National Environmental Science Program

Sustainable Community and Waste Hub research plan 2026 – Attachment B project plans

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Project IP3.06.01 – Assessing the Ecotoxicity of Whole Leachates from Tyre Wastes

Project type: • Hub research project	
• **Project status:** New project submitted for approval	
Cross-cutting initiative:	No
Project start date: 02/03/2026	**Project end date**: 31/12/2026
Project leader details:	Name: Prof Frederic Leusch Organisation: Griffith University

Project summary

Our research project aims to explore the suitability of using effect-based methods for assessing the ecotoxicity of end of life tyre products. After a systematic literature review, laboratory generated end of life tyre leachates, as well as tyre waste impacted field samples, will be tested in a comprehensive battery of *in vitro* bioassays. Leachates will also be tested in conventional *in vivo* assays to confirm the predictive power of *in vitro* testing. Our project will provide a better understanding of the risks associated with chemical mixtures in end of life tyre products and can support decision making on the safe re-use, storage and export of waste tyre products.

Pathway to impact

Outcomes
The mixture of chemicals leached from end of life tyre products and their potential adverse effects on organisms are poorly understood. Using a combination of effect-based methods and chemical analysis, this project will provide a greater understanding of the ecotoxicity of end of life tyre products and help identify key drivers of toxicity. By comparing the response *in vitro* to conventional *in vivo* assays, the project will also determine the suitability of using *in vitro* bioassays for assessing the risks associated with tyre waste products. This can help reduce the need for animal testing, with *in vitro* bioassays faster, cheaper and more ethical. To assist decision makers, the project will also develop a framework to assess whether an end of life tyre product would be considered hazardous or not. For example, thresholds in selected *in vitro* bioassays would be established, with further testing recommended if the leachate from an end of life tyre product exceeded a threshold.

Research-user	**Engagement and communication**	**Impact on management action**	**Outputs**
DCCEEW (Chemicals, Atmosphere and International Branch, on behalf of other branches within the Circular Economy and Environmental Permitting and Compliance Divisions)	Findings will be communicated regularly through monthly email progress reports, *ad-hoc* project meetings, draft and final reports, and presentations.	The research findings from the project will (a) support policy makers and regulators by establishing whether leachates from waste tyre products can be considered hazardous to the environment, and (b) inform Government and industry on how any risks from waste tyres products could be rapidly and cost-effectively assessed.	Systematic literature review and report on the ecotoxicity of end of life tyres based on both *in vitro* and *in vivo* methods. Decision making framework for assessing if end of life tyre products are hazardous or not. Tabulated chemical analysis data and ecotoxicity data on tyre leachates in a re-usable electronic data format produced according to FAIR data principles.

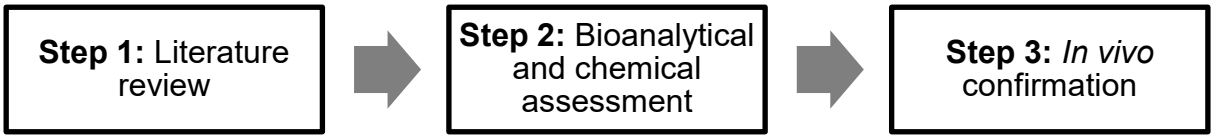
Project description

Project description

Introduction and objective: The safe reuse of waste materials is essential for achieving a circular economy. However, waste materials, such as end of life tyres, electrical and electronic equipment, and batteries, can contain a wide range of chemicals [1]. These chemicals can leach out of waste and products containing recycled waste, resulting in the likely exposure of organisms to this complex chemical mixture. Targeted chemical analysis can only detect known chemicals and cannot account for mixture effects. As a result, New Approach Methodologies (NAMs), including effect-based methods (EBM), are considered critical to assess the effects of the complex mixture of chemicals in wastes [1]. EBM, which are also known as bioanalytical tools, include cell-based *in vitro* bioassays and well plate-based *in vivo* assays. EBM can detect the mixture effects of all active chemicals in a sample, including unknown chemicals, such as transformation products, and chemicals below analytical limits of detection [2]. Further, EBM are faster, cheaper and have a lower ethical cost than conventional *in vivo* assays, with previous studies finding a good correlation between *in vitro* cell viability data and acute toxicity in whole organisms for fish [3] and daphnia [4]. Therefore, there is considerable scope for the use of EBM in waste risk assessment.

