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FINAL REPORT

Research to provide data to support application for a minor use permits for chemicals including trichlorfon, sodium and calcium hypochlorite, copper sulphate, hydrogen peroxide, benzalkonium chloride for control of disease including White Spot Syndrome Virus (WSSV) in Australian prawn farms.

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Abbreviations

APFA	Australian Prawn Farmers Association
FFVS	Future Fisheries Veterinary Service
APVMA	Australian Pesticide and Veterinary Medicine Authority
BKC	Benzalkonium chloride
$CuSO_4$	Copper sulphate
H_2O_2	Hydrogen peroxide
LC50	Lethal concentration 50%
EC50	Effective concentration 50%
PAA	Pre-application Assistance
MUP	Minor Use permit

See further in Appendices

Executive Summary

In late 2016, an exotic viral disease, White Spot Disease (WSD), was diagnosed in multiple Australian prawn farms on the Logan River catchment, Queensland. The subsequent disease response program resulted in the complete eradication of the seven infected farms (approximately 25% of the Australian prawn farming industry). Following this exotic disease outbreak an intensive review of farm-level biosecurity practices on Australian prawn farms was performed to safeguard against future endemic and exotic disease incursions. The prawn farming industry are now taking a proactive step to improve the disease preventative measures on farm to reduce the likelihood and impact of disease entry, spread and exit from their farms.

Biosecurity measures offer the prospect of safeguarding against future disease incursions into farms. Extensive investment has been allocated into auditing on-farm biosecurity measures to identify weaknesses in bio-exclusion and biocontainment that could allow potential pathogens to enter and spread on farm. Audits performed consider major routes of transmission, assesses the nature and validity of perceived hazards, rank risks, and provided recommendations for managing identified risks, with the aim of reducing overall risk of pathogen entry and spread within the farm. Biosecurity audits of Australian prawn farms have identified deficiencies in the tools that are available to prevent pathogen entry and spread on farms. In order to improve on-farm biosecurity practices the industry is seeking APVMA permits for several chemical products. Trichlorfon, Hydrogen Peroxide, Calcium and Sodium Hypochlorite, Benzalkonium Chloride and Copper Sulphate which have been highlighted as the most important.

The following additional chemicals are also of utility in control of WSD risks on prawn farms: sodium/calcium hypochlorite; hydrogen peroxide; copper sulphate; and benzalkonium chloride. The project will seek to assemble and where possible generate data to support minor-use permit (MUP) applications to APVMA for their use in disease control on prawn farms. Access to trichlorfon, sodium and calcium hypochlorite; hydrogen peroxide; copper sulphate; and benzalkonium chloride will help biosecurity agencies and industry to eradicate diseases if these are encountered (as per AquaVet Plan) and also employ this chemical for prevention and control of vectors during biosecure farming operations.

Objectives

1. Compile public domain data ready for submission to APVMA for trichlorfon MUP
2. Compile public domain data ready for submission to APVMA for hydrogen peroxide MUP
3. Compile data for copper sulphate, sodium and calcium hypochlorite and benzalkonium chloride to progress towards application-ready status
4. Collect field data to support trichlorfon use as advised by APVMA

The project involved generation of chemical data packages through review and aggregation of public domain documents for each chemical (trichlorfon, hydrogen peroxide, sodium and calcium hypochlorite, copper sulphate and benzalkonium chloride). This facilitated identification of data gaps requiring further data generation and progress towards the point of APVMA minor use permit application submission. Collection of field-based data was performed to meet APVMA requirements to support use of trichlorfon under a MUP.

Review of available knowledge and public domain literature was performed for Trichlorfon, Hydrogen Peroxide and Calcium and Sodium Hypochlorite to allow completion of data packages and facilitate subsequent submission of minor-use permit (MUP) applications to the Australian Pesticides and Veterinary Medicines Authority (APVMA). Review of available knowledge and public domain literature was performed for Benzalkonium Chloride and Copper Sulphate to allow identification of additional data requirements needed to complete of minor-use permit (MUP) applications.

This project has assisted to progress of Australian prawn farms access to priority chemical products that can assist farm biosecurity, through a process that aims to ensure safe and efficacy use of chemical products on Australian prawn farms.

Introduction

In late 2016, an exotic viral disease, White Spot Disease (WSD), was diagnosed in multiple Australian prawn farms on the Logan River catchment, Queensland. The subsequent disease response program resulted in the complete eradication of the seven infected farms (approximately 25% of the Australian prawn farming industry). Following this exotic disease outbreak an intensive review of farm-level biosecurity practices on Australian prawn farms was performed to safeguard against future endemic and exotic disease incursions. The prawn farming industry are now taking a proactive step to improve the disease preventative measures on farm to reduce the likelihood and impact of disease entry, spread and exit from their farms. In order to improve on-farm biosecurity practices the industry is seeking Australian Pesticides and Veterinary Medicines Authority (APVMA) minor use permits (MUPs) for several chemical products. Trichlorfon, Hydrogen Peroxide, Calcium and Sodium Hypochlorite, Benzalkonium Chloride and Copper Sulphate which have been highlighted as the most important.

Biosecurity measures offer the prospect of safeguarding against future disease incursions into farms. Extensive investment has been allocated into auditing on-farm biosecurity measures to identify weaknesses in bio-exclusion and biocontainment that could allow potential pathogens to enter and spread on farm. Audits performed consider major routes of transmission, assesses the nature and validity of perceived hazards, rank risks, and provided recommendations for managing identified risks, with the aim of reducing overall risk of pathogen entry and spread within the farm.

Biosecurity audits of Australian prawn farms have identified deficiencies in the tools that are available to prevent pathogen entry and spread on farms. The following additional chemicals are also of utility in control of WSD risks on prawn farms: sodium/calcium hypochlorite; hydrogen peroxide; copper sulphate; and benzalkonium chloride. The project will seek to assemble and where possible generate data to support MUP applications to APVMA for their use in disease control on prawn farms. Access to trichlorfon, sodium/calcium hypochlorite; hydrogen peroxide; copper sulphate; and benzalkonium chloride will help biosecurity agencies and industry to eradicate diseases if these are encountered (as per AquaVet Plan) and also employ this chemical for prevention and control of vectors during biosecure farming operations.

Objectives

1. Compile public domain data ready for submission to APVMA for trichlorfon MUP
2. Compile public domain data ready for submission to APVMA for hydrogen peroxide MUP
3. Compile data for copper sulphate, sodium and calcium hypochlorite and benzalkonium chloride to progress towards application-ready status
4. Collect field data to support trichlorfon use as advised by APVMA

Method

1. Assemble data packages through aggregation of public domain documents for each chemical (trichlorfon, hydrogen peroxide, sodium/calcium hypochlorite and progress towards permits for copper sulphate and benzalkonium chloride).
2. Prepare trichlorfon and hydrogen peroxide data to be submission ready to APVMA.
3. Prepare data for sodium/calcium hypochlorite and progress towards permits for copper sulphate and benzalkonium chloride.
4. Field-based data collection to meet APVMA requirements to support use of trichlorfon under a MUP.

Results, Discussion, Conclusion

Future Fisheries Veterinary Service (FFVS) have progressed the status of the data required for MUP applications for the priority chemicals highlighted by the Australian Prawn Farmers Association (APFA),

including: Trichlorfon, Hydrogen Peroxide, Calcium and Sodium Hypochlorite, Benzalkonium Chloride and Copper Sulphate.

