Project Order, Variations and Research Progress

Project Title: Microbial degradation of chemical compounds used in onshore gas production in the SE of South Australia

This document contains three sections. Click on the relevant section for more information.

1 Original Project Order

Project Order

Proforma 2018

1. Short Project Title

Microbial degradation of chemical compounds used in onshore gas production in the SE of South Australia

Long Project Title	Chemical compounds used in onshore gas production in the SE of South Australia: microbial degradation, microbial community impact and indicator taxa.
GISERA Project Number	W.15
Proposed Start Date	1 October 2018
Proposed End Date	30 June 2019
Project Leader	David Midgley

2. GISERA Region

☐ Queensland	☐ New South Wales	☐ Northern Territory
☒ **South Australia**	☐ Western Australia	☐ Victoria

3. GISERA Research Program

☒ **Water Research**	☐ GHG Research	☐ Social & Economic Research
☐ Biodiversity Research	☐ Agricultural Land Management Research	☐ Health Research

4. Project Summary

Objective

Onshore gas activities, including exploration and production, uses a range of chemical products. The risks associated with these chemicals have been the focus of numerous reviews, which have identified potential environmental and human health impacts[1, 2, 3]. While the risks of these chemicals have been identified, less is known regarding the migration and degradation of these chemicals in relevant edaphic and subsurface environments. In broad terms, this study adds to the other GISERA South Australia study on potential groundwater contamination causes, pathways and vulnerability to better understand onshore gas water quality impacts. The aim is to better understand the impacts and residual risk of these chemical compounds to environmental contamination over time in natural environments.

Specifically, this project seeks to understand whether compounds used in the production of onshore gas are degraded by microbes in relevant southeast South Australian soils and subsurface aquifers, in both oxic and anoxic conditions. In addition, the project seeks to examine the impact of these compounds on microbial communities. This will provide additional information on (a) those microbes involved in degradation and (b) changes in microbial consortia. The latter data may be useful for environmental monitoring and is being used in ecosystem health assessments as indicators of disturbance and change.

Description

This project is part of a series of linked projects that seek to holistically characterize the migration and degradation of chemicals used in onshore gas activities that have been previously identified to pose an environmental or human health risk. The individual components of this series of projects are:

1. Movement (W.13) Onshore gas and water contamination: causes, pathways and risks
2. Breakdown (this project) *Microbial degradation of chemicals used in onshore gas production*
3. Toxicology (Pending the outcomes of the above projects).

On completion of this project the ultimate fate of onshore gas-related chemicals will be established. This will be combined with the results from project W.13 which inform the likelihood of contaminant migration. Taken together data from these two projects will establish residual risk for each chemical under specific South Australian conditions and those compounds that pose a potentially significant residual risk will be subject to further analysis using standard toxicological screening against a range of model organisms.

This project will examine the microbial degradation of approximately 20, previously identified hazardous chemical compounds associated with onshore gas activities[1, 2, 3]. Degradation will be investigated under a mixed oxic and anoxic matrix (soil), as well as in an anoxic water matrix (subsurface aquifer). Both of these

experiments will be conducted in replicated microcosms (~500mL). Microcosms will be incubated under field-like conditions and temperatures. Statistical comparisons will be undertaken to confirm chemical degradation by microbial communities. The project will use 16S and ITS amplicon sequencing to describe effects on the microbial community.

The final report for this project will include data on the quantum of microbial degradation for individual chemicals used in onshore gas activities in South Australia, along with impacts on microbial communities and potential indicator taxa for contamination events.

Need & Scope

The community has raised concerns about potential risks of chemical contamination from onshore gas activities to water quality in South Australia[4]. These chemicals have multiple contaminant migration pathways for entry into the environment, both on the surface and in the subsurface[5, 6]. These pathways are the subject of research in project W.13 (Onshore gas and water contamination: causes, pathways and risks). While the microbial degradation of some of the chemical compounds previously identified as hazardous to environmental and human health have been studied, this has typically been undertaken in only a handful of microbial species. Little is known about the potential fate of these compounds in likely and in South Australian conditions and locations (soil, holding ponds and in the subsurface). Soils and subsurface aquifers are host to an array of microbial taxa with considerable catabolic potential. Microbial degradation may thus constitute an important risk mitigant. That is, chemicals that are readily degraded by microorganisms are unlikely to pose significant long term impacts on environmental and human health.

