure than sequence-based abundance, as it focuses on living cells. However, improvements in standardisation and interpretation are still needed.

Endpoint Category
Community function endpoints

Recommendations

Apply functional endpoints as supporting lines of evidence, with priority given to those directly linked to ecosystem processes (e.g. nutrient cycling), while advancing method standardisation, exposure realism, and quantitative thresholds for integration into DGV derivation.

Endpoint	Mean score for ecological relevance[b]	Support for use in DGVs (% of participants)[c]	Summary
Decrease in microbial biomass	6.8		Considered a broadly relevant integrative endpoint reflecting overall microbial presence, respondents noted that it lacks specificity and may be influenced by abiotic factors. Improvements are needed to better link biomass changes to ecological function.
Inhibition of microbial respiration/ fermentation	6.7		Respondents recognised this as a functionally relevant endpoint reflecting microbial activity and decomposition processes, but high uncertainty may indicate challenges in interpretation and variability across systems. Clearer links to ecological outcomes are needed.
Inhibition of microbial esterase activity	4.6		This was generally viewed as a weak proxy for microbial activity, with limited ecological interpretability. Respondents noted that such biochemical assays lack clear linkage to ecosystem processes and are not suitable as standalone endpoints.
Carbon digestion inhibition (e.g. EcoPlates)	5.3		Respondents considered this endpoint to be based on artificial substrate systems that do not reflect natural carbon cycling processes. As a result, it was not regarded as ecologically relevant without substantial validation.
Nitrogen fixing and nitrification/ denitrification inhibition	6.8		This endpoint was strongly supported due to its direct link to ecosystem-level nutrient cycling processes. However, uncertainty reflects the need for improved standardisation and demonstration of consistent responses under realistic environmental conditions.
Sulfate reduction capacity inhibition	5.6		Respondents viewed this as relevant in specific environments such as anoxic sediments, but not broadly applicable across all systems. Its ecological relevance is therefore context-dependent, contributing to moderate uncertainty.

Endpoint	Mean score for ecological relevance[b]	Support for use in DGVs (% of participants)[c]	Summary
Extracellular enzyme activity inhibition	5.0		Considered potentially relevant for nutrient cycling processes, respondents noted that enzyme activity measurements can be difficult to interpret ecologically. Uncertainty reflects the need to better link these measurements to ecosystem-level outcomes.
Inhibition of biofilm formation	5.9		Biofilm formation was recognised as ecologically important due to its role in habitat structure and microbial interactions. However, respondents highlighted variability in methods and interpretation, leading to moderate uncertainty.
Inhibition of protein synthesis	4.5		Most respondents were unfamiliar with the endpoint, but several noted its potential for assessing microbial function, and one pointed to a recent Nature article to support its use. Concerns focused on limited standardisation, technical complexity, and uncertain relevance, suggesting use within a WoE approach.
Inhibition of substrate-coupled microbial growth	6.5		Respondents were generally unfamiliar but noted the endpoint's promise for linking microbial growth to function. Concerns about cost, complexity, scalability, and lack of standardisation suggest it is better suited as a complementary, research-focused approach rather than routine use.
Growth-inferred resistance (SELECT assay)	3.1		This endpoint was generally not supported for DGVs when using sewage-based consortia as test organisms, with respondents questioning its ecological relevance and applicability to natural systems. High uncertainty reflects limited knowledge of the method and its validation. Note, modification of this assay to use environmental consortia will significantly increase its ecological relevance and utility in DGVs. (See also potential applicability of SELECT for context specific approaches in 'The Parking Lot', below.)

Endpoint Category
Amplicon-informed community structure endpoints

Recommendations

Advance molecular and genomic approaches through validation and standardisation to enable their eventual use in data generation for DGVs.

Endpoint	Mean score for ecological relevance[b]	Support for use in DGVs (% of participants)[c]	Summary
Change in microbial community richness	6.6		Respondents considered richness a useful indicator of biodiversity change but noted that it has limited ecological meaning on its own. Its relevance improves when interpreted alongside composition and functional endpoints.
Change in microbial abundance (via sequence reads)	7.4		This endpoint was seen as promising but limited by the fact that sequence reads do not directly represent true abundance. Respondents highlighted the need for improved quantification and ecological interpretation.
Change in microbial community composition	8.7		Support for this endpoint was based on its ability to capture whole-community responses to stressors. Respondents strongly supported its ecological relevance, particularly when linked to functional outcomes.

Endpoint Category

Community functional endpoints informed by omics (metagenomic and metatranscriptomic)

Recommendations

Prioritise the development and validation of directly measured functional omics approaches, as these provide strongest links to ecosystem processes, while addressing challenges related to cost, standardisation, and data interpretation.

Endpoint	Mean score for ecological relevance[b]	Support for use in DGVs (% of participants)[c]	Summary
Functional group/process inhibition (C, N, P, S cycling, etc.)	7.4		These endpoints were considered highly relevant when directly measured, as they link to ecosystem services and biogeochemical processes. However, uncertainty reflects challenges in interpretation, cost, and methodological consistency.
Change in resistomes (ARG abundance)	7.3		Respondents saw this as potentially important due to links with contaminant-driven selection and broader risk, with several noting it to be the most effective method for indicating AMR risk. However, relatively high uncertainty reflects lack of familiarity with the methods, and for some, an unclear connection to ecological harm and the need for better interpretive frameworks.
Contaminant-induced ROS	4.8		Despite high level of unfamiliarity with this endpoint, those who did respond were not supportive, as the endpoint was not considered ecologically relevant, reflecting a mechanistic stress response rather than an ecological outcome. Respondents noted it may be useful as supporting information.

Endpoint Category

Community endpoints informed by metabolomics, proteomics and multi-omics endpoints

Recommendations

Advance as emerging tools for capturing biological responses and linking structure to function. Prioritise improvements to interpretability, standardisation, and validation against ecologically meaningful outcomes before being considered suitable for regulatory application.

Endpoint	Mean score for ecological relevance[b]	Support for use in DGVs (% of participants)[c]	Summary
Change in microbial protein expression profiles	5.3		Proteomic endpoints were considered potentially informative as they capture realised biological responses. However, uncertainty reflects challenges in linking these changes directly to ecological outcomes.
Change in microbial metabolite profiles	5.8		Respondents viewed metabolomics as promising for capturing physiological responses, but uncertainty and opposition to this endpoint in DGVs reflect complexity of data, difficulties in interpretation and lack of standardisation.
Integrated multi-omics response	7.0		Multi-omics approaches were seen as conceptually powerful, integrating multiple biological levels. However, high uncertainty reflects their complexity and lack of readiness for regulatory application.

Endpoint Category

Predictive and model-based endpoints

Recommendations

Restrict the use of predictive models to hypothesis generation and supporting interpretation and prioritise rigorous validation against empirical data before considering their use in ecological relevance assessments or DGV derivation.

Endpoint	Mean score for ecological relevance[b]	Support for use in DGVs (% of participants)[c]	Summary
Molecular Initiating Events (MIEs, AOP framework)	6.4		Respondents saw MIE-based approaches as useful mechanistically but noted that links to ecological outcomes are not yet well established. This contributes to high uncertainty.
Functional inference from taxonomy (e.g. PICRUSt2, FAPROTAX)	3.6		Respondents strongly distrusted predictive approaches based on taxonomy alone, noting that they rely heavily on assumptions. These methods were not considered ecologically relevant without empirical validation.
Metagenomic pathway prediction (e.g. KEGG inference)	6.5		Similar to taxonomy-based inference, respondents considered these approaches insufficient on their own. Ecological relevance depends on validation against measured functional activity.
Genome-scale metabolic models (GEMs) / community models	6.1		These models were viewed as highly theoretical, with limited demonstrated applicability to real ecosystems. High uncertainty reflects the need for substantial validation before use in ecological assessment.

[a] Shading indicates the majority response on DGV suitability: Yes (dark green), Yes with modifications (light green), No (red), Unsure (blue). Rows are also shaded light green where Yes + Yes with modifications indicates overall support (but modifications likely required).

[b] Mean of participant-assigned scores between 1 and 10, where 1 was not ecologically relevant and 10 was highly ecologically relevant.

[c] Shading in bar graphs for this column indicate participant responses on DGV suitability: Yes (dark green), Yes with modifications (light green), No (red), Unsure (blue)

Topic 3: Perceived barriers and enablers to microbial data integration

Workshop discussions identified a range of perceived barriers and enabling factors influencing whether and how microbial endpoints are incorporated into derivation of DGVs. These perceptions are summarised in Table 2 and provide inputs for the gap analysis and synthesis in the next section.

Table 2. Analysis of barriers and enablers from workshop discussions on microbial data integration

Perceived Barrier / Enabler	Description	Implications for integration of data to derive DGVs	Further notes, gaps and actions
CATEGORY: PROTECTION GOALS			
Barrier (conceptual, regulatory)	Lack of clarity on what DGVs aim to protect (species, functions, ecosystem integrity, resilience)	Creates uncertainty when choosing appropriate microbial endpoints to endorse and limits confidence in incorporating community level data	The question of ecosystem protection goals for DGVs was raised throughout discussions. These are separate from the wider management goals which are identified in Step 2 of the Water Quality Management Framework. This raises the question of what DGVs are intended to protect. Levels of protection for DGVs are currently aimed at protecting a certain % of species within a receiving ecosystem. This assumes that loss of a species (which in some instances represents an entire taxonomic group and potentially also trophic level) will cause impact to the ecosystem. So, the overarching protection goal is of the ecosystem. However, there is lack of clarity for deriving high reliability DGVs using whole-community endpoints (not species-level or taxon-level), that don't necessarily relate to % species protection values. Research Objective and Guidance Action: Research required to support regulatory guidance, including use-cases for microbial data using whole community-level and functional endpoints. Guidance should also be designed to support uptake by environmental regulators, addressing current limitations in familiarity with environmental microbiology and providing sufficient context to ensure endpoints are applied appropriately and not used indiscriminately across assessment frameworks.
Enabler	Ability to explicitly redefine or expand ecosystem protection goals of DGVs	Provides framework for justifying microbial and functional endpoints alongside species data	

Perceived Barrier / Enabler	Description	Implications for integration of data to derive DGVs	Further notes, gaps and actions
CATEGORY: SSD FRAMEWORK			
Barrier (conceptual)	Community and functional endpoints do not fit the "species" sensitivity distribution definition.	Raises concerns about redefining SSD intent and interpretation of PC95, which can no longer be linked back to species protection goals.	ANZECC/ARMCANZ (2000) previously valued community exposure data as high-tiered and allowed for an assessment factor (AF) approach to derive high reliability DGVs without specifying a protection level based on % of species (as done for the pesticide esfenvalerate). Due to the inherent subjectiveness of the assessment factor (AF) approach, ANZG (2018) now stipulates that application of an AF to derive a DGV, regardless of the data type it is applied to, results in a DGV of unknown reliability. Warne et al. (2025) now explicitly states that higher taxon-level data than species (i.e., taxon-level, whole community-level) are not to be included in SSDs with single species data. These must be used as separate lines of evidence. Participants identified functional (whole-community) measures as the most ecologically relevant microbial endpoints. If lab-based microcosms can be used to estimate these endpoints with the same level of control and quality assurance as single-species exposures, approaches will be needed to integrate them with single-species data in a way that ensures they are appropriately considered rather than sidelined, particularly where microcosms cover only one taxonomic level. Research Objective: Explore and compare various options for deriving a combined, all lines of evidence DGV, as well as exploring alternative metrics and derivation approaches for different types of data (community, functional). This would be applied to several key toxicants, as supporting evidence for potential inclusion of microbial whole-community endpoints. In addition, the statistical methods underpinning microbial (or other mesocosm) whole community endpoints should be explored in the context of how well they can be linked to ecosystem protection goals in such a way that they are analogous to the current SSD methodology. Research Objective/Guidance Action: Explore key justifications that would be required to enable microbial community-level data use, and which types of communities will be acceptable (surface waters, sediment, biofilms, holobiont-associated microbiomes). Guidance action: flow chart that identifies current guidance and gaps for use of microbial endpoints to derive DGVs.
Enabler	Acceptance of alternative approaches or parallel distributions under current guidance.	Enables use of separate microbial distributions, microbial endpoints within SSDs for SSGVs (King et al., 2024) and/or weight-of evidence approaches. Supports the proposal and development of alternative metrics that define the level of risk to the community that can be readily translated into a risk assessment with the same level of precautionary confidence as the current % species protection method.	
Enabler	Provision for special cases to use alternative endpoints in DGVs.	Enables use of alternative endpoints if justified. Note, how this justification is assessed is unclear.	
Enabler and barrier (technical)	Precedent for using community data for DGVs.	The DGV for the pesticide esfenvalerate is based on community endpoints only (at taxon-level and whole community-level). However, under updated ANZG methodology, the AF approach used for esfenvalerate in 2000 would no longer be acceptable for a high reliability DGV.	

Perceived Barrier / Enabler	Description	Implications for integration of data to derive DGVs	Further notes, gaps and actions
CATEGORY: COST AND EFFORT			
Barrier (technical)	Multiple lines of evidence (MLoE) approaches are more costly, complex, and resource intensive than combining all data in one distribution.	Raises feasibility concerns for use of MLoE in routine DGV development and review cycles. This further risks marginalising microbial endpoints as non-essential, unless guidance stipulates their importance and requirement for inclusion.	Research Objective: As part of the above actions, consider cost benefit scenarios to each approach. Research Objective: Justifications for inclusion and/or other use of microbial whole-community endpoints that might be applicable for special case applications. Research Objective: Develop a decision matrix for prioritising toxicants which require DGVs (for waters and/or sediments) with microbial data.
Enabler	Phased and prioritised implementation, leveraging the flexibility of the current ANZG guidance to allow use of alternative endpoints, if justified.	Allows incremental adoption without overburdening guideline processes. How 'justification' is assessed, however, is unclear.	
CATEGORY: ENDPOINT EQUIVALENCY			
Barrier (technical)	No agreed thresholds defining ecologically relevant change in microbial community-level endpoints.	Limits comparability with NEC/NSEC/EC10 values and regulatory acceptance.	Research Objective: Use AOPs and conceptual models to identify meaningful thresholds for microbial community-level endpoints. Compare these to existing thresholds used in other jurisdictions and situations (e.g., NEPM thresholds of 25% for functional endpoints, also use of 5% community effect levels to align with 95% species protection in a field- and mesocosm-based SSGV for magnesium (Humphrey & Chandler 2018). Research Objective: Assess the ecological relevance of incorporating increasing (not only decreasing) changes in absolute taxon abundance or molecular responses, as both directions may indicate community dysfunction (Thomas et al., 2023; 2025b).
Enabler	Use of AOPs and conceptual ecological models.	Could help translate microbial molecular or functional changes to higher order ecological outcomes and identify thresholds that are ecologically meaningful.	
Enabler	Precedence of NEPM methods for setting thresholds for functional endpoints.	NEPM assign 25% change in function as a threshold for soil function assays.	

Perceived Barrier / Enabler	Description	Implications for integration of data to derive DGVs	Further notes, gaps and actions
CATEGORY: SPECIES FUNCTION			
Barrier (conceptual, regulatory)	Tension between protecting species versus protecting ecosystem function.	Generates disagreement over whether and how both types of endpoints can coexist in GV derivation.	The limitations of using species loss to represent impacts on ecosystems are known, however, species has thus far been the most straightforward metric to use and manipulate for (i) generating reliable cause-effect data, and (ii) developing models (SSDs) that are used to derive DGVs. Research Objective: Acknowledging the consensus in workshops that both functions and species are important, develop a research project that explores how both functions and species can be protected by DGVs, e.g., exploring the use of both SSDs and functional SDs with the same datasets for all levels of taxa. Note that the current focus on species for SSDs assumes that by protecting most species, that most functions are also protected. This research would test that concept.
Enabler	Recognition that both species and function matter.	Supports combined distributions, or complementary approaches addressing both types of impact to inform ecosystem health.	
CATEGORY: METHOD STANDARDISATION			
Barrier (technical, regulatory)	Lack of validated, standardised, accredited microbial assays for waters and sediments with robust QA/QC.	Restricts regulatory application and consistency across published data available for use to derive DGVs.	Research Objective: Develop, or adopt, standardised methods for ecologically relevant endpoints. Consider existing protocols and assays to adapt and include key criteria within ANZG quality assessments. Research Objective/Regulatory Action: Specifically for omics-informed endpoints, work is required to explore the QA/QC needs that will enable these data to move from research to regulatory implementation. While examples of good practice are available, guidance for regulators remains insufficient, and there are no established assessment criteria equivalent to that used for ecotoxicology.
Enabler	Existing researchgrade methods ready for adaptation. Existing methods (e.g. OECD, 2000a, b) for soils that could be adapted for sediments and waters.	Provides foundation for prioritised method validation programs. Also, existing quality assessment criteria in current guidance can be adapted to ensure any future microbial data published contain the minimum requirements to enable their use in DGVs.	