Progress against project objectives are described below and in Figure 1. This includes details on the minor use permit progression during FRDC 2018-099 and current approval status of target chemicals: Trichlorfon, Hydrogen Peroxide, Calcium and Sodium Hypochlorite, Benzalkonium Chloride and Copper Sulphate.

1. Compile public domain data ready for submission to APVMA for trichlorfon MUP
    a. **Trichlorfon**: FFVS generated the data package for sodium hypochlorite and calcium hypochlorite through extensive literature assessment and review and designed a proposed use pattern of the chemical product for the farms (See appendix 1 and 2). This allowed APFA to submit an item 21 APVMA MUP application on 08/10/18.

2. Compile public domain data ready for submission to APVMA for hydrogen peroxide MUP
    a. Hydrogen Peroxide: FFVS generated the data package for sodium hypochlorite and calcium hypochlorite through extensive literature assessment and review and designed a proposed use pattern of the chemical product for the farms (See appendix 3 and 4). This allowed APFA to submit an item 21 APVMA MUP application on 13/03/19.

3. Compile data for copper sulphate, sodium and calcium hypochlorite and benzalkonium chloride to progress towards application-ready status
    a. **Copper sulphate**: FFVS generated the data package for copper sulphate through extensive literature assessment and review (See appendix 7). Gap analysis performed on the data package identified a range of data deficiencies considered necessary to complete the data package required by APVMA for submission with an application for a minor-use permit (See appendix 7).
    b. **Calcium and Sodium Hypochlorite**: FFVS generated the data package for sodium hypochlorite and calcium hypochlorite through extensive literature assessment and review and designed a proposed use pattern of the chemical product for the farms (See appendix 5). This allowed APFA to submit an item 21 APVMA MUP application on 05/12/19.
    c. **Benzalkonium chloride:** FFVS generated the data package for benzalkonium chloride through extensive literature assessment and review (See appendix 6). Gap analysis performed on the data package identified a range of data deficiencies considered necessary to complete the data package required by APVMA for submission with an application for a minor-use permit (See appendix 6).

4. Collect field data to support trichlorfon use as advised by APVMA
    a. FFVS collected and analysed water samples 12 days following treatment of a body of water with 0.5 mg/L trichlorfon on a commercial Australian prawn farm (as per requirements of PER87229). Samples were collected on 30/01/19 and 27/02/19. Testing was performed at the Chemical Residues Laboratory, Queensland Department of Agriculture and Fisheries using an in-house testing method. Two samples were tested, with no detection of trichlorfon or dichlorvos residues (Limit of detection = 0.02 µg/L). This data was provided to APVMA. Test results demonstrate (limited current sample number) that trichlorfon and metabolite, dichlorvos, appear to degrade within 12 days of treatment.
    b. Due to these unforeseen circumstances, following the guidance of APVMA, the commissioning and commencement of the Ecotox laboratory studies on trichlorfon were not commenced. Guidance provided by APVMA requested submission of compiled public domain data prior to commencing any laboratory or field trials.

This project has assisted to provide Australian prawn farms improved access to priority chemical products that can assist farm biosecurity. Detailed review of available knowledge and public domain literature to complete data packages and fill information gaps has assisted the completion of minor-use permit (MUP) applications to the APVMA for Trichlorfon, Hydrogen Peroxide and Calcium and Sodium Hypochlorite, while identifying additional data requirements needed to complete of MUP applications for Benzalkonium Chloride and Copper Sulphate. This process ensures safe and efficacy use of chemical products on Australian prawn farms.

Table 1. Minor use permit progression during FRDC 2018-099 and current approval status of target chemicals, Trichlorfon, Hydrogen Peroxide, Calcium and Sodium Hypochlorite, Benzalkonium Chloride and Copper Sulphate

Minor-use Permit Progression	**Trichlorfon**	**Hydrogen Peroxide**	**Calcium and Sodium Hypochlorite**	**Copper Sulphate**	**Benzalkonium Chloride**
1. Data collation (Peer reviewed, public domain and industry data)	Complete	Complete	Complete	Complete	Complete
2. Data Gap analysis and review	Complete	Complete	Complete	Complete	Complete
3. Update data package and satisfy data gaps in data package	Complete	Complete	Complete	Incomplete	Incomplete
4. Draft minor-use permit	Complete	Complete	Complete	Incomplete	Incomplete
5. Specific Module Data Package criteria	Complete	Complete	Complete	Incomplete	Incomplete
6. APVMA Online application	Complete	Complete	Complete	Incomplete	Incomplete
7. APVMA Review and Assessment	Complete	In Progress	In Progress	Incomplete	Incomplete
8. Additional Peer review or public domain data requirements or MUP review from APVMA	Complete	Incomplete	Incomplete	Incomplete	Incomplete
9. Additional Trials and data generation (Lab or field)	Complete	Incomplete	Incomplete	Incomplete	Incomplete
10. APVMA approved Minor use Permit	Complete	Incomplete	Incomplete	Incomplete	Incomplete

Summary

Percentage Complete	100%	60%	0%	20%	20%
Percentage In progress	0%	10%	20%	0%	0%
Percentage Incomplete	0%	30%	80%	80%	80%
Comments	Data collation completed. MUP application completed. APVMA review completed. MUP Issued.	Data collation completed. MUP application completed and submitted, pending APVMA review.	Data collation completed. MUP application completed and submitted, pending APVMA review.	Initial Data review and gap analysis performed. Advise submit this with a PAA to guide progression to MUP	Initial Data review and gap analysis performed. Advise submit this with a PAA to guide progression to MUP
FRDC Project 2018-099 Status	Complete	Complete	Complete	Complete	Complete
Comments	Data collation completed to facilitate MUP application and submission	Data collation completed to facilitate MUP application and submission	Data collation completed to facilitate MUP application and submission	Data collation completed. Additional generation data required to complete data package to point of submission	Data collation completed. Additional generation data required to complete data package to point of submission

Implications

This project has assisted to provide Australian prawn farms improved access to priority chemical products that can improve farm biosecurity and disease response. The project has facilitated submission of Trichlorfon, Hydrogen Peroxide and Calcium and Sodium Hypochlorite MUP applications to the APVMA, while identifying additional data requirements needed to complete MUP applications for Benzalkonium Chloride and Copper Sulphate.

This process ensures safe and efficacy use of chemical products on Australian prawn farms.

Recommendations

It is recommended that:

1. Additional efficacy and environmental data are collected alongside all use of trichlorfon under PER87229 to facilitate variations to the current MUP conditions, renewal and progress to full product registration.
2. Additional efficacy and environmental data are collected alongside all use of hydrogen peroxide and calcium and sodium hypochlorite, should that satisfy APVMA MUP requirements, to facilitate variations to future MUP conditions, renewal and progress to full product registration.
3. It is recommended that the current benzalkonium chloride and copper sulphate data packages be drafted into a APVMA PAA for submission to determine if the APVMA considers the identified additional data requirements sufficient to complete the data package and/or identify any further data requirements that that may be required to complete the application.
4. All MUP chemical product users record all use details to assist compliance and future permitting.

Further development

Copper sulphate and benzalkonium chloride data review identified areas where additional data is considered required to complete the relevant data assessment. To complete this data requirement and progress to the point of APVMA MUP submission, additional funding may be required.