This project will provide specific local information on microbial degradation of important compounds used in onshore gas activities, i.e. if a hazardous chemical compound is readily and rapidly biodegraded, this compound's residual risk to environmental contamination is low. This information is an important input for practical risk assessment and regulatory protocols of industry processes in South Australia, particularly as southeast South Australia is a South Australia EPA water protection area.

This study will focus on ~20 identified compounds and will examine biodegradation in two settings (soil and subsurface water) relevant to onshore gas activities. Additionally, the present study will ascertain the impacts on microbial community profiles in the presence of these compounds. This latter piece of work may provide important information to stakeholders on a) the specific microbes responsible for degradation, and b) microbes that may serve as environmental health indicators for organisms at higher trophic levels.

Method

This project will begin with a literature and policy review (Task 1) that will ascertain prior knowledge of the degradation of the compounds of interest and their breakdown mechanisms. A short review will help refine experimental design for this project, minimise time in the laboratory by identifying potential experimental issues and understand the pathways of compound breakdown. In addition, current regulatory and management policies for chemicals investigated in this study will be reviewed through consultation with the relevant government and industry stakeholders. Finally, taken together, information from literature, and consultations with government and industry stakeholders, will be used to inform industrially relevant starting concentrations for the chemicals to be studied.

Soil and subsurface water samples will be collected from a relevant South Australian site under oxic and anoxic conditions (Task 2). Replicated microcosms (both anoxic and oxic) will be used to measure soil or aquifer degradation of individual chemicals using either analytical techniques (Task 3) or microbial growth assays (Task 4; see Figure 1). Chemicals with existing analytical methods are shown in Table 1. Where no analytical method exists for a chemical compound, sole carbon source growth trials will be conducted on solid media and in anoxic water samples. Growth assays provide evidence that microbes are able to grow on the chemical compounds as a sole source of carbon, however the rates of degradation and the residual compounds of degradation cannot be ascertained from the growth assay.

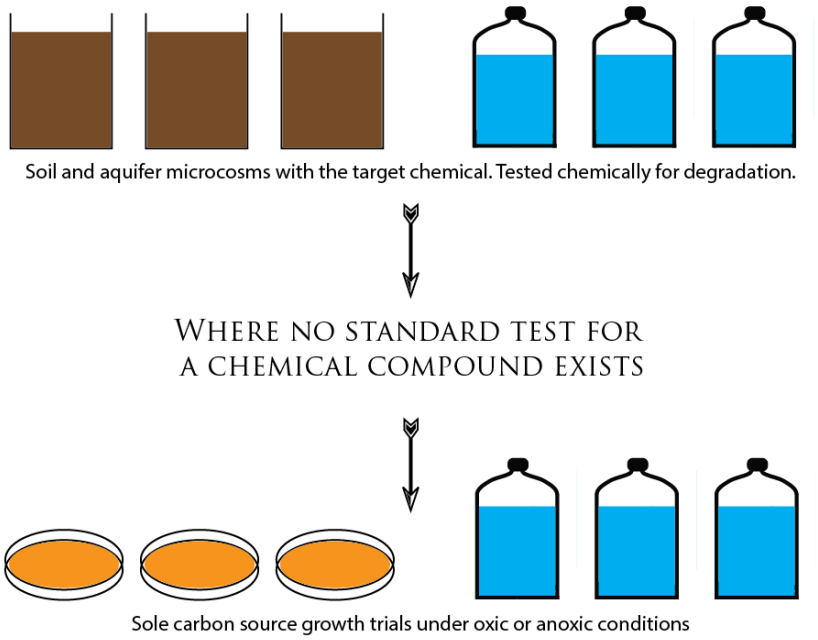

Figure 1: Schematic of workflow for onshore gas production chemical compound testing