Perceived Barrier / Enabler	Description	Implications for integration of data to derive DGVs	Further notes, gaps and actions
CATEGORY: DATA AVAILABILITY			
Barrier (conceptual)	Limited baseline data for microbial communities.	Increases uncertainty in defining effect thresholds and reference conditions.	The perceived issue raised on several occasions in discussions, of having limited baseline data is not a true barrier to development and use of microbial endpoints for cell-based assays, microcosms or mesocosms. It may present an issue for field studies, where reference sites are crucial. However, in the context of a lab assay, if whole consortia are sourced from one relatively unimpacted site, all responses are standardised to the control response. This is also discussed earlier in this white paper. Research Objective/Broader Activity: Leverage existing surveillance activities to help identify priority toxicants and establish natural baselines. Research Objective: Meta-analysis to establish baseline datasets to inform natural structure and function. This is only important if considering field studies e.g., along a toxicant gradient, to inform a DGV.
Enabler	Expanded monitoring and surveillance.	Builds baselines and evidence to prioritise toxicants for new DGVs.	
Enabler	Precedent for using field collected test organisms within current DGV databases.	The use of field-collected test organisms is widely accepted, and microbial communities as test organisms will be no different. Controls and treatments all have aliquots from the same homogenised mixture of microbes. Therefore, the baseline for each assay is the control.	
CATEGORY: ANALYTICAL CONSTRAINT			
Barrier (technical)	Difficulty measuring concentrations of toxicant at very low concentrations, and in very small volumes as used in microbial assays.	Limits the ability to confirm exposure concentrations, a crucial requirement for DGVs.	Research Objective: Develop and standardise affordable approaches within assays to address the issue of low concentrations and small volumes, e.g., larger volume vials for chemical assessment only, extrapolation of measured concentrations as lower-cost means of estimating the true exposure concentration Collaboration: Identify research groups who have the analytical tools to do trace-level analyses in small volumes and build collaborative projects to develop microbial effects databases.
Enabler	Rapid advances in analytical tools.	Provides the means for addressing low volume or trace-level analysis but will come at a cost.	

Perceived Barrier / Enabler	Description	Implications for integration of data to derive DGVs	Further notes, gaps and actions
CATEGORY: ENVIRONMENTAL CONTEXT			
Barrier (conceptual)	Surface water GVs will not translate to groundwater, polar or extreme systems.	This is no different to current DGVs and is already addressed in current guidance. ANZG DGVs are provided for surface waters, sediments and for primary industries (animal drinking water).	Action for white paper: Clarify the known limitations of DGVs for non-surface water environments and the role of site-specific GVs for these settings. Also highlight the body of work already underway in trying to address this for groundwater, noting that microbial endpoints used for DGVs will be beneficial for enabling better GVs for groundwater, particularly where higher-trophic level receptors are limited, or absent and ecosystem function is largely mediated by microbial communities. Research Objective: Much of the groundwater research for DGVs has focused on higher organisms e.g., stygofaunal crustacea. Surveillance of microbial communities in groundwater is underway and a shift in focus is needed to consider the microbial communities as useful in DGVs.
Enabler	SSGVs and scenariobased approaches.	SSGVs for site-specific scenarios are encouraged in place of relying on DGVs if the context is inappropriate. However, when resources and funding is limited, there is still a reliance on surface water DGVs for other systems such as groundwater. There is research underway to generate GVs for groundwater.	
CATEGORY: MIXTURES AND CUMULATIVE EFFECTS			
Barrier (conceptual)	Additional complexity when considering mixtures.	Known problem with using DGVs as the only line of evidence in a water quality assessment.	Action for white paper: Clarify the known limitations of using DGVs for mixtures. Reiterate the official guidance of using DTAs for risk assessments of mixtures. For mixtures, the existing guidance for DTAs is still applicable with inclusion of microbial endpoints. Any assay used to generate microbial endpoints for a DGV can be used in a DTA for a mixture.
Enabler	Use of microbial endpoints in direct toxicity assessment (DTA).	Using the same paradigm that already exists, microbial endpoints and assays used to derive DGVs can also be used as part of a suite of assays in a DTA. Noting the issue of single-species vs community endpoints remains.	
CATEGORY: CHRONIC EXPOSURE DEFINITIONS			
Barrier (technical, conceptual, regulatory)	Difficulty defining chronic exposure for microbes with variable growth rates.	Reduces consistency. There will need to be an accepted definition for chronic.	Guidance update: Revisit the definition of “chronic” exposure in the context of microbes to determine whether revision is required and, if so, prioritise development of an updated definition. Similar to the definitions assigned as chronic to other test organisms, there will need to be a balance between environmental relevance of the exposure time, required length of exposure to detect an effect, labour and cost.
Enabler	Existing flexibility in chronic definitions.	Creates space for microbialspecific metrics (e.g., doubling time, turnover).	

Perceived Barrier / Enabler	Description	Implications for integration of data to derive DGVs	Further notes, gaps and actions
CATEGORY: REGULATORY ACCEPTANCE			
Barrier (conceptual)	Perceived reluctance to adopt DGVs that use microbial data by jurisdictional regulators.	Slows uptake despite technical feasibility.	Action for white paper: Provide the compelling case for the ecological importance of microbial communities and the use of appropriate endpoints to represent them. Ongoing collaboration and codesign: Ensure end-users continue to be included in ongoing discussions around microbial endpoints and their use in DGVs. Continued engagement with regulators and their advisory, technical and working groups. Research Objective: A targeted case-study program, co-developed with regulators, could provide practical examples for how microbial datasets can be assessed and accepted. Complementary QA/QC checklists (particularly for omics datasets) would further support consistent decision-making.
Enabler	Current guidelines explicitly allow nontraditional endpoints.	Supports inclusion where ecological relevance is demonstrated.	
CATEGORY: AMR PRIORITISATION			
Barrier (conceptual)	AMR is inconsistently prioritised across jurisdictions and portfolios.	Limits appetite for AMRbased endpoints.	As above but extend to One Health and AMR. Noting that awareness and ownership of responsibility for actions relating to AMR are expected to increase over the course of the Action Plan, therefore it is important to plan for a future in which AMR is widely recognised as a priority.
Enabler	One Health framing and economic arguments. International (e.g. UNEP) guidance and knowledge sharing.	Strengthens case for prioritisation of AMR endpoints. Increases awareness and identifies responsibility that fall to environmental regulatory agencies with respect to AMR.	
CATEGORY: STAKEHOLDER PERCEPTIONS			
Barrier (conceptual)	Microbes perceived as ubiquitous, non-beneficial resilient, or lowrisk.	Reduces perceived value of microbial protection.	Ongoing collaboration and codesign: Continued engagement in workshops, projects and presentations. Develop a communication plan to effectively reframe microbial impacts as ecosystem impacts.
Enabler	Framing around ecosystem services and cascading effects.	Makes microbial impacts tangible to regulators and communities.	

Perceived Barrier / Enabler	Description	Implications for integration of data to derive DGVs	Further notes, gaps and actions
CATEGORY: SKILLS AND CAPACITY			
Barrier (technical)	Knowledge and skills gaps across disciplines.	Constrains method development and implementation.	Regulatory Action: Develop a transdisciplinary expert working group. Establish a roadmap of activities that the expert working group will be tasked with guiding and reviewing.
Enabler	Transdisciplinary expert working groups.	Enables coordinated, credible progression of microbial integration.	
CATEGORY: CROSS CUTTING IMPLEMENTATION			
Barrier (technical, regulatory)	Constraints related to data governance, limited computational and bioinformatic capabilities, challenges in characterising and propagating uncertainty, and difficulty interpreting and communicating complex microbial, multi-omics and/or modelled outputs for regulatory decisionmakers.	Reduces transparency, reproducibility and comparability of microbial datasets; limits regulator confidence in advanced endpoints; constrains reuse of data and defensible incorporation of omics, sensor and modelling outputs into WoE/MLoE and DGV derivation.	Research / Guidance Action: Develop or adopt shared data governance principles, reproducible analytical workflows, guidance on uncertainty treatment and communication of complex outputs, and clearer pathways for transitioning methods from research to regulation.
Enabler	Availability of emerging standards for data stewardship, increasing access to opensource bioinformatics tools, modelling frameworks, and growing experience with transparency and uncertainty treatment in other regulatory science domains.	Supports consistent interpretation, reproducibility and informed regulatory uptake of complex microbial datasets and integrated approaches across jurisdictions.	Guidance Action: Leverage existing bestpractice frameworks (e.g. FAIR data principles, reproducible workflows, uncertainty reporting) and embed these within microbialspecific guidance; use case studies and worked examples to build regulator confidence.



Gap Analysis for Microbial Data Use to Derive DGVs

This gap analysis synthesises issues identified across the engagement process and maps them against currently available pathways for incorporating microbial data into DGV derivation, where relevant (Figure 3). Perceived barriers and enablers documented in Topic 3 (Table 1) are translated into specific conceptual, technical, and regulatory gaps relative to current guidance and practice.

Multiple lines of evidence (MLoE) approach for deriving DGVs

ANZG provides guidance for deriving GVs using a MLoE approach. The approach involves deriving several "candidate" GVs based on different types of data (e.g., laboratory and field) or different analyses and integrating them in some way to determine a final GV. However, to date, the MLoE approach has not been applied to derive DGVs for Australia and New Zealand. Warne et al. (2025) allows flexibility for other approaches (including MLoE) to be used to DGV derivation, but this appears to be intended on a case-by-case basis rather than as a standard method. Formalising the use of MLoE for DGV derivation would provide clearer pathways for incorporating microbial endpoints.





Conceptual gaps

Several key workshop themes translate into conceptual gaps that currently constrain the routine use of microbial endpoints in deriving DGVs for toxicants. Within the water quality management framework, higher level management goals may include the need to protect a range of community values (e.g., cultural and spiritual values, drinking water, recreational water and ecosystem protection), which help inform management options. Where ecosystem protection is a priority, this implies safeguarding taxa, communities, functions and processes, alongside broader attributes such as ecological integrity and resilience.

Although ANZG strongly recommends the use of multiple lines of evidence to inform risk assessment, in practice, DGVs are often the sole tool applied. Furthermore, DGVs are fundamentally centred on species-level protection. This simplified approach is accepted globally and assumes that loss of a species (which in some instances represents an entire taxonomic group and/or trophic level) will impact the ecosystem. This creates a misalignment with microbial ecology, where the most ecologically relevant endpoints are typically expressed at the level of communities and functions. This misalignment is compounded by the conceptual assumptions underpinning the SSD approach, which are inherently species-based.

These foundational issues interact with governance and legitimacy constraints as raised by participants: microbes are often perceived as ubiquitous and resilient (and therefore at low risk of impact), AMR is not consistently prioritised across jurisdictions, and there is perceived reluctance to include microbial communities in DGVs, reducing appetite to treat microbial protection on par with protection of higher organisms. Addressing these issues will require clearer conceptual guidance coordinated capacity-building to support data generation, and consistent interpretation and uptake of microbial endpoints and DGVs that include microbial data.

Technical and regulatory gaps across three current pathways for microbial data use in ANZG DGVs

The pathway-specific gaps below provide the technical and regulatory detail that sits behind the perceived barriers and enablers summarised in Table 1. Note that ANZG (2018) and Warne et al. (2025) specify minimum data, data quality, and applicability requirements for data use, but not all caveats and criteria are covered in this summary.

Pathway 1 Species-level candidate DGV (single-species endpoints)

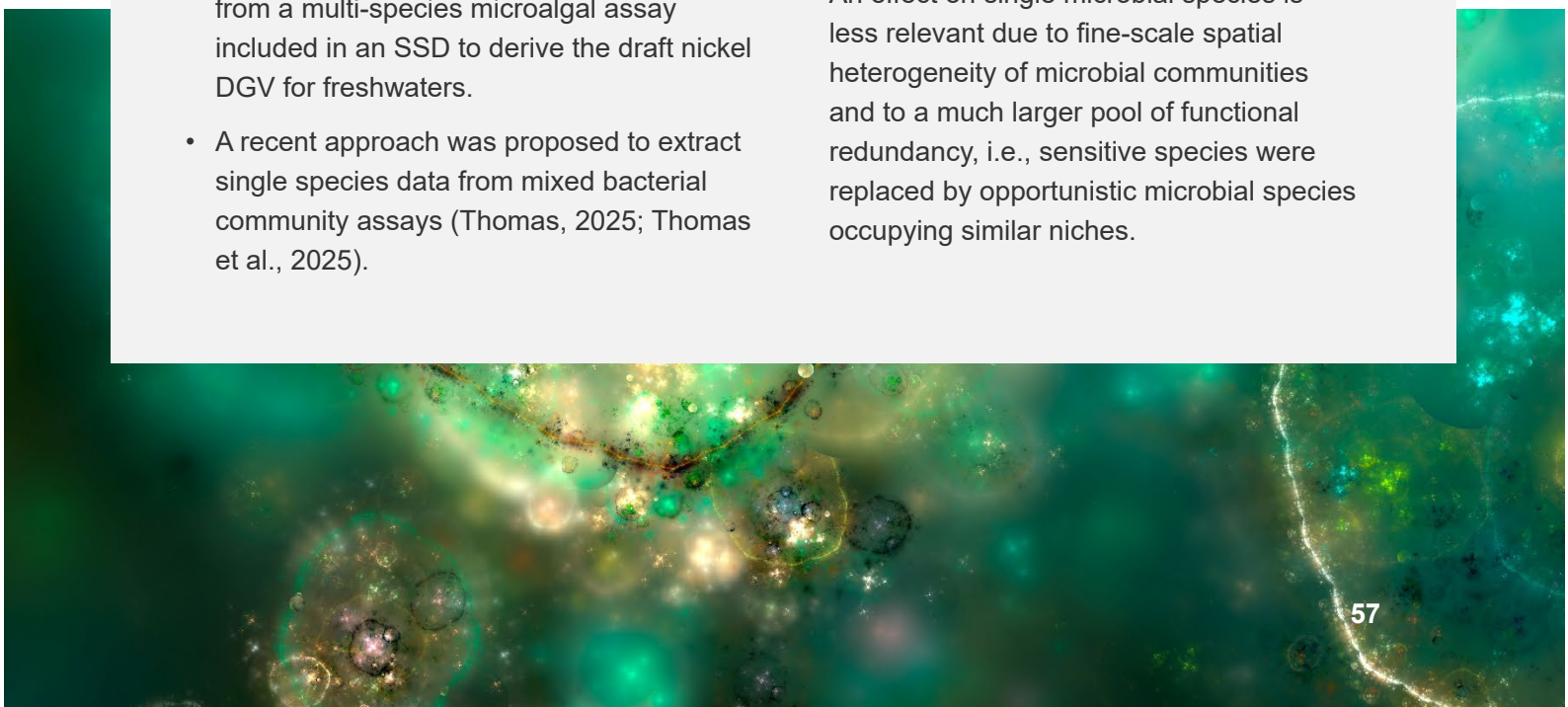
Single-species microbial endpoints (if deemed 'ecologically relevant' under the Warne et al. (2025) definition) from single-species assays, multi-species assays, microcosms, or mesocosms can be combined with single-species toxicity data for other taxa to derive a candidate DGV, preferably via an SSD or, for smaller datasets, via an assessment factor (AF) approach. If data for fewer than three species from three taxonomic groups are available, a species-level candidate DGV cannot be derived, but the data can still be used as supporting evidence for other candidate DGVs.

Examples include:

- Microbial population growth endpoints (bacterium, fungus, microalgae) included with other taxa in an SSD to derive the draft copper DGV for freshwaters.
- Species-specific population growth rates from a multi-species microalgal assay included in an SSD to derive the draft nickel DGV for freshwaters.
- A recent approach was proposed to extract single species data from mixed bacterial community assays (Thomas, 2025; Thomas et al., 2025).

Gaps/issues

- Large datasets from a single microcosm or community study may dominate an SSD, giving disproportionate weight relative to the available single-species datasets. While this was raised as an issue by Batley et al. (2018), practical guidance on managing this "over-weighting" risk is urgently required, as methods such as developing 'omics' approaches generating large datasets.
- Applying the conventional SSD paradigm to bacteria may overemphasise highly sensitive single-taxon responses, even where the affected taxon has limited ecological relevance or its function may be replaced by other microbial taxa. Guidance is needed on how to interpret sensitive microbial species-level endpoints within SSDs, particularly in light of microbial functional redundancy and community turnover.
- An effect on single microbial species is less relevant due to fine-scale spatial heterogeneity of microbial communities and to a much larger pool of functional redundancy, i.e., sensitive species were replaced by opportunistic microbial species occupying similar niches.