Using end of life tyres as a case study, this project will evaluate the suitability of EBM for chemical risk assessment of waste products. Laboratory generated leachates from end of life tyres and tyre waste runoff samples collected in the field will be tested in a battery of bioassays with the aim of establishing linkages between effects observed in the laboratory and real-world conditions. The activity in *in vitro* bioassays will also be compared with conventional *in vivo* assays commonly used for ecotoxicity assessment. The project is a priority as it will identify relevant and reliable *in vitro* assays that can routinely be used to assess the ecotoxicity of end of life tyres without the need for animal testing.

Approach: The project will be undertaken in three steps (Figure 1):



1) Literature review: A systematic literature review on the ecotoxicity of tyre wastes including both *in vitro* and *in vivo* approaches will be conducted. The review will identify available relevant and reliability *in vitro* and *in vivo* ecotoxicology data and identify key drivers of ecotoxicity in end of life tyre leachates. This review will not include modelled or quantitative structure activity relationship (QSAR) data.

2) *Bioanalytical and chemical assessment of end of life tyre products:* Laboratory generated end of life tyre leachates will be tested using a battery of *in vitro* bioassays and targeted chemical analysis. Tyre derived products, such as crumb rubber (<1 mm), buffings (<2 mm) granules (2-15 mm) and shred (50-80 mm), will be obtained, and chemical contaminants leached following standard methods (e.g., SA AS 4439.3 - Wastes, sediments and contaminated soils, Part 3: Preparation of leachates - Bottle leaching procedure). In addition to the leachate, which will contain both inorganic and organic contaminants, organic chemicals will be extracted from the leachate using solid-phase extraction (SPE). The whole samples and

SPE extracts will be tested in a battery of bioassays indicative of bacterial toxicity (BLT-Screen), viability, oxidative stress and genotoxicity in both fish cells (RTgill-W1) and insect cells (C6/36), algal toxicity (IPAM assay), estrogenic activity (ERα GeneBLAzer) and aryl hydrocarbon receptor activity (AhR-CAFLUX). All assays are established at Griffith University and are regularly used for water quality monitoring [5]. The chemical composition of the leachates will also be assessed using targeted chemical analysis for metals and organic chemicals.

Runoff from sites impacted by end of life tyre waste, such as tyre storage facilities and tyre recycling facilities, will also be collected and characterised using both bioanalysis and chemical analysis. This will allow comparison of the laboratory generated leachate bioassay fingerprint with the real-world samples and will further our understanding of the risks of tyre waste products under environmental conditions.

Finally, bioanalysis will be combined with chemical analysis using an approach called iceberg mixture modelling [6] to identify which chemicals are driving the effect in end of life tyre leachates.

3) *In vivo assessment*: Selected tyre leachates (e.g., leachates showing high bioactivity in specific assays) will be tested in whole organism tests, such freshwater algae growth inhibition (eg, OECD Test No. 201 or equivalent), *Daphnia* or *Ceriodaphnia* acute toxicity test (eg, OECD Test No. 202 or equivalent), and a fish acute toxicity test (eg, OECD Test No. 203 or equivalent). While *in vivo* testing has a higher (ethical) cost and is more time consuming than *in vitro* testing, it is included in the project to confirm that *in vitro* testing of tyre leachates does not result in false positive or false negatives.

Both the literature review and the experimental components of the project will be published as peer reviewed journal articles.

The project will link to other research conducted as part of Impact Priority 3 “Hazardous waste and pollutants” and will utilise resources such as “guidance on sampling complex waste materials”.

Research team: Prof Frederic Leusch (H-index 52) and Dr Peta Neale (H-index 41) (both Griffith University) have extensive experience in the validation and application of EBM. They have worked together for the past 12 years, publishing 48 papers in this time, including co-authoring the book “Bioanalytical tools in water quality assessment” [2]. While their research primarily focuses on water (e.g., wastewater, surface water, drinking water), EBM can be applied to other matrices, including leachates from end of life tyre products. Leusch also led an ARC Linkage Project (LP180100600) that adapted EBM to test whole water extracts [7], an approach that will be utilised in the current project.

Outcomes: The outcomes of the project can help to support decision making and on-ground action. Firstly, testing end of life tyre leachates in both *in vitro* bioassays and conventional *in vivo* assays will help assess the suitability of *in vitro* bioassays assessing the risks associated with the complex mixture of chemicals in tyre waste. If the results from EBM agree well with the *in vivo* assays, this will limit the need for animal testing in the future. Secondly, based on the findings from both Step 2 and Step 3 of the project, the researchers will develop a decision making framework to help identify whether a product containing end of life tyres is potentially hazardous or not.