Table 1: Onshore gas production chemical compounds of interest

Compound	Testing method	Use in onshore gas activities
Bronopol	Sole carbon source growth assay	Biocide
Methylchloroisothiazolinone	Sole carbon source growth assay	Antimicrobial preservative
Polyacrylamide / polyacrylate copolymer	Sole carbon source growth assay and biodegradation assay followed by acrylamide testing	Friction reducer
Acrylamide	Commercial method	Friction reducer
Xanthan gum	Sole carbon source growth assay	Viscosity management
Polyoxypropylene diamine	Sole carbon source growth assay	Pipework/Epoxy resins
Hexahydro- 1,3,5-tris(2-hydroxyethyl)-sym-triazine	Sole carbon source growth assay	Biocide
Glyoxal	Sole carbon source growth assay	Drilling additive
2-aminoethanol	Commercial method	Drilling additive
Limonene	Commercial method	Surfactant
2-methylphenol (o-cresol)	Commercial method	Biocide
Naphthalene	Commercial method	Corrosion inhibitor
Acetic acid	Commercial method	Buffer, stabiliser, solvent
Alcohols, C6-12, ethoxylated	Sole carbon source growth assay	Surfactant
Alkanes, C12-26 branched and linear	Sole carbon source growth assay	Surfactant
Benzisothiazolinone	Sole carbon source growth assay	Biocide
2-Butoxyethanol	Commercial method	Surfactant
Diethylene glycol ethyl ether	Sole carbon source growth assay	Solvent
Ethanol	Commercial method	Surfactant
Ethanolamine	Commercial method	Surfactant
Ethylene glycol	Commercial method	Viscosity management
Glutaraldehyde	Sole carbon source growth assay	Biocide
Isopropanol	Commercial method	Surfactant
Methanol	Commercial method	Surfactant
Methylisothiazolinone	Sole carbon source growth assay	Biocide
Pigment Red 5	Sole carbon source growth assay	Viscosity management

Triethanolamine	Sole carbon source growth assay	Viscosity management
Propylene glycol	Commercial method	Viscosity management
2-Ethylhexanol	Commercial method	Surfactant

DNA ecogenomic profiling of microbial communities after exposure to individual chemicals will be carried out to ascertain impacts on microbial communities and identify putatively useful indicator taxa for monitoring environmental impacts (Task 5).

The final report for this project will collate data on microbial degradation, microbial community impact and useful indicator taxa for individual chemicals used in onshore gas activities in South Australia (Task 6). These data will be combined with results from project W.13 to inform requirements for future toxicological studies and will provide information for a range of stakeholders.

5. Project Inputs

Research

The list of chemical compounds used in onshore gas activities (Table 1) pose a potential risk of accidental release, spills or leaks resulting in residual concentrations contaminating the environment due to failure of storage ponds, spills or infrastructure failures. Biodegradation of many of these chemical compounds have been partially investigated in other settings, however, South Australia has unique edaphic conditions, and degradation of compounds under these conditions are unknown. This work will provide information about how these compounds are degraded under specific South Australian conditions. While other research will examine the complete process – from where chemicals originate and their transportation – the effort in this project will focus on quantities and risks that remain after appropriate accepted management and regulatory processes are put in place (i.e. residual risks).

This project has been developed in collaboration with Dan O'Sullivan and Sreekanth Janardhanan, along with contributions from the CSIRO GISERA team.

Resources and collaborations

Researcher	Time Commitment (project as a whole)	Principle area of expertise	Years of experience	Organisation
David Midgley	30 days	Microbial Ecology & Catabolism	>20 years	CSIRO
Tania Vergara	30 days	Analytical chemistry	~5 years	CSIRO
Nai Tran-Dinh	23 days	Microbial Ecology	>20 years	CSIRO
Richard Schinteie	36 days	Geology / Geochemistry	>15 years	CSIRO

Subcontractors (clause 9.5(a)(i))	Time Commitment (project as a whole)	Principle area of expertise	Years of experience	Organisation
ALS	1-2 weeks turnaround on receipt of samples.	Testing	Many. Commercial laboratory.	ALS. NATA accredited laboratory.
Sequencing service provider	6-8 weeks turnaround on receipt of samples.	DNA sequencing, microbiomes.	Many. Commercial DNA sequencing facility.	MR DNA (Molecular Research LP) 503 Clovis Rd Shallowater, TX 79363

GISERA
Gas Industry Social and Environmental Research Alliance

Project Budget Summary

Source of Cash Contributions	2017/18	2018/19	2019/20	% of Cash Contribution	Total
GISERA	-	$176,703.00	-	75%	$180,453.00
- SA Government	-	$88,351.50	-	37.5%	$90,226.50
- Federal Government	-	$88,351.50	-	37.5%	$90,226.50
Total Cash Contributions	**-**	**$176,703.00**	**-**	**75%**	**$180,453.00**
Source of In-Kind Contribution	**2017/18**	**2018/19**	**2019/20**	**% of In kind Contribution**	**Total**
CSIRO	-	$58,901	-	25%	$60,151
Total In-Kind Contribution	**-**	**$58,901**	**-**	**25%**	**$60,151**