Pathway 2 Taxon-level candidate DGV (taxon-level community endpoints)

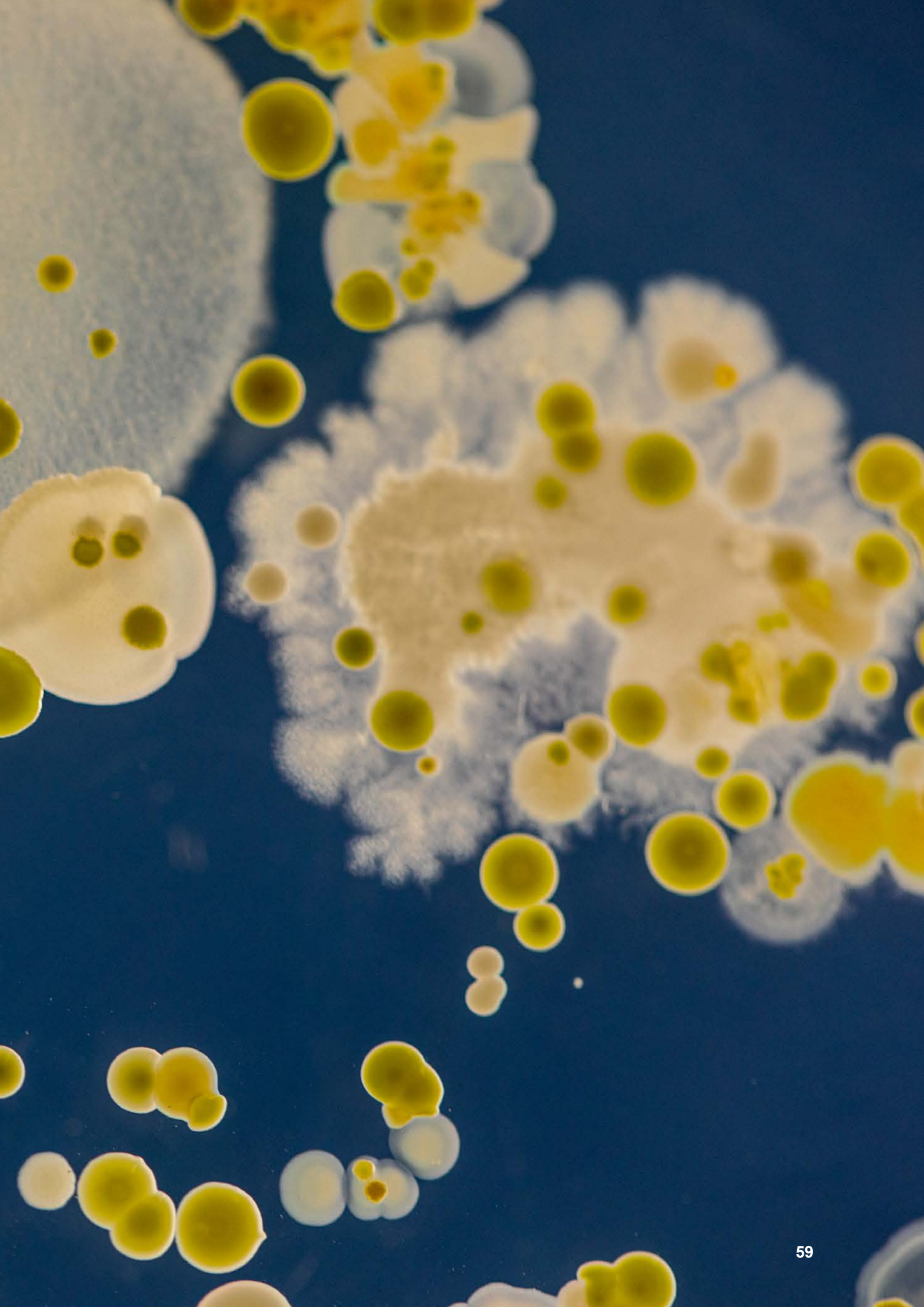
Although not explicit, guidance (Batley et al., 2018) suggests that species-based approaches may be applied to higher taxonomic endpoints. For microbes, this is most relevant to taxon-level community data from microcosms, mesocosms, and field studies, which must be described at a consistent taxonomic organisation when combined within a distribution (e.g., broad groupings such as phytoplankton, bacteria, fungi etc. or a consistent rank such as genus/family/order). Therefore, following the approach for single species, if sufficient data are available a cumulative frequency distribution approach can be used, or, alternatively, an AF applied, or the data used as supporting evidence.

Examples of taxon-level GVs (non-microbial, but indicative of potential microbial application) include:

- Community taxon-level endpoints used to derive a high reliability DGV for the pesticide, esfenvalerate (ANZECC/ ARMCANZ 2000). Note, under updated DGV methodology the AF approach used for this DGV would no longer be of high reliability (Warne et al., 2025; Batley et al., 2018).
- Field-based and mesocosm taxon-level community data used to derive a SSGV for magnesium near Ranger Uranium mine (NT) in a MLoE approach (Humphrey and Chandler 2018). Field and mesocosm taxon-level community endpoints were plotted in respective sensitivity distributions to define protective concentrations at the 1st and 5th percentiles.

Gaps/issues

- Updated acceptance criteria and data use rules for microbial microcosm data are required. Microbial microcosms may represent only one or two taxonomic groups (e.g., bacteria and archaea) and therefore fail current minimum criteria for microcosm/ mesocosm data use (Warne et al., 2025). However, given these microbiomes may comprise over 40 different phyla within the one taxonomic group (Thomas et al., 2025), it is likely that the minimum criteria of three functional roles would be met by different phyla within this microcosm. Fit-for-purpose criteria are likely to sit between those for single-species assays (with elements from both ‘plant’ and ‘non-plant’ criteria) and those for mesocosm exposures.
- Clarification is needed on minimum data requirements for community endpoints (e.g., number of community studies). While Warne et al. (2025) does not specify this, earlier guidance (ANZECC/ARMCANZ, 2000) suggested three studies as a minimum requirement. Importantly for microbial communities, their strong heterogeneity at small scales will need to be considered.
- If genomic approaches are to be used, appropriate QA/QC is needed for the protocol development, experimental design, sample processing, bioinformatics and data interpretation. Specific QA/QC guidance for genomic studies is needed for regulatory acceptance.



Pathway 3. **Supportive evidence (multiple lines of evidence)**

Microbial effects data at the single-species, taxon-level, or whole-community level that meet minimum use criteria but fall outside the integration pathways above can only be used as supporting evidence under current guidance. In practice, such data may be used to justify the addition of a caveat to a GVs reliability rating and/or be considered during the sense-check phase.

Examples (non-microbial, illustrative):

- Taxon-level data for invertebrates from three mesocosm studies were used as supporting evidence in a DGV for chlorpyrifos.
- At the sense-check phase, taxon-level field data for invertebrates were used to support use of the 99% species protection value for endosulfan in slightly to moderately disturbed systems, in place of the high reliability 95% species protection value.

Overall gaps in the three pathways for microbial data use:

- Based on commentary and reference in Batley et al. (2018) and Warne et al. (2025), the above approach (pathway 2) for taxon-level data would also be applicable to whole-community endpoints, however this could be resource intensive to generate sufficient data to fit the current requirements.
- In an earlier section of this white paper, there was a strong consensus in the participant group to shift towards functional endpoints for highest ecological relevance. However, where and how these types of endpoints fit within the current DGV framework is unclear, including whether these data types would fall into the same category as whole community endpoints for data use and integration. In addition, functional endpoints are not well suited to the application of statistical distribution methods because of ambiguity in defining functional “units”.
- Guidance is needed to advise on the best use of whole-community endpoints identified in Topic 2 as high priority for informing DGVs, including the setting of thresholds to define impact to whole communities.
- The current requirement is that distributions should only combine data collected at the same level of taxonomic organisation (i.e., species or a higher taxon level, but not a mix of both), to ensure interpretability. However, this risks marginalising high quality evidence from different study types as supportive data only, limiting the ability to derive DGVs. There is a significant gap in alternative derivation methods that use whole community and functional data and still align with % protection.
- AMR presents a specific challenge for microbial endpoint integration because it is related to, but distinct from, conventional toxicity. AMR endpoints may not fit cleanly within current species-level or taxon-level DGV pathways, and may instead be considered as functional or whole-community endpoints within a multiple-lines-of-evidence framework. This is particularly important because AMR selection is not only driven by antibiotics, but also by other toxicants such as metals, biocides and complex mixtures that promote co-selection. Further guidance is needed on how AMR endpoints should be interpreted in guideline value derivation, including whether they should inform DGVs, SSGVs, caveats, or separate AMR-focused risk thresholds.

Final DGV selection

Following derivation of multiple candidate DGVs, the MLoE approach may be applied to select the 'best' value or combine values (e.g., geometric or weighted mean), whilst also using supporting evidence to inform reliability, protection levels and final DGV selection. Worked examples are available at www.waterquality.gov.au/anz-guidelines/guideline-values/derive/mloe.

Gaps/issues:

- Reliability criteria for DGVs derived from community-based endpoints require reconsideration. In ANZECC/ARMCANZ (2000), community-based DGVs were classified as high reliability even though a percentage level of species protection could not be assigned, reflecting recognition of the high ecological relevance of community endpoints. In later guidance, high-reliability classification for these endpoints was removed (see Batley et al. 2018). In practice, because community datasets were rarely available, the AF was typically applied to the limited mesocosm data. Therefore, given the limitations of this approach (and related methods), it was no longer considered sufficiently robust to support a high-reliability value (Batley et al., 2018).
- Improved approaches are needed to ensure that ecologically relevant community-level data can contribute meaningfully to high-reliability DGVs.

Shared challenges across all non-species level endpoints

Many of the conceptual and methodological issues identified in Topics 1, 2 and 3, are not unique to microbial endpoints. Similar limitations arise for a wide range of micro- and mesocosm experiments involving eukaryotes, where responses are expressed at the level of communities, functions, or interacting taxa rather than as effects on single, isolated species. Traditional species-based frameworks struggle to accommodate such endpoints, particularly where they represent emergent properties or ecosystem processes. Consequently, improving the integration of microbial endpoints provides an opportunity to address broader limitations in DGV derivation methods. Developing transparent, consistent approaches for incorporating community-level and functional data will strengthen the ability of DGVs to reflect ecologically meaningful responses across taxa, rather than treating microbial endpoints as a special case. Additionally, examining international approaches to community endpoints will help inform fit for purpose pathways for their use in DGV derivation



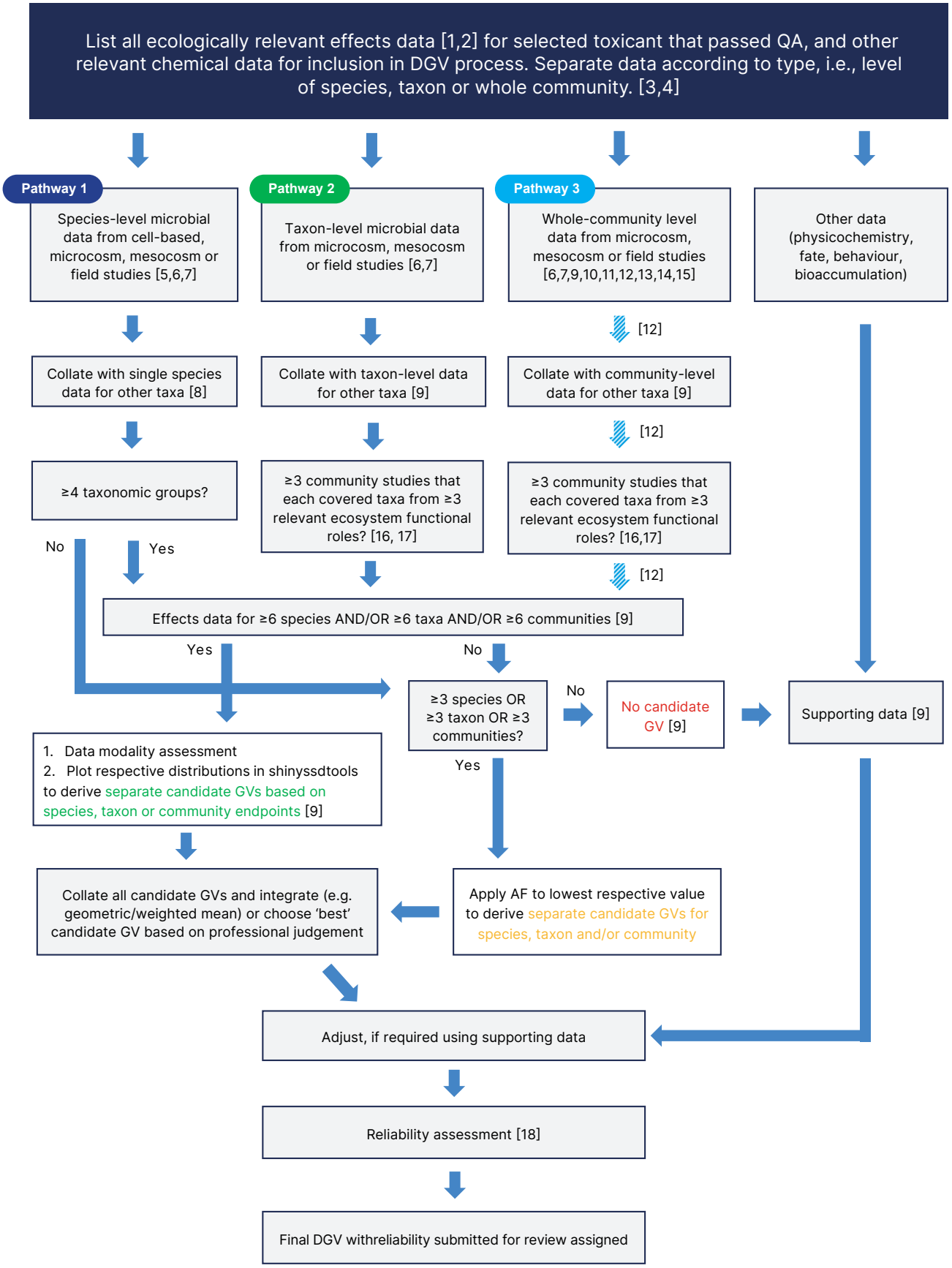


Figure 3. Current possible pathways for microbial endpoint use to derive DGVs. Note, this flow-chart represents a MLOE approach, predominantly designed for use in deriving SSGVs. This approach would be deemed acceptable for DGVs but assessed on a case-by-case basis. We suggest a significant enabler would be to recognise MLoE as a formal DGV process. Arrows indicate pathways based on data types and associated criteria.