Extension and Adoption

The project outputs facilitated the submission of an APVMA MUP application for trichlorfon, hydrogen peroxide, sodium hypochlorite and calcium hypochlorite. The trichlorfon MUP application has since been approved by the APVMA provide access of this chemical to Australian prawn farms. The hydrogen peroxide, sodium hypochlorite and calcium hypochlorite MUP applications are current pending assessment. The copper sulphate and benzalkonium chloride project outputs are available to help guide generation of further research to complete the necessary data assessments for future MUP applications.

The development of trichlorfon, hydrogen peroxide, sodium and calcium hypochlorite, copper sulphate and benzalkonium chloride data packages will assist the APFA progress and obtain MUPs to facilitate access of important biosecurity chemical tools to member farms. The project outcomes fast-tracking data assessment and MUP applications for these chemicals in different use patterns throughout the broader aquaculture sector providing important biosecurity tools.

Project outputs can extend to the broader aquaculture industry as these documents and permit applications can be drawn upon to fast-track equivalent MUP applications.

Project coverage

Nil

Project materials developed

Data packages for Trichlorfon, Hydrogen Peroxide, Calcium and Sodium Hypochlorite, Benzalkonium Chloride and Copper Sulphate (See Appendix 1-7).

Appendix 1: Trichlorfon Environmental Data Assessment

Application Overview

In late 2016, an exotic viral disease, White Spot Disease (WSD), was diagnosed in multiple Australian prawn farms on the Logan River catchment, Queensland. The subsequent disease response program resulted in the complete eradication of the seven infected farms (approximately 25% of the Australian prawn farming industry). Following this exotic disease outbreak an intensive review of farm-level biosecurity practices on Australian prawn farms was performed to safeguard against future endemic and exotic disease incursions. The prawn farming industry are now taking a proactive step to improve the disease preventative measures on farm to reduce the likelihood and impact of disease entry, spread and exit from their farms. In order to improve on-farm biosecurity practices the industry is seeking APVMA permits for several chemical products. Trichlorfon, Hydrogen Peroxide, Calcium and Sodium Hypochlorite, Benzalkonium Chloride and Copper Sulphate which have been highlighted as the most important.

One of the major risk pathways for pathogens to enter a prawn farm is through water and crustaceans entrained within that incoming water. Water is brought onto farms from local rivers and waterways to fill ponds and tanks prior to stocking prawns (Landos, 2017) (Diggles, 2017). This risk can be reduced by destroying any pathogen carrying crustacean vectors, prior to filling production ponds and stocking with prawns. Currently there are no APVMA registered or approved products that provide efficacious destruction of carrier crustaceans potentially infected with or carrying disease-causing organisms in influent water, prior to use on farm.

A second major risk pathway for the spread of pathogens within a prawn farm stems from an outbreak within the index culture unit. This risk can be reduced through early detection and rapid eradication of the infected and potentially infected populations. Rapid eradication reduces the amplification of the pathogen, thereby reducing the potential for spread around the farm and to neighbouring environments. Currently there are no APVMA registered or approved products that provide efficacious destruction of farm stock (*Penaeus monodon* and *Fenneropenaeus merguiensis*) infected with or exposed to disease-causing organisms.

The ability of this industry to remain viable and expand will in part depend on its ability to strengthen on-farm biosecurity measures. An essential element of these measures is considered to be the permit for the use of trichlorfon for water treatment and control of future disease outbreaks.

Proposed use:

Situation	**Purpose**	**Rate**
Aquaculture earthen ponds and tanks	Destruction of carrier crustaceans potentially infected with or carrying prawn disease in influent water. Treated water must be spelled, prior to use in production ponds.	1 ml product 1000 L^{-1} water (0.5 mg L^{-1} active trichlorfon)
	Destruction of farm stock infected with or exposed to disease-causing organisms. Water must be spelled for minimum 12 days prior to release.	4 ml product 1000 L^{-1} water (2 mg L^{-1} active trichlorfon)

Pre-Application Assistance (PAA)

A pre-application assistance (PAA) was completed to assist this application in 2017. PAA ID: DCPAA2-62653145A (assistance tier 2), Product number: 70118. Product name: IMTRADE TYRANEX 500 SL INSECTICIDE & NEMATICIDE

This suggested minor-use permit application (item 21) would be addressed as follows;

Module 1: Preliminary Assessment

Module 7.3 Environment

Module 8.1 Efficacy and Safety

Module 11.2 Finalisation

Data Assessment

We are seeking to use peer reviewed, published, and publicly available literature to provide information to demonstrate safety, efficacy and trade criteria of trichlorfon and its active metabolites for this application. Imtrade Australia have also provided access to the supportive data package to progress this minor-use permit application. Please advise where additional data may be needed for a successful minor-use permit application.

Module 7 - Environment

The proposed use pattern differs from current APVMA permits and the label registered use pattern of the product. However, the use pattern is similar to previous emergency-use APVMA permits (PER83675 and PER84088). Therefore, the environmental risk is considered similar to these latter two permits.

Following use of the product the water will be held for a period that is considered sufficient to allow all trichlorfon and hydrolyzed actives (dichlorvos) to be broken down, prior to using the water for either; stocking prawn larvae for grow-out or discharge be into adjacent water ways. Peer-reviewed, scientific literature and scientific argument have been used to demonstrate the proposed will mitigate risk of environmental exposure to active chemical. The environmental effects of trichlorfon has been review in detail in the overseas report 'Environmental Health Criteria 132: trichlorfon' published by the World Health Organization in 1992 (World Health Organisation, 1992) and the 1997 US EPA Re-registration Eligibility Decision (RED) report (United States Environmental Protection Agency, 2006). The applicant considers that data generated under Australian conditions should not be required as the environmental effect the product will be the same as overseas, and there are no environmental differences between Australia and the overseas countries that would be relevant to the toxicological effect of the product.

A summary of the environmental impacts of the product will also be provided using the range of published literature available across a range of taxa, and ecotoxicology data will be provided through the chemical supplier (Imtrade Australia) in support of this application.

The toxicology is not considered to differ from current use of trichlorfon approved by APVMA in the agriculture industry, so this information is not considered to be a novel requirement of this application. Additionally, the environmental effects of dichlorvos (the active metabolite generated from breakdown of trichlorfon) has been review in detail in the Australian report 'DICHLORVOS: Environmental Assessment' published by Australian Pesticides & Veterinary Medicines Authority in 2008 (Australian Pesticides & Veterinary Medicines Authority (APVMA), 2008). The applicant considers that data generated under Australian conditions in this report should be a relevant dossier to cover the environmental effect the breakdown product dichlorvos. A summary of the environmental effects of the product will also be provided using the range of published literature available across a range of taxa, and ecotoxicology data will be provided through the chemical supplier (Imtrade Australia) in support of this application.

Due to proposed the use of trichlorfon into bodies of water bodies as a liquid chemical and emphasis has been made on assessment of environmental toxicology.