6. Project Impact Pathway

Activities	Outputs	Short term Outcomes	Long term outcomes	Impact
Literature and policy review	Short literature review outlining the published research into the biodegradation of chemicals of interest. Identification of any research gaps. Consultation with government and industry stakeholders to determine regulatory and management policies for chemicals used in onshore gas activities.	Provide information on the biodegradation of chemicals involved in onshore gas activities and the perturbation caused by chemicals involved in onshore gas activities. Additionally, the project will provide information on (a) the changes to microbial communities caused by chemical exposure and (b) provide information on indicator taxa.	The findings generated from this research will directly inform Governments, regulators and policy-makers on the chemicals used in onshore gas activities in southeast South Australia, and the gas industry as a whole. Provide additional information on microbial species that may be indicators of chemical exposure. Improved industry practice and decision making to minimize the risk of groundwater contamination due to leaks and spills of chemicals related to onshore gas activities. Improved community awareness about the environmental impacts of onshore gas activities.	The research generated will provide information for the benefit of all Australian communities in onshore gas regions and industry. This research will help to inform decision-making at the government policy-making level, in the area of surface and groundwater contamination. The onshore gas industry operates in a socially, economically and environmentally sustainable way.
Determination of the risk reductions due to microbial degradation of chemicals used in onshore gas activities.	Technical report outlining the results from projects tasks investigating biodegradation of chemicals involved in onshore gas activities, microbial growth on chemicals used in onshore gas activities and changes in microbial communities due to exposure to chemicals used in onshore gas activities.			
Development of a set of chemical-specific indicator microbial taxa for environmental health.	Technical report outlining the results from projects tasks that identify microbial taxa that are sensitive to individual chemicals that could be used as indicators for environmental health.			
Work synergistically with the “Onshore gas and water contamination: causes, pathways and risks” project	Coordination and integration of technical report from this study with project W.13. Together with results from project W.13, identify chemicals with high residual risk and provide direction for future toxicological studies.			
Develop fact sheets with key findings	Completed fact sheet(s) with key findings for distribution via the GISERA website and at community engagement events.			

Prepare and submit scientific manuscripts for publication in peer-reviewed journals	Manuscript submission to peer-reviewed journals.			

7. Project Plan

Project Schedule

ID	Activities / Task Title (should match activities in impact pathway section)	Task Leader	Scheduled Start	Scheduled Finish	Predecessor
Task 1	Literature and policy review	Richard Schinteie	October 2018	November 2018	
Task 2	Sample collection- soil and water	Nai Tran-Dinh	November 2018	November 2018	
Task 3	Compounds with commercial methods of measurement	David Midgley	October 2018	February 2019	Task 2
Task 4	Compounds with no commercial methods of measurement	David Midgley	October 2018	February 2019	Task 2
Task 5	DNA ecogenomic profiling	Nai Tran-Dinh	March 2019	May 2019	Tasks 2, 3 & 4
Task 6	Final Report	David Midgley	May 2019	June 2019	All other tasks.

Task description

Task 1
TASK NAME: Literature and policy review
TASK LEADER: Richard Schinteie
OVERALL TIMEFRAME: October 2018 to November 2018
BACKGROUND: Knowledge of prior studies on the degradation of the compounds of interest is required to understand breakdown mechanisms. A review of this literature will help in focusing experiments for this project, minimise time in the laboratory by identifying potential experimental issues and understand the pathways of compound breakdown. In addition, current regulatory and management policies for chemicals investigated in this study will be reviewed through consultation with the relevant government and industry stakeholders. Taken together, information from literature, and consultations with government and industry stakeholders, will be used to inform industrially relevant starting concentrations for the chemicals to be studied.
TASK OBJECTIVES:
1) Scan literature on prior breakdown studies of the compounds of interest;
2) Describe the methods of these studies and list breakdown mechanisms and products;
3) Consult government and industry stakeholders for current regulatory and management policies around the chemicals investigated in this study; and
4) Determine concentrations of chemicals to be investigated in Tasks 3 and 4.
TASK OUTPUTS AND SPECIFIC DELIVERABLES: Short written report listing the findings as described above in 'Task Objectives'.