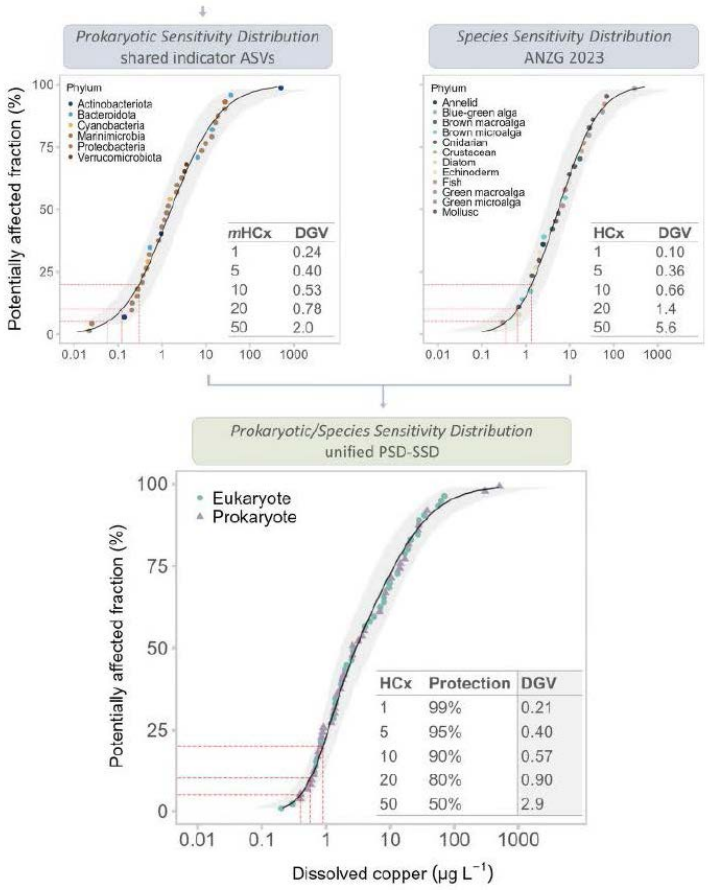
Numbered items in the diagram relate to key issues and gaps highlighted in this white paper:

1. Updated definition for 'ecologically relevant endpoints' needed (Topic 1)
2. Which microbial endpoints are most ecologically relevant and ready to use, and how will antimicrobial resistance effects be captured (Topic 2 and Topic 3)
3. Chronic effects preferred, but current definition of >24 h for microbial endpoints may not be appropriate. GAP
4. Difficulty measuring toxicant concentrations at very low concentrations and in very small volumes used in microbial assays – will restrict the development of data using measured values – need affordable standardised approaches for trace-level low-volume analyses. GAP
5. Single species microbial assays identified as low ecological relevance, yet this pathway for microbial data use is best described.
6. Updated microcosm QA criteria and data use rules required. If microcosm data are assessed using criteria for 'microcosms, mesocosms and field data', many will fail due limited taxonomic level coverage (e.g., bacterial microbiome). Lab-based QA criteria do not currently address microcosm data types as elements of both 'plant' and 'non-plant' criteria. This excludes studies reporting whole-community endpoints for communities with 1-2 taxonomic groups (e.g., bacterial microbiomes) from being used in deriving DGVs. GAP.
7. Field data gap: limited microbial (including AMR) baseline data for reference conditions. GAP
8. Lack of clarity on how to solve the issue of a single study dominating an SSD, putting more weighting on that study than others from single-species assays in the distribution. Raised in Topic 2 and 3. Guidance needed – GAP.
9. Data can only be combined for use in a distribution, if they are at the same level of taxonomic description, e.g., broad groupings like 'phytoplankton, bacteria, fungi' or specific taxonomic groupings like genus, family or order level. Current guidance states that data for different levels of taxonomic organisation cannot be combined in one distribution. This presents a risk of no DGV if sufficient data for a distribution or AF approach at the one taxonomic organisation are not available. All data would be regarded as supportive but insufficient to derive a DGV. Further research is needed to inform possible alternative approaches to derive DGVs that include all relevant data when other methods cannot be used. Discussed in Topic 3. Requires further research to inform - GAP.
10. Lack of clarity over which endpoints are acceptable to represent whole-community effects (TOPIC 2).
11. Missing data standards, thresholds and QA/QC for community endpoints, particularly omics-based endpoints
12. It is unclear if all whole community endpoints and community functional endpoints discussed in Topic 2 will all fall into this category, and how they can be combined. The lighter shaded arrow for this pathway indicates uncertainty that requires further research to inform future guidance. GAP.
13. AMR endpoints are a distinct problem not captured by standard ecotoxicity endpoints. Assays will need to capture both resistance and ecotoxicity endpoints.
14. AMR assessment currently lacks regulatory GV pathway (though work is underway – Binet et al., 2025).
15. Metagenomics seen as the only direct tool to measure resistance endpoints in microbial communities; however, research is needed to establish such assays and appropriate thresholds for use in DGV derivation.
16. Warne et al (2025) does not specify how many community-based studies are required, but ANZECC/ARMCANZ (2000) specified three studies. This requires clarification in future guidance.
17. One of the acceptability criteria for microcosm, mesocosm and field data is that they are "representative of the taxa distribution and trophic structure in the ecosystem being assessed and should contain at least invertebrates, phototrophs and organisms associated with nutrient cycling". Here, we have simplified this to being three ecosystem functional roles but have italicised the words to denote this is not the official wording in the guidance, which is open to interpretation. If this change in wording was adopted, it could enable microcosm data use, provided microcosm effects data could be related to functional roles.
18. Process for determining reliability is very clear for DGVs based on single-species endpoints, but those for community (taxon-level and whole community-level) data pathways require further clarification. ANZECC/ARMCANZ (2000) considered any DGV estimated using community-based endpoints as higher-tier and would result in a high reliability DGV. However, this was removed in Warne et al. (2025). – GAP.

How have researchers attempted to address some of these gaps?

Researchers have begun exploring approaches that allow community-level microbial datasets to be analysed alongside (and, where defensible, combined with) single-species toxicity data to support candidate values that are protective of both microbial communities and higher-order taxa (Figure 4). These provide exemplary alternative pathways for microbial data integration and use when deriving DGVs

Figure 4. Case study examples of alternative methods to integrate microbial data with traditional single-species toxicity data to inform hazard assessments. Reproduced, with permission from Thomas (2025); Binet et al. (2025); King et al. (2024) and Fajana et al. (2023).

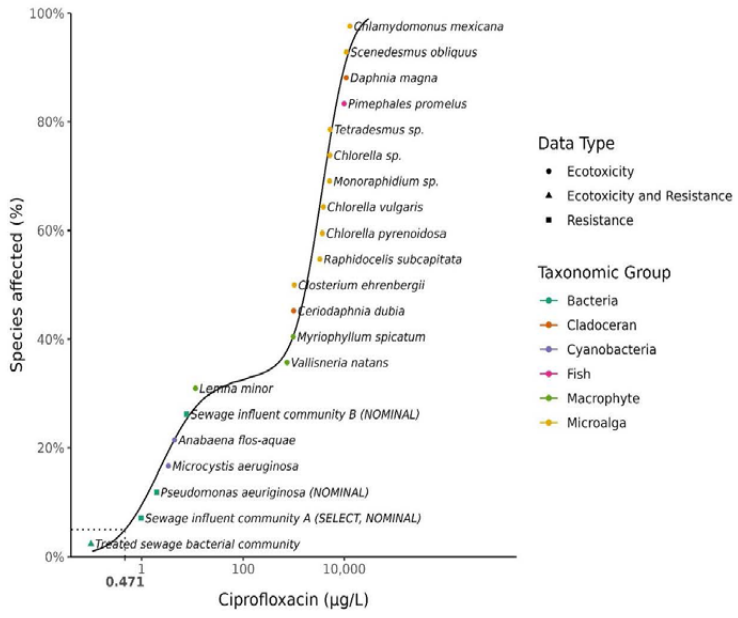


Case Study 1

Thomas (2025) explored the option of combining data extracted at the level of amplicon sequence variants (assumed to be species-level) from exposures of aquatic microbiomes to copper with existing single species copper data used in the ANZG Cu DGV.

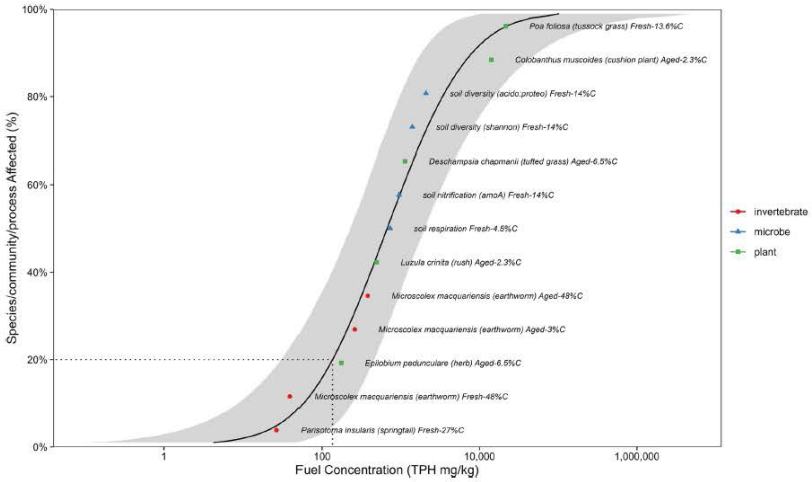
Case Study 2

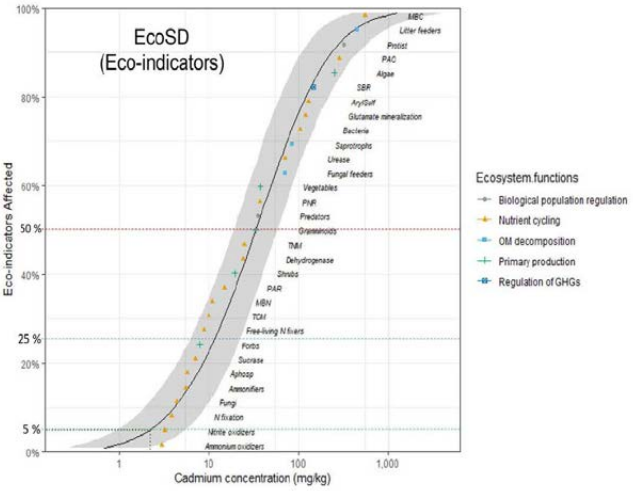
Binet et al. (2025) proposed a framework to enable microbial endpoints, including those related to AMR to be combined with traditional toxicity-based endpoints in a combined sensitivity distribution. This would enable protection of aquatic ecosystems from two types of impact that can result from anthropogenic pollution: direct toxicity and emergence and spread of environmental AMR. The authors argue this as being particularly important for antimicrobials, which are specifically formulated to kill microbes, but for which there are no current DGVs.

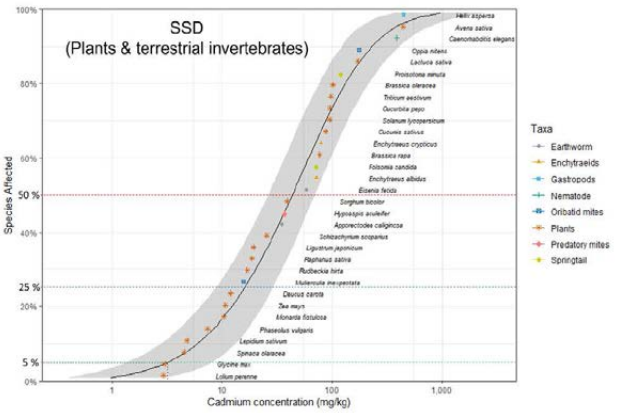


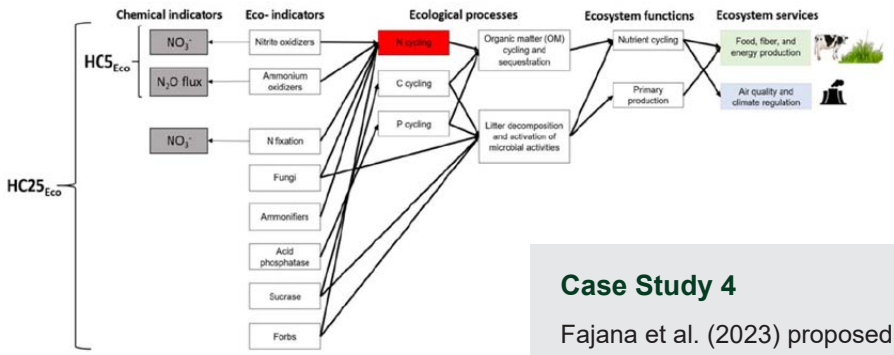
Case Study 3

King et al. (2024) derived SSGVs for sub-Antarctic soils on Macquarie Island combining single-species endpoints with microbial community and functional endpoints in an SSD. An adapted set of criteria, based on expert judgement was used to qualify data usage and enable use of non-traditional endpoints. Importantly, it enabled inclusion of microbial community- and process-based tests which the authors argue are more ecologically relevant to the soil community and overall soil health, particularly in these polar environments where diversity is relatively low and availability of traditional endpoints with standard test taxa are limited.



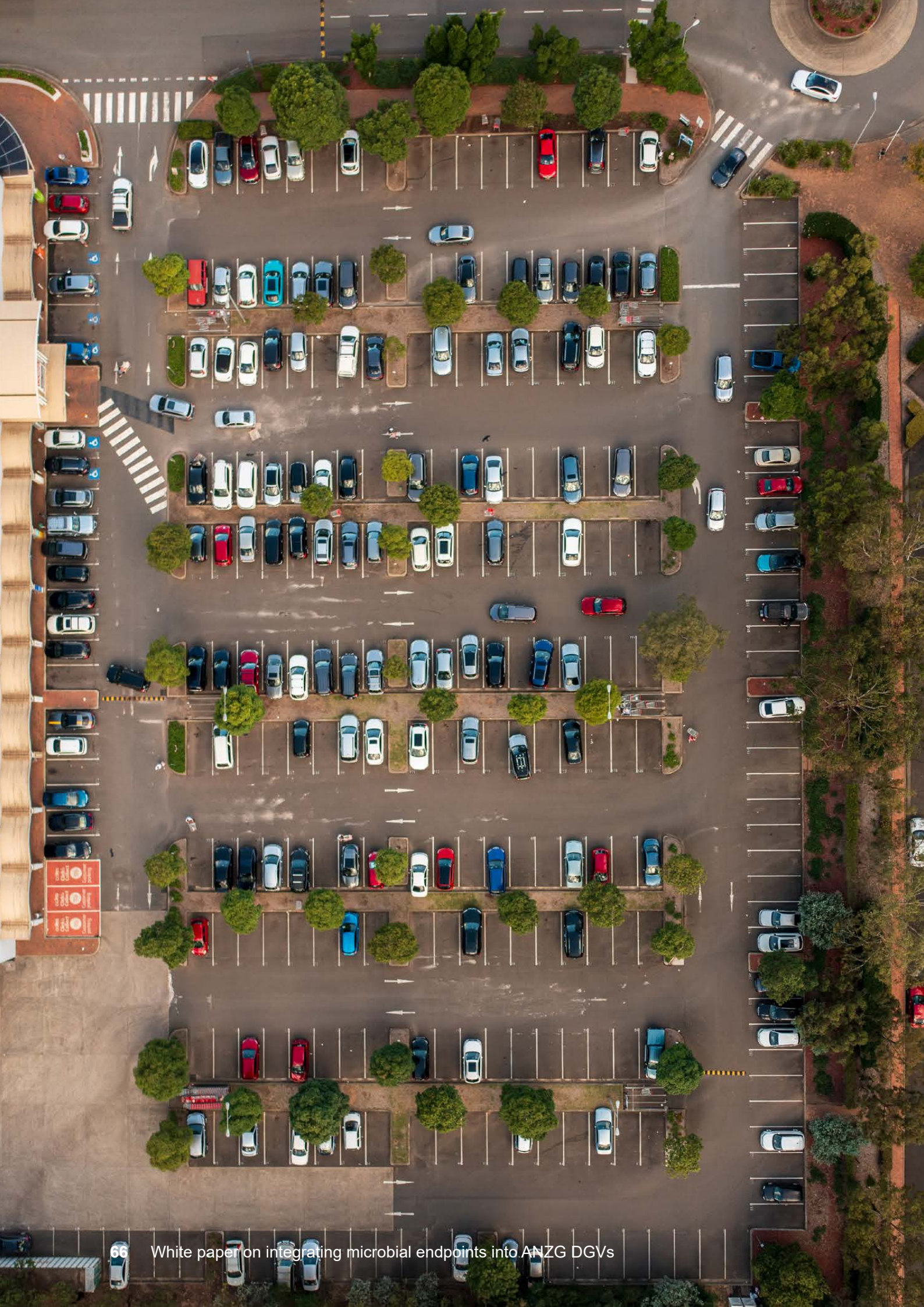






Case Study 4

Fajana et al. (2023) proposed the use of eco-indicator sensitivity distributions (EcoSD) for enabling more holistic risk assessment of soils using a process that informs impact to ecosystem services, as opposed to traditional SSD approaches that inform impact to particular species. The EcoSD fits “eco-indicators” as representatives of ecological functional groups and soil processes instead of individual species responses to a statistical distribution. The authors related eco-indicators directly to critical ecosystem functions that drive ecosystem services.



The ‘Parking Lot’

During consultation, several issues were identified as important but outside the scope of this white paper. They are recorded here in this ‘parking lot’ to acknowledge stakeholder input and to signal topics that require separate consideration in future work. Key themes included how DGVs are applied in practice versus their intended use, and how microbial endpoints for deriving DGVs interface with broader ERA and regulatory decision-making processes. These topics are noted for future, dedicated work alongside implementation of microbial endpoints for inclusion in DGV derivation for toxicants.

Local water quality and exposure considerations

The need to account for local context and scenario-specific considerations was also raised (e.g., differing discharge types, receiving environments and exposure pathways). Many of these points align more directly with processes for developing SSGVs rather than DGVs, the primary focus of this paper; however, microbial endpoints established for DGV derivation are expected to be adaptable for use within SSGV frameworks. Also, it is worth highlighting that many of the novel methods used to derive SSGVs could eventually find their way into formal DGV processes, across all endpoint types. In this way, SSGV derivation can operate as a ‘test bed’ for potential new DGV generation methods (van Dam et al., 2019).

Context-specific microbial endpoints, such as those that use sewage-based consortia (e.g., SELECT assay; Murray et al., 2020) have been proposed to inform environmental AMR risk assessments for sewage effluents and antimicrobial manufacturing wastewaters. Whilst such an approach is not applicable within the ANZG (2018) framework for toxicants (see below, Mixtures) and sits outside the scope of the current white paper, there may be merit in considering context-specific approaches to address specific risk profiles relevant to discharge types. This could vary according to discharge types, noting, however, that there has been a shift away from this kind of approach in Australia and New Zealand, with effluent management guidelines currently available as historical information resources only, and no longer form part of formal guidance. Current approaches for complex mixtures are addressed below.