1. Environmental Exposure and Breakdown
    a. General
        i. The major routes of trichlorfon dissipation in water are via hydrolysis and aerobic metabolism (USEPA, 1997).
        ii. Trichlorfon is initially hydrolysed to dichlorvos, but is 100% hydrolyzed in approximately 24 hours to nontoxic products, at pH 8 and 37.5°C. (United Nations, 1985)
        iii. Hydrolysis half-lives (at 22°C) of trichlorfon were reported as 46 hours (pH 7), and <30 minutes (pH 9) (Tomlin, 2003). The pH of inlet sea water in Australian Prawn farms is typically 7.5 to 8.5 (The Department of Primary Industries and Fisheries (DPI&F), 2006) [See also Figure 1]. This may vary if ponds are in areas of acid sulphate soils. To ensure sufficient conditions to facilitate breakdown pH is to be maintained above pH 7 prior to and for duration of conditioning period of proposed treatment.
    b. Atmosphere
        i. If released to air trichlorfon has a vapor pressure of 7.80×10^{-6} mm Hg at 20°C, which indicates that trichlorfon will exist in both the vapor and particulate phases in the atmosphere
            1. The vapor-phase of trichlorfon will be degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals.
            2. The half-life for this reaction in air is estimated to be 5.2 days (US National Library of Medicine, 2006).
            3. The particulate-phase of trichlorfon will be removed from the atmosphere by wet or dry deposition (US National Library of Medicine, 2006).
    c. Aqueous
        i. Under UV radiation, trichlorfon in aqueous solution undergoes photochemical conversion to dichlorvos, but this occurs slowly (Tomlin, 2003) (Floesser-Mueller & Schwack, 2001).
        ii. If released into water, trichlorfon is not expected to adsorb to suspended solids and sediment (US National Library of Medicine, 2006)
        iii. Based on vapor pressure (7.80×10^{-6} mm Hg) (Freed, 1977) and water solubility (1.20×10^{5} mg L^{-1}) (Lopes, et al., 2006), volatilization of trichlorfon from water surfaces is not expected (Lyman & Reehl, 1990) (Freed, 1977)
        iv. Potential for bioconcentration in aquatic organisms is low (Meylan, et al., 1999) (Franke, et al., 1994) (Douett, et al., 2009).
        v. Rate of breakdown of trichlorfon is temperature and pH dependent and decomposition is increased in alkaline and warmer solutions (O'Neil, 2001 p. 1716) (Douett, et al., 2009).
            1. The half-life of trichlorfon at 4.5-15°C, seawater is from 1.2 to 6.3 days (Samuelsen, 1987).
            2. Complete biodegradation of trichlorfon in river water occurred within 5 days at 10 mg/L, 13 days at 20 mg L^{-1}, and 20 days at 30 mg L^{-1}. (K, 2001)
            3. Trichlorfon breakdown increases with high temperature or high pH (above pH 6), but decreases with low temperature or low pH (below pH 5) (Tomlin, 2003) (K, 2001) (Wu, et al., 2002)
        vi. In activated sludge inoculum at 20°C aerobic biodegradation rate of trichlorfon was determined to be 0.28 day^{-1} with a half-life of 2.5 days (Kawamoto K, 1989)
        vii. Trichlorfon applied at 1.0 mg L^{-1} to pond water and incubated for 24 hours (USEPA, 1997).
            1. At pH 5.0 trichlorfon did not degrade significantly.
            2. At pH 8.5 trichlorfon degraded rapidly to dichlorvos and could not be detected after 8 hours.
            3. Dichlorvos concentrations peaked at 0.56 mg L^{-1} after 8 hours of incubation and declined to 0.39 mg L^{-1} after 24 hours.

viii. When 0.5 mg L^{-1} trichlorfon was added to water, 95-100% trichlorfon was broken down by 1 hours post addition of 0.5 mg L^{-1} (Ekachote, 1999)
    1. 0.011-0.016 mg L^{-1} dichlorvos detected in water 5 days following 0.5 mg L^{-1} trichlorfon addition. (Ekachote, 1999)
    2. Both trichlorfon and dichlorvos were not detected in pond soil after 5 days (at pH 7.5, 8.0, 8.5) (Ekachote, 1999)

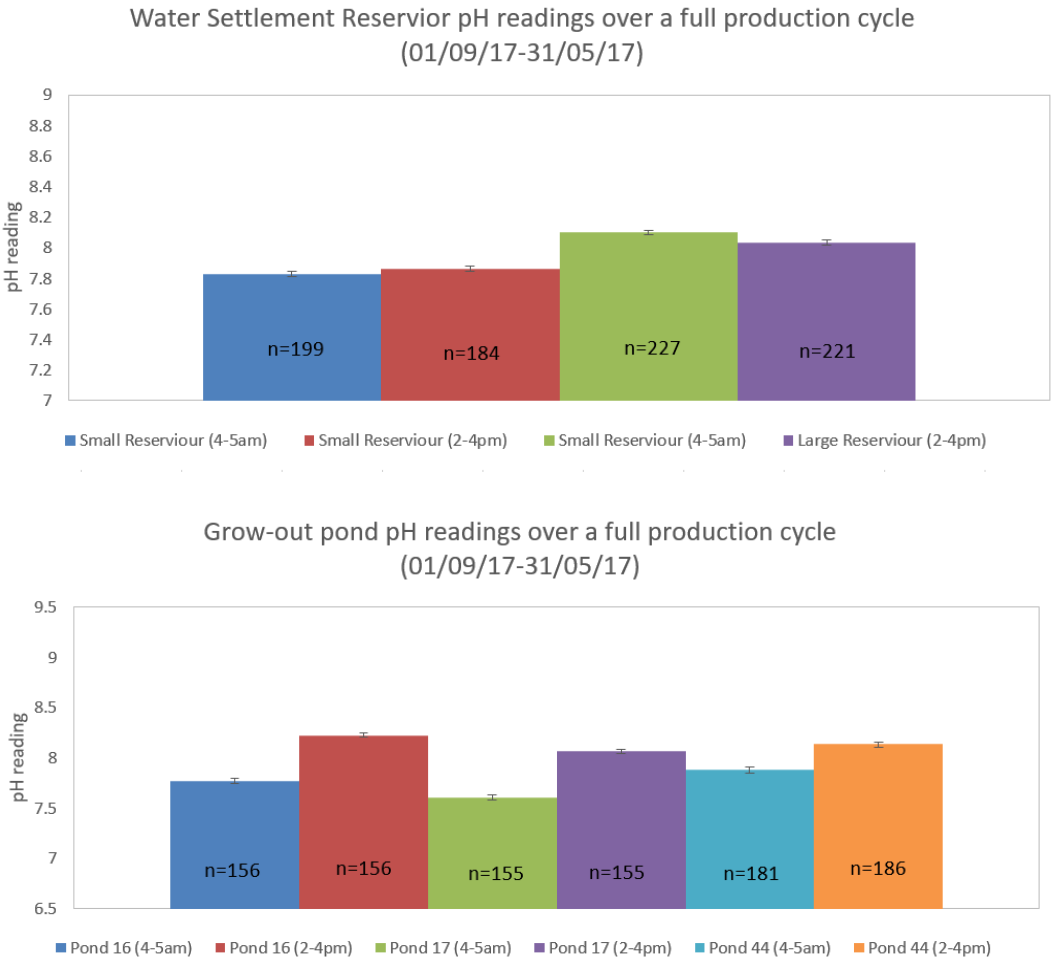

Figure 1. *Water Reservoir and grow-out pond am and pm pH reading during an entire production cycle at an Australian Prawn Farmers Association (APFA) member farm. The number of water samples assessed for each pond and time point are shown in over the coloured column, and error bars are displayed as standard error (standard deviation/square-root of the number of samples). Source data set is available for analysis of additional ponds if required.*

d. Terrestrial
    i. If released to soil, trichlorfon is expected to have high to very high mobility (Odanaka, et al., 1994).
    ii. Persistence of trichlorfon in soils has been reported as 2 weeks or less and is degraded by ammonifying microorganisms occurs (K, 2001).
    iii. In creeping bentgrass, the dissipation half-life of trichlorfon was found to be 1.1 to 6 days (Wu, et al., 2002).
    iv. The degradation half-life of trichlorfon at soil depths of 0-2 cm (6.6% organic carbon content), 2-5 cm (3.4% OC), and below 5 cm (0.3% OC) were 1.0, 7.7, and 0.5 days, respectively (Wu, et al., 2002)
    v. In aerobic soils, the reported trichlorfon half-life is between 3 and 27 days in aerobic soils, with an average of 10 days (Wu, et al., 2002).