Task 2
TASK NAME: Sample collection- soil and water
TASK LEADER: Nai Tran-Dinh
OVERALL TIMEFRAME: November 2018
BACKGROUND: Anoxic water collection is required by CSIRO staff with appropriate sampling equipment.
TASK OBJECTIVES: To collect oxic and anoxic samples:
1) Soil from locations proximal to putative production locations. Sampling will be carried out from the top 50 cm of soil,
2) Subsurface water from drilled wells.
Two CSIRO staff will drive to the Otway Basin with appropriate sampling equipment (pumps, bailers, purge gas and other equipment) to collect the samples to ensure the survival of microbial communities. NB: Due to anoxic collections it is required that the samples not be shipped via air for both scientific and logistic reasons.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: Collection of oxic and anoxic samples to establish microcosms.

Task 3

TASK NAME: Compounds with commercial methods of measurement

TASK LEADER: David Midgley

OVERALL TIMEFRAME: October 2018 to February 2019

BACKGROUND: Using samples collected during Task 2, replicated soil or aquifer water microcosms will be established to quantify biodegradation of compounds of interest that be measured using a NATA accredited test.

TASK OBJECTIVES: The task will include the following objectives:

1) Establish replicated microcosms;
2) Spike microcosms with target compounds at realistic* concentrations;
3) Incubate at realistic conditions for 90 days. i.e. for soil microcosms, incubate at field relevant conditions (local temperatures and day/night cycle will be reproduced in the laboratory) for aquifer water microcosms relevant subsurface temperature will be used in the absence of light.
4) Harvest all treatments and prepare samples for commercial analysis.
5) Statistical analyses of the resultant data.

**spike concentrations will be determined considering both the limit of detection of the analytical method and the amount routinely used by the onshore gas industry.*

TASK OUTPUTS AND SPECIFIC DELIVERABLES: Replicated Experimental data on the degradation of target compounds.

Task 4

TASK NAME: Compounds without commercial methods of measurement

TASK LEADER: David Midgley

OVERALL TIMEFRAME: October 2018 to February 2019

BACKGROUND: Growth experiments will be established using chemical compounds of interest as a sole carbon source, in both solid media and anoxic water. Where possible estimates of biodegradation will be measured by loss of mass of the chemical of interest, otherwise growth of microbes will be quantified and used as a proxy for biodegradation.

TASK OBJECTIVES: The task will include the following objectives:

1) Establish sole carbon source experiments;
2) Incubate at a relevant field conditions for 90 days (see Task 3 for conditions);
3) Determine the mass of chemical degraded by microbes;
4) Inspect cultures for visual signs of growth and where possible (in the aquifer cultures) measure biomass.

5) Statistical analyses of the resultant data.
TASK OUTPUTS AND SPECIFIC DELIVERABLES: Experimental data on the ability of microbes to grow on the chemicals of interest as a sole source of carbon, where applicable information on mass of degraded compound or a quantitation of biomass produced.

Task 5
TASK NAME: DNA ecogenomic profiling
TASK LEADER: Nai Tran-Dinh
OVERALL TIMEFRAME: March 2019 to May 2019
BACKGROUND: Microbial community profiling will be performed on soil and water aquifer samples collected in Task 2. Microbial community profile changes will be assessed for both biodegradation experiments (Task 3) and for sole carbon source growth experiments (Task 4).
TASK OBJECTIVES: The task will include the following objectives:
1) Extract DNA from soil and water samples collected in Task 2 to determine initial microbial community profiles;
2) Establish soil and aquifer microcosm storage controls without any chemical addition;
3) Incubate all treatments and controls at a relevant field conditions for 90 days (Tasks 3 and 4);
4) Extract DNA from all treatments and controls to determine microbial community changes;
5) Statistical and bioinformatics analyses of the resultant data.
TASK OUTPUTS AND SPECIFIC DELIVERABLES: Experimental data on the microbial community changes in response to exposure to onshore gas activity-related chemicals.

Task 6
TASK NAME: Final Report
TASK LEADER: David Midgley
OVERALL TIMEFRAME: May 2019 to June 2019
BACKGROUND: Critical evaluation of the results is needed to understand the experimental outcomes of this study.
TASK OBJECTIVES: The task will include the following objectives:
1) Reporting and updating the literature review in Task 1;
2) Reporting results and analyses from Tasks 3-5;
3) Critically discuss the results in light of prior studies (Task 1);
4) Integration of this studies results with those of project W.13; and
4) Provide recommendations of chemicals with potential high residual risk requiring toxicological studies.
TASK OUTPUTS AND SPECIFIC DELIVERABLES: Final written report encompassing all the tasks outlined above and integration with its sibling project (W.13).