Mixtures

Mixtures were highlighted as a practical consideration because most point-source discharges to the environment occur as complex combinations of stressors. Under ANZG guidance, mixtures can be addressed using DTAs, and other ecosystem indicators to inform risk (ANZG, 2023). Accordingly, any microbial endpoints developed and approved for use in deriving DGVs should also be considered for application within DTAs, and as additional broader ecosystem indicators. Future guidance should make this linkage explicit. Equally, toxicity identification evaluations (TIEs) which are used to inform the causative toxicants within mixtures, could be adapted for use with microbial endpoints.

Sediments and other non-surface water reservoirs for microbiomes in aquatic ecosystems

This white paper focuses primarily on water quality guideline value derivation, while recognising that sediments are an important component of aquatic ecosystems and are considered throughout the document where relevant. Microbial diversity and abundance are often greatest on and within living and non-living surfaces including sediments, biofilms, detritus and suspended particles. Further consideration is required to identify which microbiomes should be represented in DGVs and how microbial endpoints can be coherently addressed across sediment and surface water guidelines and their respective DGVs.

Application to groundwater ecosystems

Groundwater was identified as a distinct application area where surface-water DGV derivation approaches may not be directly transferable. However, microbial endpoints and methods developed through the roadmap in this white paper are likely to be informative for groundwater assessments, where microbial taxa are often dominant. While groundwater quality guideline value development is being progressed through other work programs, much of this is focussed on higher organisms, e.g., stygofaunal crustaceans (Hose, 2004). Surveillance of microbial communities in groundwater is underway and a shift in focus is needed to consider microbial communities as useful for informing DGVs. The importance of separately assessing the applicability of the microbial endpoints explored in Topic 2 to groundwater was also highlighted, as current research in New Zealand indicates a need for more sensitive early indicators, such as enzyme-based responses.

Policy and systems issues

Contributors highlighted related policy and systems considerations, including biosolids management (and the potential limitations of assessing chemical-AMR linkages one chemical at a time) and the broader One Health/One Water framing of managing water as a connected resource across sectors. While these considerations are closely connected to risk management for water quality, water reuse and AMR, developing One Water pathways and associated policy settings is beyond the scope of this white paper.

Other ‘parked’ issues

Additional out-of-scope topics included the following:

- Specific methodological development, including challenges associated with field-based endpoints where reference communities may change rapidly over time.
- Applicability of the definition of “ecological relevance” within the broader context across all biota (beyond microbes).
- Wider limitations of existing DGVs, including those that we well known and accepted as a limitation for lab-based assays, e.g., the removal of environmental microbial communities from their natural environment for use in microcosm assays in the laboratory. The limitations of lab-based assays, microcosms, mesocosms and field data for use in DGVs is addressed sufficiently in Warne et al. (2025) and Batley et al. (2018).



Priority Actions and Research Needs

Governance, engagement and implementation

- Establish a transdisciplinary expert working group, including end-users (e.g., regulators, consultants) to guide method development, interpretation and implementation, including prioritisation of actions and targeted funding opportunities.
- Develop and implement an engagement and communication plan to ensure ongoing end-user involvement and co-design, support end-user capacity building, data access and stewardship principles for microbial and AMR datasets, and to reframe microbial impacts as ecosystem-level impacts. End-users include (but are not limited to) regulators, water utilities, First Nations, environmental consultants, environmental managers and researchers.

Protection goals and conceptual framing

- Define ecosystem protection goals that explicitly incorporate microbial community and functional endpoints, and that can be translated into risk assessments with the same level of precautionary confidence as the current percentage-of-species protection approach.
- Update guidance to provide clearer use-cases and integration pathways for microbial whole-community and functional endpoints within the Water Quality Management Framework, including an explicit statement of their ecological relevance.

SSD framework and integration pathways

- Explore and compare alternative approaches to integrating microbial data for selected toxicants (e.g., separate microbial SDs, combined SDs, and WoE/MLoE approaches), and assess the impact of their inclusion on resultant DGVs.
- Develop justifications for including microbial whole-community endpoints as “special case” applications where appropriate.
- Develop a decision matrix to prioritise toxicants for which microbial data-informed DGVs are urgently required.
- Clarify rules for combining data collected at different taxonomic levels (species, taxon, whole community), noting current guidance limitations.

Microcosms, mesocosms and community data use

- Establish acceptance criteria specifically for microbial microcosms, particularly those covering only one or two taxonomic levels (e.g., bacterial microbiomes).
- Clarify minimum data requirements for community-based endpoints, including the number of community studies required (e.g., revisit the “three studies” historical guidance).

Chronic exposure and exposure verification

- Review “chronic exposure” for microbial assays, beyond the current “>24 h” definition, through expert consultation that balances feasibility with ecological relevance for microbes.
- Develop and standardise affordable exposure-verification approaches for small-volume and trace-level microbial assays.
- Identify and connect laboratories with trace-level analytical capability to support microbial effects testing.

SSD and data handling

- Develop practical guidance for integrating SSDs, including rules for handling and balancing large, high-resolution community/omics datasets (e.g., weighting, aggregation) to prevent disproportionate influence of individual studies.

Community-level and functional endpoints

- Use AOPs and conceptual ecological models to define meaningful thresholds for microbial community-level and functional endpoints.
- Establish comparability between microbial community/functional thresholds and traditional ecotoxicity metrics (e.g., NEC, NSEC, EC10).
- Clarify which whole-community and functional endpoints are acceptable to represent ecologically relevant impacts in DGV derivation.
- Identify priority microbiomes to target for inclusion in DGVs, considering surface waters, benthic, biofilm and host-associated communities and relative functional groups.

Omics and genomic endpoints

- Develop or adopt QA/QC and metadata standards for genomic and omics-based microbial endpoints to support regulatory acceptance.
- Clarify the regulatory role of amplicon, metagenomic and metatranscriptomic endpoints, including their use as primary vs supportive evidence.
- Develop guidance for assessing functional inference from genomic data, including confidence and uncertainty.

Reliability classification and final DGV selection

- Review and revise reliability criteria for DGVs derived from community-based endpoints, noting changes from ANZECC/ARMCANZ (2000) to Warne et al. (2025).
- Clarify how community-based candidate GVs contribute to final DGV selection, including use within MLoE frameworks.

AMR-specific actions

- Build on earlier work (Binet et al., 2025) to define the policy framing and decision context for AMR endpoints in guideline derivation.
- Identify AMR-relevant microbial endpoints for DGVs, recognising AMR as distinct from, but crucially linked to conventional toxicity.
- Establish and validate assays and thresholds for resistance endpoints, particularly metagenomic approaches.
- Collect targeted AMR data for priority contaminants, including non-antimicrobial drivers.

Data quality, baselines and regulatory readiness

- Establish minimum reporting, metadata and QA/QC requirements for microbial and AMR datasets used to derive or support DGVs and SSGVs.
- Develop criteria and decision pathways for transitioning microbial endpoints from research-grade to regulatory-ready status.
- Recognise computational and bioinformatic capability as core capacity, including access to validated, reproducible analytical workflows.
- Use existing microbial and AMR monitoring datasets, where suitable, to inform endpoint selection, baseline variability, study design and interpretation.
- Undertake targeted meta-analyses of suitable published and grey-literature datasets to characterise natural variability in microbial community structure, function and AMR profiles, particularly where field-based endpoints are proposed.





Action Plan and Roadmap

All actions identified within this white paper have been consolidated into an action plan (Figure 5) and associated roadmap (Figure 6). There are four overarching objectives, fifteen key actions, and within each of these actions are specific targets. All targets deliver outcomes through foundational and/or research pathways to inform guidance. The action plan will be delivered through a suite of activities, workshops, projects and partnerships. Recognising the breadth of the plan, an early priority is to establish an advisory committee to guide prioritisation of the most essential actions.

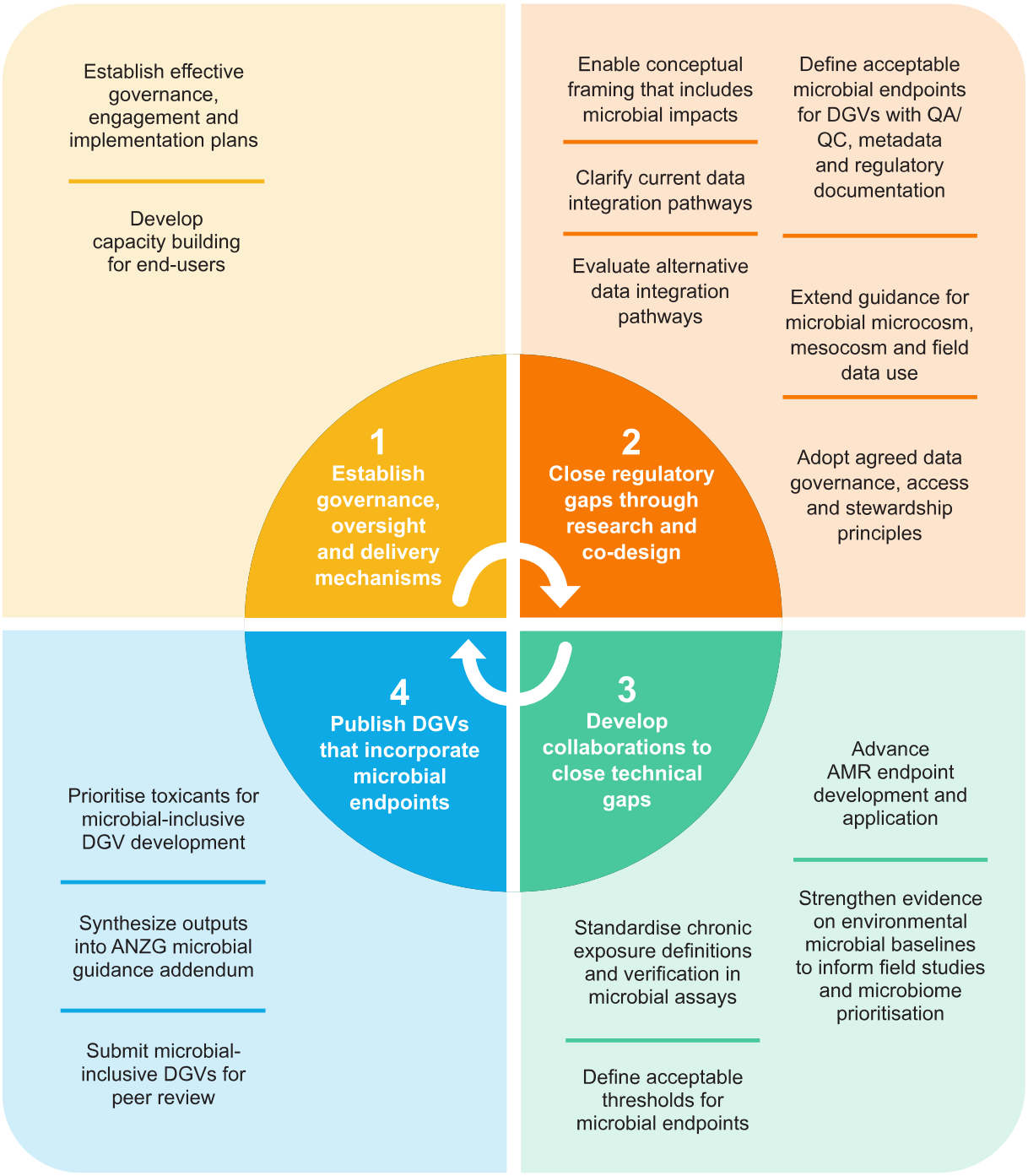
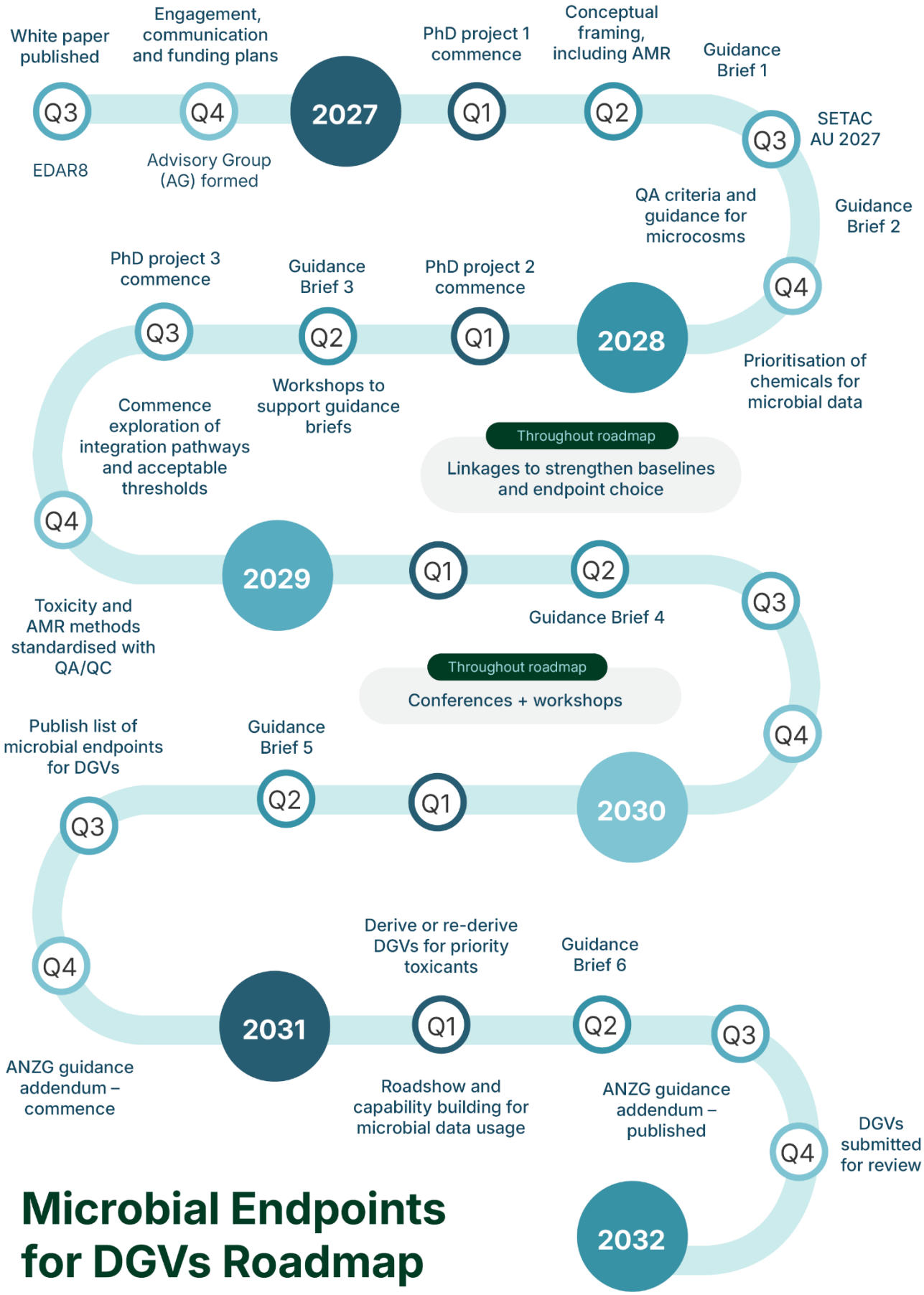


Figure 5. Action plan for enabling microbial endpoint use in ANZG DGVs for toxicants.



Microbial Endpoints for DGVs Roadmap

Figure 6. Roadmap for deriving DGVs that include microbial endpoints for toxicity and AMR. The staged actions shown here also address current limitations in data availability, methods, and regulatory readiness identified in this white paper.