vi. Trichlorfon at initial concentration of 2 mg L^{-1} in soil degraded by 100% in 1.5 months at pH values of 3 to 4.6, and in 0.5 months at pH values of 8.7 to 9.05 (US National Library of Medicine, 2006)
vii. Half-lives in clay (pH 7.9) and calcerous (pH 8.1) soils have been determined to be 1.15 and 1.05 days, respectively (Guirguis MW, 1975)

2. Non-target species toxicology:
    a. Rats and Mice
        i. 80-90% of consumed trichlorfon is excreted in in the first 4 hours via urine, faeces, and expired air, with only 2% found in tissues after 96 hours (USEPA, 1997).
        ii. The plasma half-life of trichlorfon is approximately 80 mins (Bingham, et al., 2001).
        iii. There are no negative effects observed at concentrations less than or equal to 100 mg/kg (World Health Organisation, 1992), and sub-chronic exposure is not reported to have a cumulative effect (World Health Organisation, 1992).
        iv. LD_{50} of 600 mg/kg for an oral dose (United States Environmental Protection Agency, 2006)
        v. LD_{50} of greater than 2000 mg kg^{-1} for a dermal dose (United States Environmental Protection Agency, 2006)
        vi. LD_{50} of greater than 533 mg m^{-3} for a 4-hour inhalation dose (United States Environmental Protection Agency, 2006)
    b. Fish
        i. Toxicity increases with pH and decreases with temperature (Howe, et al., 1994).
        ii. Rainbow trout (*Salmo gairdneri*)
            1. LC_{50} 96-hour exposure at 12°C in saltwater with a pH 7.5 is 4.85 mg L^{-1} (United States Environmental Protection Agency, 2006).
        iii. Cutthroat trout (*Salmo clarki*)
            1. LC_{50} 96-hour exposure at 12°C in saltwater with a pH 6.5 is 4.75 mg L^{-1} (United States Environmental Protection Agency, 2006).
            2. LC_{50} 96-hour exposure at 12°C in saltwater with a pH 7.5 is 3.25 mg L^{-1} (United States Environmental Protection Agency, 2006).
            3. LC_{50} 96-hour exposure at 12°C in saltwater with a pH 8.5 is 0.375 mg L^{-1} (United States Environmental Protection Agency, 2006).
        iv. Estuarine Sheephead minnow (*Cyprinodon variegatus*)
            1. LC_{50} of 96 hour exposure is 13-19 mg L^{-1} (Brecken-Folse, et al., 1994)
    c. Dogs
        i. No acute negative effects reported at concentrations of 50 mg kg^{-1} or below (Bingham, et al., 2001).
        ii. Cholinesterase depression was observed following 12-26 weeks of oral administration of trichlorfon at 1.25mg Kg^{-1} day^{-1} (National Health and Medical Research Council (NHMRC), 2011).
    d. Birds
        i. European starling (*Sturnus vulgaris)*
            1. LD_{50} oral dose is 37-75 mg kg^{-1} (United States Environmental Protection Agency, 2006).
    e. Bivalves
        i. Trichlorfon is rapidly diluted and degraded in water to dichlorvos, so the potential effect appears limited to bivalves reared within or in close contact with fish farms (Le Bris, et al., 1995). No mortality with 1 mg L^{-1} dichlorvos exposure, but relaxation of adductor muscles occurs at 0.1 mg L^{-1} and 1 mg L^{-1} exposure (Le Bris, et al., 1995).
3. Cleanup Methods:
    a. Environmental considerations:
        i. Contain a spill and absorb with clay, sand, soil or proprietary absorbent (Imtrade Australia Pty Ltd, 2016) (Imtrade Australia Pty Ltd, n.d.)

ii. Contaminated earth (after a spill) can be treated with lime to increase the pH and hasten decomposition of the active ingredient (Imtrade Australia Pty Ltd, 2016) (Imtrade Australia Pty Ltd, n.d.)
iii. Refer to MSDS for response to accidental spills (Imtrade Australia Pty Ltd, 2016)

4. Disposal Methods:
    a. Recycle any unused portion of the material for its approved use or return it to the manufacturer or supplier.
    b. Plastic liners, and paper or card containers should be crushed and buried well below top soil or burned, preferably in an incinerator (Stellman, 1983).
    c. Trichlorfon initially hydrolyzes to the more toxic compound dichlorvos at pH 8 and 37.5°C, but is essentially 100% hydrolyzed in approximately 24hr to nontoxic products which can be mixed with a portion of soil rich in organic matter and buried. Recommendable methods include Incineration, hydrolysis, & landfill. Peer-review: Large amount of trichlorofon should be incinerated at high temp in a unit with effluent gas scrubbing (International Register of Potentially Toxic Chemicals (IRPTC) Expert Consultation, 1985 p232).

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Appendix 2: Trichlorfon Efficacy Data Assessment

Application Overview

In late 2016, an exotic viral disease, White Spot Disease (WSD), was diagnosed in multiple Australian prawn farms on the Logan River catchment, Queensland. The subsequent disease response program resulted in the complete eradication of the seven infected farms (approximately 25% of the Australian prawn farming industry). Following this exotic disease outbreak an intensive review of farm-level biosecurity practices on Australian prawn farms was performed to safeguard against future endemic and exotic disease incursions. The prawn farming industry are now taking a proactive step to improve the disease preventative measures on farm to reduce the likelihood and impact of disease entry, spread and exit from their farms. In order to improve on-farm biosecurity practices the industry is seeking APVMA permits for several chemical products. Trichlorfon, Hydrogen Peroxide, Calcium and Sodium Hypochlorite, Benzalkonium Chloride and Copper Sulphate have been highlighted as the most important.

One of the major risk pathways for pathogens to enter a prawn farm is through water and crustaceans entrained within that incoming water. Water is brought onto farms from local rivers and waterways to fill ponds and tanks prior to stocking prawns (Landos, 2017) (Diggles, 2017). This risk can be reduced by destroying any pathogen carrying crustacean vectors, prior to filling production ponds and stocking with prawns. Currently there are no APVMA registered or approved products that provide efficacious destruction of carrier crustaceans potentially infected with or carrying disease-causing organisms in influent water, prior to use on farm.

A second major risk pathway for pathogens to spread within a prawn farm is following an outbreak within a farm production unit. This risk can be controlled through early detection and eradication of infected and potentially infected stock. This will reduce the amplification and spread of disease within and from a farm system. Currently there are no APVMA registered or approved products that provide efficacious destruction of farm stock (*Penaeus monodon* and *Fenneropenaeus merguiensis*) infected with or exposed to disease-causing organisms.

To assist the strengthening of Australian prawn farms biosecurity, we are seeking a minor-use permit for the use of trichlorfon (Imtrade Australia Tyranex 500 SL, containing: 500 gL^{-1} Trichlorfon) on Australian Prawn Farmers Association (APFA) member farms to assist control of disease outbreaks and allow treatment of incoming water to destroy potential vectors of disease. For this industry to remain viable and grow into the future will depend, in part upon, the ability to strengthen on-farm biosecurity. The acquisition of a minor-use permit for trichlorfon for water treatment and control of disease outbreaks is considered an essential component of these biosecurity improvements.