Project Gantt Chart

Task	Task Description	Task Leader	Oct-18	Nov-18	Dec-18	Jan-19	Feb-19	Mar-19	Apr-19	May-19	Jun-19
1	Literature and policy review	Richard Schinteie									
2	Sample collection- soil and water	Nai Tran-Dinh									
3	Compounds with commercial methods of measurement	David Midgley									
4	Compounds with no commercial methods of measurement	David Midgley									
5	DNA ecogenomic profiling	Nai Tran-Dinh									
6	Final Report	David Midgley									

8. Technical Reference Group

The project will establish a Technical Reference Group (TRG) aimed at seeking peer-to-peer technical advice on contextual matters and to discuss research needs as well as outputs as the project progresses. The TRG will include the project leader and a group of different stakeholders as appropriate.

9. Communications Plan

Stakeholder	Objective	Channel	Timeframe
Government and industry	To facilitate a deeper understanding of research findings and implications for policy, programs, planning, and other initiatives	Knowledge transfer session	Towards completion
Wider public	To communicate key findings from the research	GISERA-facilitated fact sheets	Towards completion
Scientific community	To publish results in international peer-reviewed journals	Manuscript for submission to journals	At completion

10. Budget Summary

Expenditure	2017/18	2018/19	2019/20	Total
Labour	-	$151,104	-	$151,104
Operating	-	$34,500	-	$34,500
Subcontractors	-	$55,000	-	$55,000
Total Expenditure	-	**$240,604**	-	**$240,604**

Expenditure per Task	2017/18	2018/19	2019/20	Total
Task 1	-	$28,999	-	$28,999
Task 2	-	$17,073	-	$17,073
Task 3	-	$92,488	-	$92,488
Task 4	-	$34,200	-	$34,200
Task 5	-	$26,054	-	$26,054
Task 6	-	$41,790	-	$41,790
Total Expenditure	-	**$240,604**	-	**$240,604**

Source of Cash Contributions	2017/18	2018/19	2019/20	Total
SA Government (37.5%)	-	$90,226.50	-	$90,226.50
Federal Government (37.5%)	-	$90,226.50	-	$90,226.50
Total Cash Contributions	-	**$180,453**	-	**$180,453**

In-Kind Contribution from Partners	2017/18	2018/19	2019/20	Total
CSIRO (25%)	-	$60,151	-	$60,151
Total In-Kind Contribution from Partners	-	**$60,151**	-	**$60,151**

	Total funding over all years	Percentage of Total Budget
SA Government Investment	$90,226.50	37.5%
Federal Government Investment	$90,226.50	37.5%
CSIRO Investment	$60,151	25.0%
TOTAL	**$240,604**	

Task	Milestone Number	Milestone Description	Funded by	Start Date (mm-yy)	Delivery Date (mm-yy)	Fiscal Year Completed	Payment $ (excluding CSIRO contribution)
Task 1	1.1	Literature review document	GISERA	Oct-18	Nov-18	2018-19	$21,749.25
Task 2	2.1	Sample Collections- soil and water	GISERA	Nov-18	Nov-18	2018-19	$12,804.75
Task 3	3.1	Compounds with commercial methods of measurement	GISERA	Oct-18	Feb-19	2018-19	$69,366.00
Task 4	4.1	Compounds with no commercial methods of measurement	GISERA	Oct-18	Feb-19	2018-19	$25,650.00
Task 5	5.1	DNA economic profiling	GISERA	Mar-19	May-19	2018-19	$19,540.50
Task 6	6.1	Final report	GISERA	May-19	Jun-19	2018-19	$31,342.50

11. Intellectual Property and Confidentiality

Background IP (clause 11.1, 11.2)	**Party**	**Description of Background IP**	**Restrictions on use (if any)**	**Value**
				$
				$
Ownership of Non-Derivative IP (clause 12.3)	CSIRO			
Confidentiality of Project Results (clause 15.6)	Project Results are not confidential.			
Additional Commercialisation requirements (clause 13.1)	Not Applicable			
Distribution of Commercialisation Income (clause 13.4)	Not Applicable			
Commercialisation Interest (clause 1.1)	**Party**		**Commercialisation Interest**	
	CSIRO		Not Applicable	
	Other		Not Applicable	