Icons denote delivery types

Research task | Guidance task | Foundational task

OBJECTIVE 1: Establish governance, oversight and delivery mechanisms for the Action Plan

Action	Target / task	Output(s)	Delivery	Type
A1: Establish governance, engagement and implementation plans	T1. Establish transdisciplinary expert working group and reporting cadence	Transdisciplinary expert working group (WG) established; Terms of Reference (TOR), EOI report, quarterly reporting package, and meeting minutes produced	WG Dec 2026; TOR Feb 2027; reports – quarterly (2026–2032)	
	T2. Develop engagement, communication and funding plans. Engagement plans will consider all key stakeholders, including First Nations.	Communication, engagement and funding plans produced; conference papers and workshop reports delivered (various)	Dec 2026	
A2: Develop capacity building for end-users	T3. Develop targeted capacity building for end-users	Develop targeted capacity building training to enhance regulators' ability to integrate microbial endpoints into risk assessments and decision-making	2030-2032	

OBJECTIVE 2: Close regulatory gaps to enable generation and use of microbial data in DGV derivation

Action	Target / task	Output(s)	Delivery	Type
A3: Enable conceptual framing that includes microbial impacts	T4. Define and document ecosystem protection goals that explicitly incorporate microbial community and functional endpoints	Guidance brief	2027	
	T5. Make an explicit statement on the ecological relevance of microbial endpoints in DGV derivation	Current white paper; guidance brief	2027	
	T6. Provide clearer use cases for microbial whole community and functional endpoints within the National Water Quality Management Framework to support regulator uptake by addressing limited microbiology expertise.	Guidance brief (dependent on A3–A6)	2032	

Action	Target / task	Output(s)	Delivery	Type
A4: Adopt agreed data governance, access and stewardship principles for microbial and AMR datasets used in DGV derivation.	T7: Adopt shared data governance principles, reproducible analytical workflows, guidance on uncertainty treatment and communication of complex outputs, and clearer pathways for transitioning methods from research to regulation shift	Guidance brief	2027	
A5: Clarify current microbial data integration pathways	T8. Update guidance to include clarified current pathways for microbial data in DGV derivation (in this white paper)	Current white paper; guidance brief	Guidance brief 2027; microbial guidance addendum 2031; formal update 2032	
	T9. Clarify current rules for combining data collected at different taxonomic levels (species, taxon, whole community), noting current guidance limitations	Guidance brief, including practical decision support tools (e.g. flowcharts, decision matrices and worked examples).	2027	
	T10. Develop practical guidance for integrating SSDs, including rules for handling and balancing large, high-resolution community/omics datasets	SETAC workshop report; research paper; guidance brief	Workshop 2027; paper 2028; brief 2029	
	T11. Clarify how percentage species protection values (e.g., PC95) should be interpreted when non-species microbial endpoints are used	Research paper; guidance brief	Paper 2028; brief 2029	
A6: Evaluate alternative microbial data integration pathways	T12. Establish methods to translate thresholds for microbial endpoints into risk assessments with comparable precautionary confidence (applied to selected high-priority toxicants)	Research paper; guidance brief	Paper 2029; brief 2030	
	T13. Achieve consensus on microbial data integration methods and thresholds	Workshop report/paper; research paper; guidance brief	Workshops 2029; brief 2030	

Action	Target / task	Output(s)	Delivery	Type
A7: Define acceptable microbial endpoints for DGVs with established minimum reporting, metadata and QA/QC requirements for microbial endpoints used to derive or support DGVs and SSGVs.	T14. Clarify which whole-community and functional endpoints are acceptable to represent ecologically relevant impacts in DGV derivation, and which microbiomes within an aquatic ecosystem to prioritise for inclusion in DGVs (considering surface waters, benthic, biofilms and host-associated, as well as functional groupings)	Current white paper; research papers/workshop outputs; guidance brief	White paper 2026; paper 2027; workshop report 2026; brief 2028	
	T15. Develop QA/QC and metadata standards for genomic and omics-based microbial endpoints	Research paper(s); guidance brief	Papers 2031; briefs 2031	
	T16. Clarify the regulatory role of amplicon, metagenomic and meta-transcriptomic endpoints (primary vs supportive evidence)	Research paper(s); guidance brief	Papers 2031; briefs 2031	
	T17. Develop guidance for assessing functional inference from genomic data, including confidence and uncertainty	Research paper(s); guidance brief	Papers 2031; briefs 2031	
A8: Extend guidance for microbial microcosm, mesocosm and field data use	T18. Establish quality assessment criteria specifically for microbial microcosms	Research paper; guidance brief	Paper 2027; brief 2028	
	T19. Clarify minimum data requirements for community-based endpoints (e.g., revisit the “three studies” precedent)	Guidance brief	Brief 2030	
	T20. Review and revise reliability criteria for DGVs derived from community-based endpoints	Guidance brief	Brief 2030	

OBJECTIVE 3: Deliver collaborative research to address technical gaps and leverage existing programs

Action	Target / task	Output(s)	Delivery	Type
A9: Standardise chronic exposure definitions and verification in microbial assays	T21. Redefine “chronic exposure” for microbial assays beyond the current “>24 h” definition	Guidance brief	Brief 2028	
	T22. Develop and standardise affordable exposure-verification approaches for small-volume and trace-level toxicant exposures	Method + collaboration established; research paper; guidance brief	Collaboration 2027; paper 2028; brief 2029	
A10: Define acceptable thresholds for microbial endpoints	T23. Use AOPs and conceptual ecological models to define meaningful thresholds for microbial community-level and functional endpoints	Research paper; proposed list of thresholds	2028	
	T24. Establish comparability between microbial thresholds and traditional ecotoxicity metrics (e.g., NEC, NSEC, EC10)	Research paper	2028	
A11: Advance AMR endpoint development and application	T25. Define the policy framing and decision context for AMR endpoints in DGV derivation	Workshop paper; research paper; guidance brief	Workshop 2026; paper 2027; brief 2027	
	T26. Identify AMR relevant microbial endpoints	Workshop paper; research paper; guidance brief	Workshop 2026; paper 2027; brief 2027	
	T27. Establish and validate assays and thresholds for resistance endpoints (particularly metagenomic approaches)	Standard methods and papers	Methods developed/ validated 2028	
	T28. Collect targeted resistance and toxicity data for priority contaminants (including non antibiotic drivers where no DGV exists)	Papers and reports	Data published 2029	

Action	Target / task	Output(s)	Delivery	Type
A12: Strengthen evidence on environmental microbial baselines to inform field studies and microbiome prioritisation	T29. Leverage any baseline monitoring and surveillance of microbial communities and AMR to inform endpoint development (e.g. which microbiomes to use in assays for DGVs)	Papers and reports (linked to monitoring program)	Throughout (linked to monitoring program)	
	T30. Undertake meta-analyses to establish natural baseline structure and function for microbial communities (including AMR profiles). This is only useful for field studies, and less relevant for DGVs in general.	Papers and reports (linked to monitoring program)	Throughout (linked to monitoring program)	

OBJECTIVE 4: Publish DGVs that incorporate microbial endpoints

Action	Target / task	Output(s)	Delivery	Type
A13: Prioritise toxicants for microbial-inclusive DGV development	T31. Develop a decision matrix to prioritise toxicants for which DGVs with microbial data are required	Research paper; priority list for DGVs; guidance brief	Paper 2027; brief 2028	
A14: Synthesize outputs into ANZG microbial guidance addendum	T32. Produce endorsed table of microbial endpoints for use in DTAs and deriving DGVs	Addendum to Warne et al., and future updated guidance; conference/ roadshow reports	Addendum published 2032	
	T33. Collate all guidance briefs and research papers to inform an addendum guidance document for ANZG to inform microbial data use in deriving DGVs. Advisory Group to select authors and oversee delivery	Addendum to Warne et al., and future updated guidance; conference/ roadshow reports	Addendum published 2032	
A15: Submit microbial-inclusive DGVs for peer review	T34. Gap analysis for any outstanding guidance updates	Research papers	2031	
	T35. Derive DGVs that include microbial endpoints for priority toxicants	Draft toxicant guideline briefs	2032	



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Appendix A

A1: List of Abbreviations

Abbreviations are listed alphabetically. Where an abbreviation has a standard meaning, no further note is provided. Where contextual clarification is useful for readers of this white paper, a brief note is included.

Abbreviation	Full Term	Contextual Note
16S rRNA	16S ribosomal ribonucleic acid	Marker gene used in amplicon sequencing to identify and classify bacteria and archaea
ACTRA	Australasian College of Toxicology and Risk Assessment	
AF	Assessment factor	A precautionary multiplier applied to toxicity data when deriving guideline values from limited datasets
AIMS	Australian Institute for Marine Science	
AMR	Antimicrobial resistance	
AMRIA	AMR Industry Alliance	
ANZ	Australia and New Zealand	
ANZECC	Australian and New Zealand Environment and Conservation Council	
ANZG	Australian and New Zealand Guidelines for Fresh and Marine Water Quality	Historically, ANZG referred to the Australian and New Zealand Governments; however, DCCEEW have advised that ANZG is now used to refer to the Australian and New Zealand Guidelines.
AOP	Adverse outcome pathway	A conceptual framework linking molecular initiating events to adverse outcomes at the organism or population level
ARMCANZ	Agriculture and Resource Management Council of Australia and New Zealand	
ASV	Amplicon sequence variant	High-resolution DNA sequence read used in amplicon sequencing; more precise than OTUs
CSIRO	Commonwealth Scientific and Industrial Research Organisation	
DCCEEW	Department of Climate Change, Energy, the Environment and Water	
DGV	Default guideline value	
DNA	Deoxyribonucleic acid	
DTA	Direct toxicity assessment	Also known as Whole Effluent Toxicity (WET) testing in other countries.

Abbreviation	Full Term	Contextual Note
EC10	10% effect concentration	The concentration of a toxicant causing a 10% effect on the test endpoint; used as a proxy for a no-effect threshold
EOI	Expression of interest	
ERA	Environmental risk assessment	This is different to environmental health risk assessment
FAPROTAX	Functional Annotation of Prokaryotic Taxa	A database used to predict ecological functions of microbial communities from 16S rRNA gene data
HGT	Horizontal gene transfer	The transfer of genetic material between organisms other than by direct descent; important for AMR spread
GV	Guideline value	Broadly refers to any water or sediment quality guideline value (see also DGV, SSGV)
ITS	Internal transcribed spacer	Marker gene region used in amplicon sequencing to identify fungi
ISO	International Organization for Standardization	
MARA	Microbial Assay for Risk Assessment	A multi-species bacterial assay used to assess toxicity across multiple cultured microbial taxa simultaneously
MLoE	Multiple lines of evidence	An approach that integrates data from several sources (laboratory, field, mesocosm) to inform a guideline value or risk assessment
NEC	No effect concentration	A statistically derived threshold below which no significant effect on the endpoint is expected (different to a no observed effect concentration, or NOEC).
NEPM	National Environment Protection Measure	Australian regulatory instrument; referenced for its 25% change threshold for soil functional endpoints
NSEC	No-significant-effect concentration	The concentration below which no statistically significant effect is observed
OECD	Organisation for Economic Co-operation and Development	
OTU	Operational taxonomic unit	A cluster of similar DNA sequences used to approximate species in amplicon sequencing; less precise than ASVs
PC	Protection concentration (HC)	The contaminant concentration protecting a specified percentage of species or taxa (e.g. PC95 = 95% protection)
PC90	Protection concentration at 90%	The concentration estimated to protect 90% of species or taxa
PC95	Protection concentration at 95%	The concentration estimated to protect 95% of species or taxa; commonly used in ANZG DGV derivation

Abbreviation	Full Term	Contextual Note
PCR	Polymerase chain reaction	A molecular technique used to amplify specific DNA sequences; central to amplicon sequencing and viability PCR
PNEC	Predicted no-effect concentration	
PSD	Prokaryotic sensitivity distribution	A sensitivity distribution constructed from microbial (prokaryotic) taxon-level effect thresholds; analogous to SSD
qPCR	Quantitative polymerase chain reaction	A method for quantifying specific DNA or RNA sequences; used to measure functional genes and AMR genes
QA/QC	Quality assurance/quality control	
RNA	Ribonucleic acid	
SAAFE[CRC]	Cooperative Research Centre for Solving Antimicrobial Resistance in Agribusiness, Food and Environments	
SETAC AU	Society for Environmental Toxicology and Chemistry Australasian Chapter	
SOS response	SOS DNA repair response	A bacterial stress response triggered by DNA damage; can promote mutagenesis and AMR
SSD	Species sensitivity distribution	A statistical distribution of toxicity values across species, used to derive protective guideline concentrations
SSGV	Site-specific guideline value	A locally derived guideline value that accounts for site conditions; more refined than a DGV
TIE	Toxicity identification evaluation	A process used to identify which components of a mixture are causing toxicity
TOR	Terms of reference	
USEPA	United States Environmental Protection Agency	
WG	Working group	
WHO	World Health Organization	
WoE	Weight of evidence	An approach that considers multiple data types and sources together to draw conclusions about risk or ecological impact

A2: Glossary of Terms

Technical terms are defined as they are used within this white paper. Where a term has a specific meaning under the ANZG framework, that meaning is reflected here. Cross-references to related abbreviations are noted where relevant.

Term	Definition
Adverse outcome pathway (AOP)	A framework linking a molecular initiating event to adverse outcomes at the organism or ecosystem level via a chain of key events. Used to interpret mechanistic data and identify ecologically meaningful effect thresholds.
Amplicon sequencing	A method that sequences a targeted genomic region (e.g., the 16S rRNA gene for bacteria; ITS for fungi) to characterise microbial community composition across many taxa simultaneously. Produces relative or absolute abundance data but does not directly assess gene function.
Antimicrobial resistance (AMR)	The capacity of microorganisms to survive or grow in the presence of antimicrobial agents at concentrations that would normally inhibit them. In an environmental context, AMR is concerning because environmental reservoirs can facilitate the selection and spread of resistance genes with consequences for human, animal, and ecosystem health.
Assessment factor (AF)	A precautionary multiplier applied to toxicity data to account for uncertainty when deriving a guideline value from limited datasets. Under the ANZG framework, use of an AF rather than an SSD results in a guideline value of lower or unknown reliability.
Biofilm	A community of microorganisms attached to a surface within a self-produced extracellular matrix. Biofilms are ecologically important in aquatic systems and can harbour AMR genes and resistant organisms.
Cell-based endpoint	A toxicity measure at the cellular level from assays using microbial test organisms (e.g., growth or luminescence inhibition). These are reproducible and often utilise cultured organisms.
Chronic exposure	An exposure duration sufficient in length to detect sub-lethal effects on test organisms. Chronic exposure is defined in Warne et al (2025) for each taxonomic group. Chronic exposure for microbes is defined as >24h - a threshold that was challenged during the workshop and conference sessions as being too short to be ecologically meaningful.
Community functional endpoint	A measure of an integrated process performed by the whole microbial community (e.g., respiration, nutrient cycling, enzyme activity). These endpoints reflect ecosystem services directly but are context-dependent and lack universally agreed effect thresholds.
Co-selection	The process by which a contaminant (e.g., a metal or biocide) simultaneously selects for resistance to a different agent (e.g., an antibiotic), typically because resistance mechanisms are co-located on the same mobile genetic element.
Default guideline value (DGV)	A nationally derived water or sediment quality guideline value for a toxicant, produced using standardised ANZG methodology. DGVs are designed to protect a specified proportion of aquatic species (typically 95%) and form one line of evidence in environmental risk assessments.

Term	Definition
Direct toxicity assessment (DTA)	An assessment using whole-water or whole-sediment samples to test toxicity to a suite of organisms under realistic exposure conditions. Recommended under the ANZG framework for complex mixtures; can incorporate microbial endpoints.
Dysbiosis	A disruption to the composition, diversity, or function of a microbial community, typically with negative consequences for the health of a host organism or ecosystem.
Ecologically relevant endpoint	Under the ANZG framework, an endpoint that negatively affects a species' ability to increase its genes in subsequent generations. This white paper proposes a supplementary microbial definition: a measurable attribute of community structure, function, or activity whose perturbation is predictive of adverse changes in ecosystem processes or services.
Ecosystem protection goals	Defines what ecological components and functions are to be protected and the acceptable level of change, guiding endpoint selection. For microbiomes, which operate as highly dynamic, functionally redundant communities embedded within larger complex biological and environmental systems, impacts are more appropriately assessed at the community and functional level rather than through the loss of individual species.
Ecotoxicology	The study of the effects of chemical and physical stressors on organisms in controlled, semi-controlled or field exposures. Provides the toxicity data used to derive ANZG water quality and sediment quality guideline values.
Environmental microbe / microorganism	As used in this white paper: all microscopic eukaryotes (e.g., microalgae, protists, yeasts) and prokaryotes (bacteria, archaea) inhabiting natural environments. Excludes viruses, plants and animals.
Eukaryotes	For the purpose of this white paper, the use of the term eukaryotes refers to protists and fungi, but not animals or plants.
Functional gene	A gene encoding a protein with a specific role in ecosystem processes (e.g., nitrogenase for nitrogen fixation; AMR genes for antibiotic resistance). Abundance is typically measured by qPCR or metagenomics.
Functional redundancy	The capacity of multiple taxa to perform the same ecological function, so that loss of one taxon does not necessarily result in loss of that function. Relevant when assessing whether toxicant-induced changes in community composition translate to functional impacts.
Guideline value (GV)	A concentration of a substance in water or sediment at or below which adverse effects on aquatic ecosystem health are not expected. Encompasses both DGVs (national) and SSGVs (site-specific).
Holobiont	A host organism (e.g., coral, fish, plant) together with all its associated microorganisms. Disruption (dysbiosis) of the holobiont microbiome can impair host health and broader ecosystem function.
Horizontal gene transfer (HGT)	Movement of genetic material between organisms other than by parent-to-offspring inheritance. A primary mechanism for AMR spread in microbial communities; can be enhanced by environmental contaminants.