Proposed use:

Situation	**Purpose**	**Rate**
Aquaculture ponds and tanks	Destruction of carrier crustaceans potentially infected with or carrying prawn disease in influent water. Treated water must be spelled, prior to use in production ponds.	1 ml product 1000 L^{-1} water (0.5 mgL^{-1} active trichlorfon)
	Destruction of farm stock infected with or exposed to disease-causing organisms. Water must be spelled for minimum 12 days prior to release.	4 ml product 1000 L^{-1} water (2 mgL^{-1}active trichlorfon)

Pre-Application Assistance (PAA)

A pre-application assistance (PAA) was completed to assist this application in 2017.
PAA ID: DCPAA2-62653145A (assistance tier 2), Product number: 70118.
Product name: IMTRADE TYRANEX 500 SL INSECTICIDE & NEMATICIDE

This suggested minor-use permit application (item 21) would be addressed as follows;

Module 1: Preliminary Assessment

Module 7.3 Environment

Module 8.1 Efficacy and Safety

Module 11.2 Finalisation

Data Assessment

We are seeking to use peer reviewed, published, and publicly available literature to provide information to demonstrate safety, efficacy and trade criteria of trichlorfon and its active metabolite (dichlorvos) for this application. Imtrade Australia have also provided access to the supportive data package to progress this minor-use permit application.

Module 8 - Efficacy and safety

Efficacy and safety are demonstrated using peer-reviewed literature and scientific studies on trichlorfon to support assessment based on the two proposed purposes of use of this chemical. A similar use pattern is proposed to previous emergency-use APVMA permits (PER83675 and PER84088). It is anticipated efficacy assessment can also draw upon the previous assessments for the above permits. Target dose and duration are set upon the target of 100% efficacy (destruction) of crustacea in each use pattern described above.

1. Crusticide to kill prawns in pond, semi-closed grow-out systems and carrier crustaceans in effluent channels and adjacent to ponds when White Spot Syndrome Virus (WSSV) is detected in a population of farmed prawns.
    a. Target Species (Prawns)
        i. Fairy Shrimp (*Streptocephalus torvicornis*)
            1. LC_{50} of a 48-hour exposure is 0.04 mgL^{-1} (Flerov, 1979)
        ii. Giant freshwater prawn (*Macrobrachium rosenbergii*)
            1. LC_{50} of a 24-hour exposure is 0.77 mgL^{-1} (Chang, et al., 2006)
            2. LC_{50} of a 48-hour exposure is 0.35 mgL^{-1} (Chang, et al., 2006)
            3. LC_{50} of a 72-hour exposure is 0.27 mgL^{-1} (Chang, et al., 2006)
            4. LC_{50} of a 96-hour exposure is 0.26 mgL^{-1} (Chang, et al., 2006)
        iii. Freshwater Crustacean (*Gammarus pseudolimnaeus*)
            1. LC_{50} of a 96-hour exposure at pH 6.5 is 0.14 mgL^{-1} (Howe, et al., 1994)
            2. LC_{50} of a 96-hour exposure at pH 9.5 is 0.02 mgL^{-1} (Howe, et al., 1994)
        iv. Estuarine Glass shrimp (*Palaemonetes sp.)*
            1. LC_{50} of a 48-hour exposure is 0.009-0.025 mgL^{-1} (Brecken-Folse, et al., 1994)
            2. LC_{50} of a 96-hour exposure is 0.006-0.011 mgL^{-1} (Brecken-Folse, et al., 1994)
        v. Shrimp (*Acetes* sp.)
            1. LC_{50} of a 24-hour exposure at pH 7.5 is 0.027 mgL^{-1} (Ekachote, 1999)
            2. LC_{50} of a 24-hour exposure at pH 8.0 is 0.0202 mgL^{-1} (Ekachote, 1999)
            3. LC_{50} of a 24-hour exposure at pH 8.5 is 0.013 mgL^{-1} (Ekachote, 1999)
        vi. Black tiger prawn (*Penaeus monodon*) post larvae (PL 15)
            1. LC_{50} of a 24-hour exposure at pH 7.5 is 0.0044 mgL^{-1} (Ekachote, 1999)

2. LC_{50} of a 24-hour exposure at pH 8.0 is 0.0325 mgL^{-1} (Ekachote, 1999)
3. LC_{50} of a 24-hour exposure at pH 8.5 is 0.0235 mgL^{-1} (Ekachote, 1999)

2. Crusticide to kill WSSV carrier crustaceans in influent water, prior to spelling, then for use in prawn production ponds.
    a. Trichlorfon applied to ponds at the rate of 1 mgL^{-1} water destroyed the food invertebrates for fish (World Health Organisation, 1992). This treatment killed large numbers of zooplankton, rotifers, and crustacea, in the first 24 hours after treatment, whereas the benthos died during the first week. (World Health Organisation, 1992)
    b. Trichlorfon was found to be more effective than formalin, hydrogen peroxide and copper sulphate to kill marine isopods (*Caecognathia coralliophila*) (Thing, et al., 2016). For this study 0.2 mgL^{-1} trichlorfon for 24 hours or 3.2 mgL^{-1} for 60 minutes effectively killed the isopods (Thing, et al., 2016).
    c. It is recommended that any crustacean carriers are eliminated by treating pond water with chemicals such as calcium hypochlorite or trichlorfon, before ponds are filled (Fegan & Clifford, 2001). Treated water should be held for at least 12 days to ensure all trichlorfon and active breakdown products are degraded before stocking the pond. The safety period for shrimp post larvae after 0.5ppm trichlorfon application in water pH 8.0 was 12 days in aerated condition and 15 days in non-aerated conditions. (Ekachote, 1999).
    d. Trichlorfon at a dose of 1 mg/L has been shown to reduce copepod crustaceans by 100%, but does not negatively impact bacteria, rotifers, navicular algae or cyanobacterium (Ruangdetkorn, 1998).
    e. The LC_{50} for a 96-hour exposure for planktonic crustacea *Daphnia magna* is 0.29 μgL^{-1} (Coelho, et al., 2011). A 0.5 mgL^{-1} trichlorfon dose reduced *Daphnia magna* by 100% and had no significant effect on fish (*Danio rerio*) early or adult lifestages, and no significant effect on algae (*Pseudokirchneriella subcapitata* and *Chlorella vulgaris)* (Coelho, et al., 2011).

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Appendix 3: Hydrogen Peroxide Efficacy Data Assessment

Abbreviations

CFU – Colony Forming Units
APFA – Australian Prawn Farmers Association
WSD – White Spot Disease
WSSV – White Spot Syndrome Virus
HP / H_2O_2 – Hydrogen Peroxide
LC50 – Lethal dose that kills 50 percent of a test sample.
EC50 – Half maximal effective concentration
LCLo - lowest lethal concentration
NOEC - No observed effect concentration
LOEC - Lowest observed effect concentration
MAC - Maximum allowable concentration
Phys. Tox. – Physiological Toxicity
PL – Post larvae
TCBS - Thiosulphate-citrate-bile salts-sucrose agar
TSA - Trypticase soy agar
MIC – Minimum Inhibitory Concentration
MBC - Minimum Bactericidal Concentration

Chemistry

Chemical Identity

Common Name: Hydrogen Peroxide

Chemical Name: Hydrogen Peroxide

Other Names: Hydrogen Peroxide 20-60%, INTEROX® EG-ST Hydrogen Peroxide 50%, INTEROX ® ST-50, INTEROX® ST-60

Tradenames: Various

CAS Number: 7722-84-1

Molecular Formula: H_2O_2

Molecular Weight: 34.014 g/mol

Structural Formula: HO-OH

Purity of the active constituent: 50-60% hydrogen peroxide, 40-50% water.