12. References

1. Australian Government Department of the Environment and Energy (2017) National assessment of chemicals associated with coal seam gas extraction in Australia – Overview (http://www.environment.gov.au/system/files/resources/03137f85-1bea-46a4-b9e7-67d985b4aeb5/files/national-assessment-chemicals-overview.pdf)
2. Australian Government Department of the Environment and Energy (2014) Hydraulic fracturing ('fraccing') techniques, including reporting requirements and governance arrangements (http://www.environment.gov.au/system/files/resources/de709bdd-95a0-4459-a8ce-8ed3cb72d44a/files/background-review-hydraulic-fracturing_0.pdf)
3. Australian Government Department of the Environment and Energy (2017) Risk assessment guidance manual: for chemicals associated with coal seam gas extraction (http://www.environment.gov.au/system/files/consultations/81536a00-45ea-4aba-982c-5c52a100cc15/files/risk-assessment-guidance-manual-chemicals-associated-csg-extraction-australia-exposure-draft.pdf)
4. SA EPA (2014) South East Regional Groundwater Quality Monitoring, Evaluation and Reporting Program, Environment Protection Authority, 21st Oct, 2014
5. Mallants D, Bekele E, Schmid W and Miotlinski K (2017) Human and environmental exposure conceptualisation: Soil to shallow groundwater pathways, Project report prepared by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) as part of the National Assessment of Chemicals Associated with Coal Seam Gas Extraction in Australia, Commonwealth of Australia, Canberra.
6. Mallants D, Apte S, Kear J, Turnadge C, Janardhanan S, Gonzalez D, Williams M, Chen Z, Kookana R, Taylor A, Raiber M, Adams M, Bruce J, Prommer H (2017) Deeper groundwater hazard screening research, prepared by the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Canberra.

2 Variations to Project Order

Changes to research Project Orders are approved by the GISERA Director, acting with authority provided by the GISERA National Research Management Committee, in accordance with the National GISERA Alliance Agreement.

The table below details variations to research Project Order.

Register of changes to Research Project Order

Date	Issue	Action	Authorisation

3 Progress against project milestones

Progress against milestones are approved by the GISERA Director, acting with authority provided by the GISERA National Research Management Committee, in accordance with the National GISERA Alliance Agreement.

Progress against project milestones/tasks is indicated by two methods: Traffic Light Reports and descriptive Project Schedule Reports.

1. Traffic light reports in the Project Schedule Table below show progress using a simple colour code:
   - Green:
     - Milestone fully met according to schedule.
     - Project is expected to continue to deliver according to plan.
     - Milestone payment is approved.
   - Amber:
     - Milestone largely met according to schedule.
     - Project has experienced delays or difficulties that will be overcome by next milestone, enabling project to return to delivery according to plan by next milestone.
     - Milestone payment approved for one amber light.
     - Milestone payment withheld for second of two successive amber lights; project review initiated and undertaken by GISERA Director.
   - Red:
     - Milestone not met according to schedule.
     - Problems in meeting milestone are likely to impact subsequent project delivery, such that revisions to project timing, scope or budget must be considered.
     - Milestone payment is withheld.
     - Project review initiated and undertaken by GISERA Research Advisory Committee.
2. Progress Schedule Reports outline task objectives and outputs and describe, in the 'progress report' section, the means and extent to which progress towards tasks has been made.

Project Schedule Table

ID	Activities / Task Title (should match activities in impact pathway section)	Task Leader	Scheduled Start	Scheduled Finish	Predecessor
Task 1	Literature and policy review	Richard Schinteie	October 2018	November 2018	
Task 2	Sample collection- soil and water	Nai Tran-Dinh	November 2018	November 2018	
Task 3	Compounds with commercial methods of measurement	David Midgley	October 2018	February 2019	Task 2
Task 4	Compounds with no commercial methods of measurement	David Midgley	October 2018	February 2019	Task 2
Task 5	DNA ecogenomic profiling	Nai Tran-Dinh	March 2019	May 2019	Tasks 2, 3 & 4
Task 6	Final Report	David Midgley	May 2019	June 2019	All other tasks.

Project Schedule Report

TASK 1

TASK NAME: Literature and policy review

TASK LEADER: Richard Schinteie

OVERALL TIMEFRAME: October 2018 to November 2018

BACKGROUND: Knowledge of prior studies on the degradation of the compounds of interest is required to understand breakdown mechanisms. A review of this literature will help in focusing experiments for this project, minimise time in the laboratory by identifying potential experimental issues and understand the pathways of compound breakdown. In addition, current regulatory and management policies for chemicals investigated in this study will be reviewed through consultation with the relevant government and industry stakeholders. Taken together, information from literature, and consultations with government and industry stakeholders, will be used to inform industrially relevant starting concentrations for the chemicals to be studied.