Term	Definition
Metagenomics	Sequencing of total genomic DNA extracted directly from an environmental sample. Provides information on taxonomic composition and functional gene content (including AMR genes / resistome) of the whole community.
Metatranscriptomics	Sequencing of community mRNA to characterise which genes are actively expressed at the time of sampling. Reflects realised functional activity rather than functional potential.
Microbiome	The collective community of microorganisms (and their genes) inhabiting a defined environment—such as a water body, sediment, soil, or host organism—including bacteria, archaea, fungi, protists, and viruses.
Microorganism	For the purposes of this white paper, microorganisms include bacteria, archaea, fungi and protists.
Microcosm	A small-scale, controlled laboratory system replicating simplified aspects of a natural ecosystem, used to expose microbial communities or other organisms to toxicants. Compare with mesocosm.
Mesocosm	A semi-field experimental system, larger and more ecologically complex than a microcosm, that bridges laboratory control and field realism. Mesocosm data can inform candidate guideline values under the ANZG framework.
Mobile genetic element (MGE)	DNA segments capable of moving within or between genomes (e.g., plasmids, transposons, integrons). Key vectors for horizontal transfer of AMR genes across microbial cells and species.
Multiple lines of evidence (MLoE)	Integration of data from multiple sources (laboratory assays, field studies, mesocosms, chemical monitoring) to derive or refine a guideline value or risk assessment. Currently recommended for SSGVs under the ANZG framework; not yet formally adopted for DGVs.
No-effect concentration (NEC) / No-significant-effect concentration (NSEC)	Statistically derived thresholds below which no significant effect on the measured endpoint is expected. Preferred over NOECs and LOECs because they are less influenced by test design.
One Health	An approach recognising that human, animal, plant, and environmental health are interconnected and must be addressed together. Central to this white paper's framing of AMR as a cross-domain risk.
Omics	Molecular profiling approaches that measure large sets of biological molecules simultaneously. Relevant disciplines include metagenomics (DNA), metatranscriptomics (mRNA), proteomics (proteins), and metabolomics (metabolites). Multi-omics combines two or more.
Prokaryote	Prokaryotes are single-celled organisms, including bacteria and archaea, that lack a defined nucleus and membrane-bound organelles.
Quantitative microbiome profiling (QMP)	An amplicon sequencing approach that normalises data to absolute cell or gene copy numbers rather than relative abundances, enabling detection of true changes in living microbial populations.
Resistome	The complete set of AMR genes and their precursors in a microbial community or environment, characterised using metagenomic sequencing.

Term	Definition
Site-specific guideline value (SSGV)	A guideline value derived for a specific location using local data, which may differ from the national DGV. SSGVs can incorporate community-level microbial endpoints and are the primary context in which MLoE approaches are currently applied under the ANZG framework.
Species sensitivity distribution (SSD)	A statistical distribution fitted to toxicity data from multiple species, used to estimate a concentration protecting a specified proportion of species (e.g., PC95). The SSD is the standard method for deriving DGVs under the ANZG framework.
Taxon / Taxa	A group of organisms classified at a particular level of the biological hierarchy (e.g., species, genus, phylum). In microbial ecology, taxon is preferred over 'species' because microbial species boundaries are not always well-defined. Under the ANZG framework, data can be incorporated into distributions at the species, taxon, or whole-community level. Warne et al (2025) define specific ‘taxonomic groups’ for relevance to use in SSDs when deriving DGVs and SSGVs.
Viability PCR	A technique using DNA-intercalating dyes (e.g., propidium monoazide) before PCR to exclude DNA from dead or membrane-compromised cells, allowing sequencing to focus on living community members.
Weight of evidence (WoE)	Integration of multiple lines of evidence to support risk assessment or decision-making. Within the ANZG context, there are two ways this is done: (1) considering data from a range of indicators, e.g., toxicity tests, chemical data and field observations for water quality assessment https://www.waterquality.gov.au/anz-guidelines/resources/key-concepts/weight-of-evidence; (2) Using a mix of data types, e.g., field and laboratory data when deriving site-specific guideline values for water quality stressors. https://www.waterquality.gov.au/anz-guidelines/guideline-values/derive/mloe

Appendix B

B1: SETAC AU/ACTRA Conference Abstracts

Plenary: Microbial Fallout: Pollution-Driven Evolution, Ecosystem Tipping Points, and the Case for New Risk Endpoints

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Anthropogenic chemical stressors—including pesticides, metals, pharmaceuticals, and industrial byproducts—are now pervasive in environments. Together with climate change and human-mediated microbial dispersal, they are altering the evolutionary landscape of environmental microbiomes by acting on the “big three” evolutionary forces: genetic drift (via mutation, recombination, and horizontal gene transfer), competition and migration/dispersal. Yet, these dynamics remain poorly addressed in current risk frameworks.

Traditional microbial monitoring focuses on phylogenetic or taxonomic shifts, but such metrics often fail to capture ecological degradation. Mounting evidence shows that erosion of microbial functional diversity—especially in metabolic and biogeochemical processes—would better reflect the loss of ecosystem services in the Anthropocene. Chronic, low-level chemical exposures consistently reduce functional gene richness and redundancy, even when community composition appears unchanged. Core ecosystem functions such as nitrification, sulfur reduction, organic matter degradation, and maintenance of stable host-associated microbiomes in plants and animals are frequently impaired.

These impacts are exacerbated by interactions with free-living amoebae and other protozoan predators, which over the eons have shaped bacterial stress adaptations. Environmental stressors like reactive oxygen species, metals and pharmaceuticals now amplify microbial survival strategies, including SOS responses, biofilm formation, intracellular persistence, and gene transfer mechanisms—processes that evolved before human influence but now drive emerging threats, such as antimicrobial resistance (AMR) persistence and spread.

This ecological perspective reveals the inadequacy of current risk models focused on single chemical concentrations or growth inhibition thresholds. To account for the emergent properties within microbiomes (i.e., not seen in any single species) and to protect microbial function, guideline values must reflect multiple endpoints, including stress response activation, interspecies interactions, and survival associated with environmental hosts— and must consider realistic environmental exposures involving contaminant mixtures that include transformation products. To meet this paradigm shift, regulation will need to address the full spectrum of environmental stressors affecting microbial evolution, resilience, and function. Overall, preserving microbial functional diversity is essential to sustaining ecosystem services and mitigating long-term ecological risks, such as environmental propagation of AMR.

Session Theme 8B: Identifying 'ecologically relevant endpoints' for microbial processes to inform Environmental Quality Guidelines and One Health approaches

Mining impacts on biogeochemical processes: Insights from the Ranger Uranium Mine

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The Ranger Uranium Mine in the Northern Territory, Australia, is surrounded by the World-Heritage Listed, Kakadu National Park. The Office of the Supervising Scientist was established at the beginning of mining operations to conduct research and monitoring into the potential impacts of uranium mining on communities and ecosystems within the Alligator Rivers Region. A comprehensive research program has investigated the impacts of mine-derived contaminants on various ecosystem components, with the aim of deriving water and sediment quality guideline values for managing mine-water discharges and as targets for closure. Advances in Next Generation Sequencing (NGS) technologies have enabled the characterisation of impacts on microbial communities and their processes. The characterisation of sediment and groundwater biota required the use of DNA-based methods and the studies included analyses of the structure and function of microbial communities. Insightful differences were found in the responses of microbial community structures based on the contaminant type and the environmental matrix. For example, field studies showed that microbes exposed to magnesium sulfate-enriched groundwaters displayed structural community changes at low concentrations of 2 mg Mg/L, while microbes exposed to uranium spiked sediments responded at concentrations of >400 mg U/kg. The effect of these contaminants on microbial functional genes was investigated with metagenomic and metabolic pathway analyses (FAPROTAX), which indicated changes in carbon, sulfur and nitrogen cycling processes. However, the functional inferences being made from structural changes and genomic data warrant testing and validation. Our current soil study aims to validate inferred functional endpoints for nutrient cycling through employing a multiomic approach with amplicons, metagenomes and metabolomes. The ability to elucidate structure-function relationships will allow the identification of ecologically relevant endpoints, which will be useful for setting Environmental Quality Guidelines and targets for rehabilitation of mine-sites.

Inclusion of microbial endpoints in site-specific Risk Assessments, Environmental Guidelines and Remediation Targets for Antarctic soils

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Changes in microbial community composition are commonly used in Antarctic site-specific risk assessments as microbes represent the dominant and most biodiverse component of Antarctic soil ecosystems. To ensure effective restoration and environmental protection of contaminated sites in Antarctica and subantarctic Islands where Australia operates research stations, we are developing site-specific Environmental Quality Guidelines and Remediation Targets. While best practice ecotoxicological and statistical modelling methods are used (following Australia/New Zealand Water Quality Guidelines and National Environmental Protection Measure), the generally low diversity of invertebrates and plants in soils in Antarctica, limits the availability of typical toxicity test organisms by which sensitivity data can be generated for inclusion in species sensitivity distribution (SSD) models. This has necessitated the use of non-standard endpoints including microbial processes and community diversity indices alongside more traditional

endpoints based on single species tests. Here we present an example of this approach, using data compiled from over a decade of research on the impacts of fuels on sub-Antarctic biota. Four of the 13 most robust and representative sensitivity data estimates selected for inclusion in the SSD were based on microbial endpoints. These included two critical processes (soil nitrification and respiration) and two contrasting community diversity indices (Shannon diversity and acidobacteria/proteobacteria ratio), which showed a range of sensitivities to fuel. We estimated Protective Concentrations for fuel at levels appropriate to the ongoing land use status. Protection of 90% of biota (PC90) is estimated at concentrations not exceeding 72 mg TPH/kg soil, and this value is recommended for use in the assessment of ongoing risk of remediated soils in subantarctic environments. We advocate for the ecological relevance and critical importance of microbes in soils ecosystems, and the necessity therefore for the integrated use of microbial endpoints in contemporary approaches for Environmental Quality Guideline derivation for protection of these vulnerable ecosystems.

Identifying microbial indicators of pollution in freshwater urban constructed wetlands

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Freshwater ecosystems are under significant threat from anthropogenic pollution. Currently, there is a lack of consideration for impacts on microbial taxa in environmental quality guidelines, as well as an absence of microbial indicators that could complement and enhance the scope of current biomonitoring analyses. Environmental DNA is expanding the field of aquatic monitoring with the ability to assess large biological profiles quickly and reliably, and providing knowledge of how contamination affects ecosystems down to a microbial level. Improved understanding of what contamination is present in waterbodies and how it is affecting all aspects of ecosystem functioning enables better targeted mitigation measures. Our research explored the impacts of three major contaminants on sedimentary microbial assemblages, assessing the variation in assemblage structure and the identification of potential bacterial bioindicator taxa. We used lab-based microcosms to assess the impact of metal contamination on microbial communities from clean and contaminated ecosystems, and field-based microcosms to compare responses to exposure from metal, pesticide, and pharmaceutical contamination. Our results showed significant variation in microbial community structure that was correlated with contamination levels. The lab-microcosms identified significant assemblage change over a temporal and concentration gradient in response to metal contamination, and between microbes from uncontaminated and contaminated ecosystems. Seven bacterial families were identified as possible diagnostic indicators of metal contamination. The field-microcosms identified significant variation between the three contaminant types and identified potential bacterial indicators that could be diagnostic for either one, two, or all three of the contaminant types. We also discuss the ideal taxonomic resolution for the assessment of community-level variation and the identification of bioindicator taxa that have potential to be used in ecosystem health assessments.

Beyond Microtox – a bacterial toxicity assay suitable for risk assessment in tropical environments

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Bioassays using bioluminescent bacteria offer a cost-effective and efficient alternative to standard toxicity assays for assessing contaminant risks in the environment, with applications in water quality assessments and effluent screening spanning decades. However, most bacterial bioassays utilise Aliivibrio fischeri in a low-throughput format under temperate conditions, limiting their suitability for tropical marine environments. Furthermore, conventional A. fischeri assays rely on short exposure periods (e.g., 5, 15, 30 min) and luminescence as the sole toxicity endpoint, reducing their applicability for deriving water quality guideline values. This study aimed to develop a high-throughput, and cost-effective assay using the tropical bioluminescent bacterium Vibrio azureus (Lum-31), incorporating toxicity endpoints suitable for both acute and chronic contaminant assessment in tropical marine conditions. Using copper and zinc as reference toxicants, we demonstrated the repeatability of the acute luminescence assay at 15 and 30 min, with increased sensitivity compared to A. fischeri assays. In addition, integrating an ecologically relevant endpoint (specific growth rate) and a chronic exposure period (>24 h) enabled comparisons of V. azureus sensitivity with other taxa in sensitivity distributions for copper and zinc species. This approach enhances the ecological relevance of the assay and improves its applicability for future ecological risk assessments.

Research to regulation: A quantitative framework for establishing microbial sensitivity thresholds to protect marine ecosystems

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Marine ecosystem resilience is closely linked to microbiome health, yet microbial communities are typically overlooked in conservation management due to challenges in quantitatively defining microbial sensitivity thresholds. This study introduces a quantitative framework that combines 16S rRNA gene amplicon sequencing with ecotoxicology concentration-response modelling to assess marine microbial responses to contamination stress at both community and taxon levels. By applying propidium monoazide to differentiate intact from membrane-compromised cells, combined with normalisation of relative to absolute abundances via flow cytometry, no-significant-effect concentration values can be derived from individual taxon-specific stress-response curves. These thresholds inform Prokaryotic Sensitivity Distributions, enabling the derivation of microbiome Hazard Concentrations, which estimate the contaminant concentrations affecting x% of the microbial community. This approach aligns with the Australian and New Zealand water quality guideline methodology and offers a pathway to formally incorporate microbial endpoints into risk assessments. We applied this approach to planktonic microbial communities with varying disturbance histories, exposing them to increasing copper concentrations in controlled microcosm experiments. Microbiomes from ecologically valuable, low impact sites (e.g., pristine coral reefs) were more sensitive to copper stress than those from disturbed areas. The microbiome Hazard Concentrations for copper affecting 5% of the community ranged from 0.10 to 0.94 µg L-1, underscoring the need for stricter environmental protection of high-value ecosystems. Using this

case study, we also demonstrate how microbial sensitivity data could be integrated alongside eukaryotic data in future environmental risk assessments. Ultimately, establishing more robust protection thresholds that span both prokaryotic and eukaryotic taxa represents a critical step toward evaluating whole-ecosystem responses to environmental disturbances.

Predicting the combined effects of pollution and heat stress to adjust guideline values for tropical marine microbiomes

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The health and resilience of marine ecosystems are increasingly threatened by the rising frequency of heating events, which further intensify the vulnerability of species and ecosystems to chemical pollution. To effectively prioritise ecosystem protection interventions, it is essential to quantify the individual impacts of each stressor and predict their combined effects. In this study, we developed a cumulative Prokaryotic Sensitivity Distribution (PSD) for marine microbiomes in response to thermal stress over a standard exposure period, analogous to conventional Species Sensitivity Distributions. This approach enabled quantitative predictions of community-level impacts as temperatures exceeded ambient conditions. The microbiome hazard temperature (mHT5), affecting 5% of taxa in the community was 1.5°C above ambient–similar to thresholds impacting the same proportion of eukaryotic marine species and known to trigger coral bleaching–highlighting its relevance for multi-stressor risk assessments. To predict the combined effects of thermal stress and chemical pollution, we combined the thermal stress PSD with a previously derived chemical PSD for the reference stressor copper by applying the multi-substance potentially affected fraction (ms-PAF) model. Expanding the ms-PAF framework to include thermal stress impacts on microbial communities presents a promising strategy for adjusting water quality guideline values for microbiomes to account for heating events, thereby improving the protection of marine ecosystems under multiple stressors.

Session Theme 9B: Identifying 'ecologically relevant endpoints' for microbial processes to inform Environmental Quality Guidelines and One Health approaches

Antimicrobial resistance: A global health crisis with complex environmental dimensions

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Antimicrobial resistance (AMR) is one of the most pressing issues facing humanity this century. It's a complex threat that is projected to cost the global economy US$100 trillion by 2050, impacting agricultural productivity, food security, and the health of humans, animals, plants, and environments. Recent analysis shows that more than 39 million people will die from antibiotic-resistant infections between now and 2050. Antimicrobials and other drivers of AMR are used and released in myriad ways to diverse environments. As the world mobilises to mitigate the complex threat of AMR, the importance of integrating the environmental dimensions into a One Health approach to tackle the issue is increasingly emphasised. Yet how to best do that is much debated. The One Health concept recognises the inherent interrelatedness of human, animal, plant, and environmental health and has important implications for environmental management.

For example, it is evident that water, wastewater, and biosolids are important connectors in the complex web of AMR sources, amplifiers, and receptors, and there is a growing evidence base showing that improvements in water, sanitation, and hygiene management can significantly reduce the critical threat of AMR by limiting its development and spread in the environment and reducing infections and the need for antimicrobials. This presentation will demonstrate the multifaceted nature of One Health AMR, highlight some of the key challenges in assessing AMR associated water quality risks, and show why the environmental dimensions of AMR are central to mitigating this pressing global One health crisis.