HYDROGEN PEROXIDE 50% (unregistered)
Containing: 598 g/L HYDROGEN PEROXIDE as its only active constituent.

HYDROGEN PEROXIDE 60% (unregistered)
Containing: 742 g/L HYDROGEN PEROXIDE as its only active constituent.

Detailed information on the chemistry of hydrogen peroxide is considered to already be held by APVMA. Where data gaps exist please refer to the complete data package including further references (Goor, Glenneber and Jacobi, 2007).

Application Overview

Background

In late 2016, an exotic viral disease, White Spot Disease (WSD), was diagnosed in multiple Australian prawn farms on the Logan River catchment, Queensland. The subsequent disease response program resulted in the complete eradication of the seven infected farms (approximately 25% of the Australian prawn farming industry). Following this exotic disease outbreak an intensive review of farm-level biosecurity practices on Australian prawn farms was performed to safeguard against future endemic and exotic disease incursions. The prawn farming industry are now taking a proactive step to improve the disease preventative measures on farm to reduce the likelihood and impact of disease entry, spread and exit from their farms. In order to improve on-farm biosecurity practices the industry is seeking APVMA permits for several chemical products. Trichlorfon, Hydrogen Peroxide, Calcium and Sodium Hypochlorite, Benzalkonium Chloride and Copper Sulphate which have been highlighted as the most important.

One of the major drivers for disease within a prawn farm is via proliferation of bacteria in culture water and the water conditions that culture animals are subject to. Disease outbreaks usually stem from within the index culture unit. Maintaining optimal culture conditions, monitoring pathogen presence and preventing of pathogen build-up in these systems can help prevent major disease outbreaks from occurring. This risk can be reduced through disinfection and killing micro-organisms, generally in these systems, to safe and manageable levels.

Currently there are no APVMA registered products that provide safe and efficacious control of micro-organisms, generally on Australian prawn (*Penaeus monodon* and *Fenneropenaeus merguiensis*) production ponds and tank systems.

Hydrogen peroxide provides is an effective general disinfectant that can rapidly knockdown micro-organisms in prawn production ponds and tanks. The active constituent is a reserved chemical under Schedule 3C—Reserved Schedule of the Agricultural and Veterinary Chemicals Code (AgVet Code) Regulations 1995. However, this only permits a chemical product of 6% Hydrogen peroxide to be used for this purpose, which is impractical for use on Australian prawn farms.

Future Fisheries Veterinary Service (FFVS) are applying for an item 21 minor-use permit (MUP) for use of unregistered hydrogen peroxide 50-60% as a disinfectant to kill micro-organisms generally responsible for disease in farmed giant black tiger prawns (Penaeus monodon) and banana prawns *(Fenneropenaeus merguiensis*).

This unregistered chemical product has a current minor-use permit for treatment of parasitic and fungal infections in saltwater and freshwater finfish and eggs (PER83276); expired emergency-use permits for control of viral infections in prawns (PER83658); expired emergency-use permits for control of bacterial infections in prawns (PER85506). This wide range of uses demonstrates the diverse disinfection properties of this chemical.

The ability of this industry to remain viable and expand will in part depend on its ability to strengthen on-farm biosecurity measures. An essential element of these measures is the safe, efficacious and legal access and use of hydrogen peroxide to assist farm disinfection procedures and control and prevention of future disease outbreaks.

Overseas Regulatory Activity

United States of America

In 2007, 35 % PEROX-AID® (Eka Chemicals, Marietta, Georgia) was approved by the US Food and Drug Administration (FDA) for use in aquaculture. This product is supplied in 5 and 55 gallon containers, over the counter (OTC), and is permitted as an immersion treatment for control of fungal and bacteria diseases in freshwater-reared finfish eggs; and freshwater-reared salmonids, coolwater finfish and channel catfish. Hydrogen peroxide is classified as a low regulatory compound by the Food and Drug Administration of the United States.

Indications for use:

The control of mortality in freshwater-reared finfish eggs due to saprolegniasis, for the control of mortality in freshwater-reared salmonids due to bacterial gill disease associated with Flavobacterium branchiophilum, and for the control of mortality in freshwater-reared coolwater finfish and channel catfish due to external columnaris disease associated with Flavobacterium columnare (Flexibacter columnaris).

Application and dose rate:

"Freshwater-reared finfish eggs: 500 to 1000 mg/L for 15 minutes in a continuous flow system once per day on consecutive or alternate days until hatch for all coldwater and coolwater species of freshwater-reared finfish eggs or 750 to 1000 mg/L for 15 minutes in a continuous flow system once per day on consecutive or alternate days until hatch for all warmwater species of freshwater-reared finfish eggs."

"Freshwater-reared salmonids: 100 mg/L for 30 minutes or 50 to 100 mg/L for 60 minutes once per day on alternate days for three treatments in a continuous flow water supply or as a static bath." "Coolwater species of freshwater-reared finfish (except northern pike & paddlefish) and channel catfish: 50 to 75 mg/L for 60 minutes once per day on alternate days for three treatments in a continuous flow water supply or as a static bath. Coolwater species of freshwater-reared finfish fry (except northern pike, pallid sturgeon & paddlefish) and channel catfish fry: 50 mg/L for 60 minutes once per day on alternate days for three treatments in continuous flow water supply or as a static bath."*

Canada

In 2016, Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the Pest Control Products Act and Regulations, granted full registration for the sale and use of Interox M-70 Hydrogen Peroxide, Interox CPMC-50 and Interox Paramove 50, containing the technical grade active ingredient hydrogen peroxide, for the treatment of sea lice on Atlantic salmon reared in marine aquaculture sites (Health Canada Pest Management Regulatory Agency (PMRA), 2016). The registration assessment stated: *"The evaluation found that, under the approved conditions of use, the products have value and do not present an unacceptable risk to human health or the environment."*

In Canada hydrogen peroxide is used as an algaecide, bactericide, fungicide, slimicide, sanitizer and acaricide and has a broad use pattern including use in aquaculture, agriculture, industry and as a hard and soft surface sanitizer (Health Canada Pest Management Regulatory Agency (PMRA), 2018). A recent review of all the above registered hydrogen peroxide products in Canada was performed by the PMRA. The PMRA has determined that continued registration of products containing hydrogen peroxide is acceptable (Health Canada Pest Management Regulatory Agency (PMRA), 2018).

For a full list of Registered Hydrogen Peroxide Products in Canada See attachment (Health Canada Pest Management Regulatory Agency (PMRA), 2018).

Asian prawn farms

In Asian prawn farms hydrogen peroxide is used as an effective disinfectant agent, to alter the growth of some primary producer species and affect the structure of microbial communities in the pond environment (Ali *et al.*, 2018).

Europe

Hydrogen peroxide is a generally recognised as safe (GRAS) veterinary medicinal product in as declared by the European Agency for the Evaluation of Medicinal Products Veterinary Medicines Evaluation Unit 1996.

Proposed Permit Details

Efficacy Claims

Hydrogen peroxide has proven disinfectant properties against micro-organisms generally (including but not limited to bacteria, cyanobacteria, fungi, algae and parasites).