TASK OBJECTIVES:

1) Scan literature on prior breakdown studies of the compounds of interest;

2) Describe the methods of these studies and list breakdown mechanisms and products;

3) Consult government and industry stakeholders for current regulatory and management policies around the chemicals investigated in this study; and

4) Determine concentrations of chemicals to be investigated in Tasks 3 and 4.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: Short written report listing the findings as described above in 'Task Objectives'.

PROGRESS REPORT:

The milestone is now complete.

TASK 2

TASK NAME: Sample collection- soil and water

TASK LEADER: Nai Tran-Dinh

OVERALL TIMEFRAME: November 2018

BACKGROUND: Anoxic water collection is required by CSIRO staff with appropriate sampling equipment.

TASK OBJECTIVES: To collect oxic and anoxic samples:

1) Soil from locations proximal to putative production locations. Sampling will be carried out from the top 50 cm of soil,

2) Subsurface water from drilled wells.

Two CSIRO staff will drive to the Otway Basin with appropriate sampling equipment (pumps, bailers, purge gas and other equipment) to collect the samples to ensure the survival of microbial communities. NB: Due to anoxic collections it is required that the samples not be shipped via air for both scientific and logistic reasons.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: Collection of oxic and anoxic samples to establish microcosms.

PROGRESS REPORT:

100% complete. Two CSIRO staff collected soil and water samples from Penola, South Australia with the assistance of staff from Beach Energy. Subsurface water samples were collected from monitoring holes near well sites.

TASK 3

TASK NAME: Compounds with commercial methods of measurement

TASK LEADER: David Midgley

OVERALL TIMEFRAME: October 2018 to February 2019

BACKGROUND: Using samples collected during Task 2, replicated soil or aquifer water microcosms will be established to quantify biodegradation of compounds of interest that be measured using a NATA accredited test.

TASK OBJECTIVES: The task will include the following objectives:

1) Establish replicated microcosms;

2) Spike microcosms with target compounds at realistic* concentrations;

3) Incubate at realistic conditions for 90 days. i.e. for soil microcosms, incubate at field relevant conditions (local temperatures and day/night cycle will be reproduced in the laboratory) for aquifer water microcosms relevant subsurface temperature will be used in the absence of light.

4) Harvest all treatments and prepare samples for commercial analysis.

5) Statistical analyses of the resultant data.

**spike concentrations will be determined considering both the limit of detection of the analytical method and the amount routinely used by the onshore gas industry.*

TASK OUTPUTS AND SPECIFIC DELIVERABLES: Replicated Experimental data on the degradation of target compounds.

PROGRESS REPORT:

All microcosms were harvested and sent for testing in NATA accredited laboratories. Data indicates most chemicals are significantly, and rapidly degraded or lost in soil microcosms. Similar, statistically significant losses of most chemicals were also observed in anoxic water (the aquifer-like microcosms), though more slowly than for soils.

TASK 4

TASK NAME: Compounds without commercial methods of measurement

TASK LEADER: David Midgley

OVERALL TIMEFRAME: October 2018 to February 2019

BACKGROUND: Growth experiments will be established using chemical compounds of interest as a sole carbon source, in both solid media and anoxic water. Where possible estimates of biodegradation will be measured by loss of mass of the chemical of interest, otherwise growth of microbes will be quantified and used as a proxy for biodegradation.

TASK OBJECTIVES: The task will include the following objectives:

1) Establish sole carbon source experiments;

2) Incubate at a relevant field conditions for 90 days (see Task 3 for conditions);

3) Determine the mass of chemical degraded by microbes;

4) Inspect cultures for visual signs of growth and where possible (in the aquifer cultures) measure biomass.

5) Statistical analyses of the resultant data.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: Experimental data on the ability of microbes to grow on the chemicals of interest as a sole source of carbon, where applicable information on mass of degraded compound or a quantitation of biomass produced.

PROGRESS REPORTS:

Observations indicate readily microbial growth on most chemicals as a sole source of carbon. Numerous bacterial and fungal colonies were observed on our soil mimicking agar medium. Similarly, distinct turbidity was observed for most chemicals in our aquifer-like microcosms indicating compounds were readily used as a sole source of carbon.