References

[1] Boxall, N.J., et al., Understanding how chemical risk can be managed in a circular economy. Resources, Conservation and Recycling, 2026. 225: 108600; **[2]** Escher, B.I., P.A. Neale, and F.D.L. Leusch, Bioanalytical Tools in Water Quality Assessment - Second Edition. 2021, London: IWA Publishing. 462; **[3]** Fischer, M., et al., Repeatability and reproducibility of the RTgill-W1 cell line assay for predicting fish acute toxicity. Toxicological Sciences, 2019. 169(2): 353-364; **[4]** Johnson, M., et al., Validation of an in vitro bioassay using C6/36 insect cells as a model for evaluating toxicity of aquatic contaminants to invertebrates. Aquatic Toxicology, 2025. 286: 107481; **[5]** Lewis, P., et al., A bioanalytical and chemical approach for wastewater discharge: Beyond detected chemicals for water quality assessment. Environmental Pollution, 2025. 383: p. 126807; **[6]** Neale, P.A., et al., Solid-phase extraction as sample preparation of water samples for cell-based and other in vitro bioassays. Environmental Science: Processes & Impacts, 2018. 20(3): p. 493-504; **[7]** Johnson, M., et al., Adaption and application of cell-based bioassays to whole-water samples. Chemosphere, 2024. 361: p. 142572.

Is this a cross-hub project?

No

Does this project contribute to a cross-cutting initiative?

No

Indigenous consultation and engagement

Indigenous consultation and engagement are integral to our project, guided by our hub's Indigenous Partnerships Strategy. We will work with our Indigenous facilitator to identify the most effective ways to demonstrate the hub's impact for Indigenous communities and to capture the contributions of Indigenous-led projects within the hub. This approach highlights the value of Indigenous leadership and the benefits of collaboration between Western and Indigenous knowledge systems.

To ensure our work is communicated appropriately, our Indigenous facilitator will play a central role in guiding how project outcomes are shared. Their expertise will ensure communications are culturally respectful, accessible, and aligned with community priorities. This will allow us to clearly demonstrate the hub's contributions to sustainable community development and waste management.

Furthermore, all project team members will undertake *Our Mob* cultural awareness training and *Indigenous Cultural and Intellectual Property (ICIP) True Tracks* training. These requirements will help ensure that engagement with Aboriginal and Torres Strait Islander communities is informed, respectful, and aligned with best practice.

The project falls under Category 3. This is because it includes a literature review in Step 1 of the project. It is also considered niche research as effect-based methods are an emerging approach and are not yet regulated.

The project meets the following revised Three Category approach:	**Category 1** Indigenous led	**Category 2** Co-design	**Category 3** Communicate
	☐	☐	☒

Project milestones

Milestones	Due date	Responsible person
Milestone 1 – Signing of contract between hub lead and research organisations	Mar 2026	Frederic Leusch
Milestone 2 – Literature review submitted to DCCEEW	30 Jun 2026	Frederic Leusch
Milestone 3 – Draft report on toxicological (*in vitro* and *in vivo*) and chemical assessment of tyre leachates submitted to DCCEEW	2 Nov 2026	Frederic Leusch
Milestone 4 – Final report on toxicological (*in vitro* and *in vivo*) and chemical assessment of tyre leachates submitted to DCCEEW	20 Dec 2026	Frederic Leusch
Milestone 5 – Workshop to present findings of project	Early 2027	Frederic Leusch

Data and information management

Analytical chemistry data and ecotoxicity data generated under this project will be provided in a machine-readable electronic format to be agreed with DCCEEW. The data sets generated under this project will be compiled according to FAIR (Findable, Accessible, Interoperable and Reusable) data principles with the objective to make the data re-usable and readily accessible by research end-users.

Project output	**Data management and accessibility**
Literature review (Step 1)	• Literature review submitted to DCCEEW • Published in peer-reviewed open access journal
In vitro and *in vivo* assessment of end of life tyre products (Steps 2 and 3)	• Final report on toxicological (*in vitro* and *in vivo*) and chemical assessment of tyre leachates submitted to DCCEEW • Chemical and ecotoxicity data generated in Steps 2 and 3 will be provided in a machine-readable electronic format • Published in peer-reviewed open access journal • Bioassay data, such as effect concentrations or bioanalytical equivalent concentrations, and chemical concentrations will be published as part of the supporting information section

Location of research

The table below describes the scale at which the project will be working, and the location(s) where the majority of the project research will be conducted.

At which spatial scale is the project working	**National** ☐	**Regional** ☐	**Local** ☒
Location(s) – gazetted region /place name	Gold Coast		
Aboriginal or Torres Strait Islander nation or traditional place name(s)	Lands of the Kombumerri peoples, part of the Yugambeh language region		

Project keywords

ecotoxicity, effect-based methods, end of life tyres, leachate, mixture effects