Defining Meaningful Environmental Endpoints for Antimicrobial Resistance: Moving Beyond MICs to Assess Minimal Selective Concentrations for Key Endpoints

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Antimicrobial resistance (AMR) is increasingly recognized as an ecological threat, yet risk assessments remain narrowly focused on human clinical endpoints. To effectively manage AMR in aquatic and terrestrial ecosystems, we must establish meaningful environmental endpoints that capture microbial community disruption, functional losses in nutrient and carbon cycling, and increased disease susceptibility across plants, animals, and their microbiomes. This presentation advances the case for using dysbiosis, microbial functional shifts, and keystone species health as sentinel indicators of AMR impact.

Minimal selective concentrations (MSCs)—the lowest concentrations of antimicrobials that select for resistance—are currently defined by growth inhibition metrics for clinical isolates in pure culture, which fail to capture broader ecological consequences. Microbiomes, have emergent properties not seen in individual species, that emerge through cross-population interactions such as gene exchange. Hence, we propose that MSC assays must evolve to include endpoints that include stress responses, biofilm enhancement, horizontal gene transfer potential, and altered microbial community structure. Emerging tools in metagenomics, transcriptomics, and functional profiling now enable tracking of AMR gene dynamics and their cascading effects across microbiomes.

We highlight emerging methods from microbial ecology and ecotoxicology—such as metagenomic profiling, functional gene assays, and host-microbiome health indicators—that could inform more ecologically meaningful MSC determinations. Case studies from aquatic systems illustrate how AMR may contribute to disease susceptibility in seagrasses, fish, and other keystone species by disrupting microbial symbioses. Without such ecologically meaningful thresholds, AMR management will continue to underestimate its environmental burden. Addressing this gap requires interdisciplinary collaboration between environmental toxicologists, microbiologists, and policy makers to develop standardized, scalable assays and risk thresholds. As AMR continues to spread across interconnected water systems, identifying ecologically relevant endpoints is essential for aligning environmental protection with One Health goals.

Environmental drivers of AMR: investigations of associations of metal contaminants and AMR in agricultural, airstrip and microcosm soil

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Environmental drivers selecting for the development and maintenance of antimicrobial resistance (AMR) are of increasing concern from a One Health perspective. Despite this, the challenges of

sampling and modelling environments where contaminants select for AMR mean this is a neglected area of research. This presentation summarises the findings of investigations by Massey University Environmental Health researchers and collaborators on the association of heavy metal (cadmium, Cd; zinc, Zn) contamination of agricultural soil and antibiotic resistance (ABR) at sites in the Waikato and Wellington regions of New Zealand. Soils from three contaminated sites and one uncontaminated site in Waikato, and a farm airstrip near Wellington with graduated Cd contamination were sampled for the presence and proportion of total aerobic heterotrophic bacteria with metal resistance (HMR) (Cd, Zn and mercury, Hg - a positive control). Soil microcosm experiments determined the range of Cd, Zn or Hg concentrations associated with elevated numbers of ABR bacteria. Antibiotic sensitivity, 16SrDNA and horizontal gene transfer analyses were employed to investigate co-selection for HMR and ABR. In Waikato, Wellington airstrip and microcosm samples, higher levels of bacterial resistance to Cd, Zn, Hg and antibiotics correlated with higher levels of Cd or Zn in soil. Bacterial community structures were altered in soils with high HM levels. Cd resistance genes were transferred from donor bacterial isolates to recipients, and transconjugants were also resistant to Zn and/or Hg and a range of antibiotics. 16S rDNA next-generation sequencing profiling of Wellington airstrip and microcosm samples found changes in HM-exposed bacterial communities. The bacterial phyla Acidobacteria and Chloroflexi differed most in abundance between airstrip subsite samples (p

Shaping an approach for deriving water quality guideline values for antimicrobials that integrate ecotoxicity and antimicrobial resistance endpoints

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Antimicrobials pose ecological risks in aquatic environments, particularly to cyanobacteria, aquatic plants and green algae, and with the potential to disrupt microbiomes that biota rely upon. Beyond direct toxicity, these chemicals also contribute to the emergence and spread of antimicrobial resistance (AMR), posing risks to human, animal (including wildlife), and plant crop health, particularly through wastewater discharges and water reuse. Despite these concerns, Australia and New Zealand currently lack environmental water quality guideline values (GVs) for antimicrobials. Furthermore, existing guideline derivation frameworks do not currently consider endpoints to protect against AMR. This presentation will describe our approach to deriving environmental water quality GVs that integrate AMR and traditional toxicity endpoints, using a co-design approach involving industry and regulator stakeholders. Through a systematic review of international practices, we evaluated existing approaches to estimate the environmental hazard of AMR and aspects of these were considered for use within the Australian and New Zealand guideline context. The resulting framework, while developed for antimicrobials, may have applicability for other toxicants known to influence AMR, such as other pharmaceuticals, metals, pesticides and plastics. Key to the success of this project was the journey with stakeholders through the co-design process, and elements of this will be presented to showcase how collaborative input shaped the development of a fit-for-purpose framework for deriving environmental water quality guideline values.

Appendix C

Supporting detail: Critical appraisal of microbial endpoints (Topic 2)

This appendix provides supplementary detail supporting Topic 2 in the main white paper. It includes: (1) discussion of microbial groups underrepresented in the endpoint appraisal; (2) additional methodological and regulatory context for each endpoint category; and (3) the full endpoint appraisal table. References to the main text are indicated throughout.

C1. Microbial groups and scope of assessment

Although microbial endpoints were considered broadly, workshop discussions focused primarily on bacterial endpoints, reflecting their dominance in aquatic microbiomes and the greater availability of relevant toxicity methods. The scope and treatment of other microbial groups is summarised below.

Microalgae

Microalgae are already well-accommodated within existing ANZG guideline frameworks. They are typically treated as surrogates for photosynthetic eukaryotes rather than as members of microbial communities per se, and are not a primary focus of this white paper. Growth inhibition assays using microalgae are accepted for use in SSDs and contribute to several existing DGVs (e.g., draft Copper and Nickel DGVs for freshwaters).

Fungi

Fungi are ecologically important drivers of carbon and nutrient cycling, particularly in detrital systems, and their activity contributes to responses measured by existing community functional endpoints (e.g., respiration, extracellular enzyme activity, carbon transformation). However, these processes are rarely incorporated explicitly into DGV derivation for water and sediments. Dedicated fungal toxicity endpoints are not yet sufficiently standardised or validated for regulatory use. Single-cell fungi (yeasts) were discussed primarily as laboratory model organisms, with limited demonstrated relevance to ecosystem-level processes.

Free-living Protozoa (Protists)

Free-living protozoa were not considered in detail during the workshops, despite their ecological importance as hosts for bacteria and their role in virulence evolution, biofilm dynamics, and AMR development. Their exclusion represents a notable gap in current endpoint coverage. Protozoa facilitate horizontal gene transfer between bacterial cells and act as environmental reservoirs for pathogenic bacteria, making their consideration relevant to AMR risk assessment in particular.

Archaea

Archaea were not specifically addressed during the workshops. They play important roles in biogeochemical cycling (e.g., methanogenesis, nitrification) and are captured in 16S rRNA-based amplicon sequencing approaches, but their specific sensitivities to contaminants and regulatory status as endpoints remain largely undefined.

C2. Additional context by endpoint category

Cell-based endpoints: Further detail

The ecological limitations of single-species microbial assays are well recognised by the ecotoxicological community, and likely explain why (with the exception of microalgae and cyanobacteria) cell-based microbial tests have rarely been incorporated into SSDs for DGV derivation. Workshop participants emphasised that the analytical simplicity and reproducibility of these assays do not equate to ecological relevance.

While microalgal growth rate is an ecologically relevant measure of community abundance and remains useful within single-species SSDs, the relevance of growth-rate endpoints for other microbial taxa, especially under non-realistic culture conditions, remains debated. Cells maintained under laboratory conditions for multiple generations may be selected for traits unrepresentative of in situ communities. Artificial test conditions (enriched media, simplified exposure matrices) can substantially ameliorate contaminant toxicity, limiting transferability of results to natural systems. Although these limitations also apply to eukaryotic toxicity test methods, they are particularly pronounced for microbes given the greater ecological distance between cultured strains and natural communities.

Multi-species assays such as the Microbial Assay for Risk Assessment (MARA) and the multi-species algal growth assay (Stone et al., 2024) capture differential sensitivity across multiple taxa simultaneously, enabling capture of species-level interactions within a single exposure. Although still based on cultured organisms and not fully representative of in situ communities, these assays offer a pragmatic bridge toward community-level assessment and enable higher-throughput comparative screening. They remain limited in their ability to capture horizontal gene transfer, functional redundancy, and adaptive responses that characterise natural microbial systems.

Importantly, single-species toxicity estimates can be extracted from community-level assays. When combined with environmentally relevant exposure conditions (minimal nutrient supplements; realistic temperature and light regimes), such estimates can be incorporated into SSDs while departing from standard internationally prescribed approaches (OECD, USEPA). This approach was explored in Thomas et al. (2025a) using mixed bacterial community assays.

Community functional endpoints: Further detail

The regulatory precedent for community functional endpoints in soil guideline derivation (standardised OECD/ISO carbon and nitrogen transformation tests) reflects the greater environmental stability of soil systems and clearer linkages between microbial processes and ecosystem services compared with aquatic environments. The availability of defined, regulatorily defensible effect thresholds in the soil context, such as the 25% change threshold used in NEPM methods, has supported their use; equivalent thresholds are not yet established for aquatic systems.

In surface water and sediment systems, community functional endpoints are constrained by strong context dependence, limited cross-system transferability, and interpretive ambiguity in assigning effect thresholds. These factors currently limit their pathway into DGV derivation. Their greatest current value lies in: (1) serving as anchoring metrics within WoE frameworks for SSGVs; and (2) testing and validating functional inferences drawn from genomic studies, an important role given the known gap between gene presence (metagenomics) and realised function.

In the AMR context, community functional endpoints provide only indirect insight into resistance selection or dissemination. Changes in rates of nutrient cycling, for example, cannot reveal whether AMR genes are being enriched or transferred. These endpoints should therefore be supplemented with molecular approaches when AMR risk is a concern.

Amplicon-based endpoints: Further detail

Early applications of amplicon-based sequencing in ecotoxicology were limited by reliance on relative abundance data; without knowledge of total cell numbers, it is impossible to distinguish true community change from compositional shifts driven by differential taxon growth or loss. DNA sequencing technologies are also generally unable to distinguish viable from non-viable cells or extracellular environmental DNA, meaning detected sequences may originate from dead cells or cell-free DNA, reducing the ecological relevance of community profiles.

The integration of amplicon sequencing with viability PCR (using DNA-intercalating dyes such as propidium monoazide) prior to extraction addresses the viability issue. QMP, which normalises sequencing data to absolute cell counts or gene copy numbers using flow cytometry or spike-in standards, addresses the relative abundance limitation (Thomas et al., 2025a). Together, these methods reduce PCR-related compositional bias and enable derivation of taxon-specific no-significant-effect concentrations (NSECs) that can be aggregated into PSDs, an approach that closely aligns with the SSD framework used for DGV derivation (Thomas et al., 2025b).

This approach was applied in Thomas (2025a), which explored combining amplicon sequence variant (ASV)-level data from aquatic microbiome exposures to copper with existing single-species copper data used in the ANZG Copper DGV. The resulting unified PSD-SSD provided Hazard Concentrations at the 1%, 5%, 10%, 20%, and 50% levels, illustrating the potential for integrating prokaryotic community data with traditional eukaryotic toxicity data.

When combined with shotgun metagenomic sequencing, functional endpoints can be integrated alongside compositional endpoints, enhancing the ecological relevance of thresholds derived from amplicon-based approaches alone. It should be noted that standard amplicon sequencing is insufficient for AMR assessment: while it can detect known AMR-associated taxa, it cannot quantify AMR gene copy numbers or characterise resistome composition. This requires more targeted metagenomic methods.

Metagenomic and metatranscriptomic endpoints: Further detail

A key limitation of metagenomic approaches is that gene presence does not necessarily translate to realised functional activity: a gene encoding a metabolic function may be present in a community but not expressed, or expressed but not translated into active enzyme. Metatranscriptomics addresses this partially by characterising active gene expression (mRNA), but transcripts themselves do not guarantee protein production or enzymatic activity. Both functional profiles and expression patterns also exhibit high natural spatial and temporal variability, complicating the establishment of stable baselines.

The high analytical complexity, computational demands, and cost of metagenomic and metatranscriptomic analyses have historically limited their regulatory uptake. However, recent advances in concentration–response modelling using these data types, allowing derivation of thresholds for key functional genes along contaminant gradients (Webster et al., 2018), provide a pathway toward alignment with established ecotoxicological frameworks. When aggregated into cumulative functional sensitivity distributions, this approach could ultimately enable molecular endpoints to be considered alongside traditional SSD-derived values.

For AMR characterisation specifically, metagenomics is the most defensible current approach: it enables quantification of resistance gene abundance and diversity (resistome), detection of mobile genetic elements (plasmids, transposons, integrons) that facilitate gene transfer, and characterisation of co-selective pressures from non-antibiotic contaminants. However,

metagenomic data alone cannot quantify resistance risk, predict gene transfer rates, or establish ecological consequences. Integration with ecotoxicity data within a multiple-lines-of-evidence framework is therefore required. Further development of microbial ecosystem models and Adverse Outcome Pathways (AOPs) would help validate the use of metagenomic endpoints for GV derivation.

Other omics and multi-omics endpoints: Further detail

Proteomics measures protein abundance and provides evidence of enzyme translation, offering a more direct measure of functional activity than metagenomics or metatranscriptomics. Metabolomics characterises the suite of small-molecule metabolites produced by a community, reflecting realised metabolic activity. Both approaches confirm that particular metabolic pathways are active, addressing the gene-to-function gap that limits interpretation of genomic data.

Some progress has been made in applying metabolomics to ecological surveillance. For example, Beale et al. (2022) demonstrated the use of environmental metabolomics in monitoring aquatic ecosystem health. However, these methods remain highly context-specific, analytically complex, and costly. They lack defined effect threshold metrics for use in effect-based assessments, and their scalability for routine regulatory application is limited.

Multi-omics approaches, combining genomics, transcriptomics, proteomics, and metabolomics, strengthen causal inference across biological levels and have potential to establish genomic endpoints that reliably reflect ecological function. Linking molecular-level changes to ecosystem outcomes through AOPs and conceptual ecological models is a key research priority. Until validated thresholds and regulatory-grade QA/QC frameworks are established, proteomics, metabolomics, and multi-omics approaches are best applied as research and hypothesis-generating tools rather than primary regulatory endpoints.

For AMR specifically, the relevance of proteomics and metabolomics is currently indirect: changes in cellular metabolism may indicate stress responses associated with resistance selection, but these cannot be unambiguously attributed to AMR-specific processes. Metagenomic approaches remain the priority for AMR endpoint development.

C3. Full endpoint appraisal table

Table C1 provides a comprehensive appraisal of all five endpoint categories against key criteria: ecological relevance, regulatory utility under the ANZG framework, methodological maturity, AMR applicability, and primary research gaps. This table is provided as a separate excel attachment.

Survey responses from 16 workshop participants indicated strongest support for microbial endpoints that directly capture changes in community structure, viable abundance, and measured ecosystem functions, particularly where those functions relate to nutrient cycling or other core biogeochemical processes (Table C1). Community composition emerged as one of the most strongly supported endpoints, while richness, viable abundance, biomass, respiration, and functional pathway measures were generally viewed as ecologically relevant but still requiring methodological refinement, stronger chronic exposure framing, or clearer linkage to ecological outcomes. In contrast, simplified proxy assays and mechanistic biomarkers such as luminescence, ROS, and short-term metabolic tests were usually not considered sufficiently ecologically relevant on their own. A clear pattern also emerged around distrust of predictive tools: respondents were sceptical of taxonomy-based functional inference and metabolic network models unless they are empirically validated against measured functions and observed ecological change.

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This publication is produced by Australia's Cooperative Research Centre for Solving Antimicrobial Resistance (AMR) in Agribusiness, Food and Environments (SAAFE CRC) which is owned and operated by SAAFE Limited.

SAAFE CRC is supported through funding from the Australian Government as well as contributions from valued research and industry partners. We gratefully acknowledge the support of our funding partners, whose contributions have enabled the research and development presented in this work. The views and opinions expressed in this publication are those of the authors and do not necessarily reflect those of the Australian Government or any other funding bodies.

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Cooperative Research Centres Program

This project is supported by the Australian Government Department of Industry, Science and Resources through the Cooperative Research Centres Program.