Hydrogen peroxide has properties that yield rapid emergency oxygen delivery to a body of water when added directly to the water (including saltwater).

Use pattern

Proposed Directions for Use:

For use as a disinfectant to kill micro-organisms, generally, and as for emergency oxygen supplementation of production prawns in farmed giant black tiger prawns (*Penaeus monodon*) and banana prawns *(Fenneropenaeus merguiensis*) in aquaculture earthen pond sediment, water, tanks, equipment and surfaces.

Situation	**Purpose**	**Rate**
Aquaculture earthen pond sediment, water, tanks, equipment and surfaces.	Method 1: General disinfectant to kill micro-organisms in a situation where culture animals are absent.	Up to 100 mg/L (ppm) active hydrogen peroxide
	Method 2: General disinfectant to kill micro-organisms, OR, as a source of emergency oxygen in a situation where culture animals are present.	Up to 4 mg/L (ppm) active hydrogen peroxide

Methods of use

Restraint

DO NOT USE in environmental waters. Only for use in waters where the release of treated water can be controlled, following treatment.

The hydrogen peroxide product is not to be used in conjunction or mixed with any other chemical.

For disinfection purposes apply product during daytime.

For emergency oxygen supplementation apply product during day-time or night-time.

Critical Use Comments

Observe all label safety directions relevant to handling of hydrogen peroxide. Ensure all personnel handing the product are wearing correct PPE (refer to product label and MSDS).

Only apply product when the potential for undiluted product drift is minimal (e.g. sub-surface application via leaky hoses). Minimise bystander exposure from any spray drift.

DO NOT discharge or re-use treated water until the active hydrogen peroxide level is below detectable limits (<0.5 mg/L active hydrogen peroxide).

DO NOT stock post larvae into a treated production grow-out ponds until hydrogen peroxide level is safe levels (less than 4 mg/L active hydrogen peroxide).

Treatment:

Calculate the volume of concentrated hydrogen peroxide required (See Attachment 1).

Introduce the hydrogen peroxide product directly into the water body, ideally behind the water paddlewheel, to aid mixing and distribution throughout the water column. Ensure the entire water column is treated. Pre-dilution of the product will assist even mixing and distribution of the product throughout the water column.

Following the initial hydrogen peroxide addition test the active concentration from multiple locations around the water body to ensure target concentration is achieved. Where a lower than target dose is achieved perform a secondary hydrogen peroxide product addition (See Attachment 1).

Post treatment

Water must be retained to ensure complete breakdown of active hydrogen peroxide actives, prior to release or re-use.

Active hydrogen peroxide will undergo natural degradation to water and oxygen following cessation of product addition. This may vary between hours and days.

Treated water must be retained until the active hydrogen peroxide level is below detectable limits (<0.5 mg/L active hydrogen peroxide) before discharge or re-use.

Formulation, handling and disposal

Product to be used:

HYDROGEN PEROXIDE 50% (unregistered)
Containing: 598 g/L HYDROGEN PEROXIDE as its only active constituent.

HYDROGEN PEROXIDE 60% (unregistered)
Containing: 742 g/L HYDROGEN PEROXIDE as its only active constituent.

Product supplier:
Redox Pty Ltd
4 Holmes Rd
MINTO NSW 2566

Solvay Interox Pty Ltd
20-22 McPherson Street
BANKSMEADOW NSW 2019

Packaging

Concentrated hydrogen peroxide products are required due to the large volume of water required to be disinfected. Product distributed in reusable, multi-use industrial grade intermediate bulk containers (IBC) (approximate liquid volume of 950 L).

Data Assessment – Target Animal Efficacy

We are seeking to use peer reviewed, published, and publicly available literature to provide any necessary additional information to demonstrate safety, efficacy and trade criteria of hydrogen peroxide for this application. In addition, we are seeking to provide field data collected from an Australian prawn farm, under PER85506, and draw upon current and expired minor-use permits in aquaculture for this chemical active (PER83276, PER83658), for which there are large similarities in the use pattern. Efficacy data is detailed in the sections below.

There are, as of December 2018, thirteen APVMA currently registered products containing the single active constituent of hydrogen peroxide. These products consist of pool/spa sanitisers (6), pool/spa algicides (6), and a disinfectant (1), with claims to kill micro-organisms generally. They range in hydrogen peroxide concentration of 15% (g/L) to 60% (g/L). Half of the registered hydrogen peroxide products for use in domestic pools and spas are between 50-60% hydrogen peroxide concentration.

Hydrogen peroxide kills microorganisms by reacting with their surface membranes and release molecular oxygen into the water (Boyd and Tucker, 2014). The below information and additional collated data in Appendix 1-4, clearly demonstrate the efficacy of hydrogen peroxide against general microorganisms can be expected, as per the proposed use pattern (see 4. Exposure efficacy) of this minor-use permit application.

Hydrogen peroxide as disinfectant to kill microorganisms generally

The target organism to demonstrate efficacy for the proposed use pattern is the knock-down of microorganisms generally following exposure to hydrogen peroxide liquid concentrate.

The FAO published manual on Improving ‘Penaeus monodon hatchery practices’ advises periodic disinfection of hatchery pipelines with 20 mg/L hydrogen peroxide as part of the disinfection protocol (FAO Fisheries and Aquaculture Department, 2007). Additional efficacy information can be found in (European Union, 2015).

Algae

Phytoplankton species appear to display differential toxicity to hydrogen peroxide (Drábková, Admiraal and Maršálek, 2007). The ambient light or irradiance also impacts hydrogen peroxide toxicity, with toxicity increasing with increasing light irradiance (Drábková, Admiraal and Maršálek, 2007).

The addition of 50 mg/L hydrogen peroxide provided an emergency efficacious treatment to eliminate a toxic dinoflagellate bloom of *Alexandrium ostenfeldii* in a small brackish lake in the Netherlands, with algae cells abundance decreasing by 98% after 24 hours and 99.8% after 48 hours following treatment (Burson *et al.*, 2014).

Srisapoom et al., 1999 (In: Schmidt, Gaikowski and Gingerich, 2006) found that 4.19 and 7.18 mg/L hydrogen peroxide for 72 hours caused a 42% and 46% reduction, respectively, in chlorophyll of Oscillatoria spp.

The application of 1.6 mg/L hydrogen peroxide to antural seawater has been shown to be efficacious to control harmful algae blooms caused by the excessive growth of brown tide (*Aureococcus anophagefferens*) (Randhawa, Thakkar and Wei, 2012).

Bacteria and Cyanobacteria

A 4 mg/L hydrogen peroxide equivalent concentrations of sodium carbonate peroxyhydrate was shown to provide an efficacious kill of toxic cyanobacterium *Planktothrix* sp with treatment effects lasting up to 5 weeks and leaving no long-term traces of H2O2 in the environment (Sinha, Eggleton and Lochmann, 2018).

Cyanobacterium has been shown to have a 10 times greater toxicity compared to green alga and diatoms (Drábková, Admiraal and Maršálek, 2007). Efficacious dosages of 0.3 - 2 mg/L have been suggested to eliminate harmful cyanobacteria species, with little to no effects on beneficial green algae and diatoms (Drábková, Admiraal and Maršálek, 2007).

Lab trials to explore the lethal efficacy of hydrogen peroxide against cyanobacteria *Microcystis aeruginosa* found that 10.2, 51 and 102 mg/L hydrogen peroxide caused disruption of the cell membrane integrity in approximately 14%, 54% and 49%, 1 day following hydrogen peroxide administration, respectively (Fan *et al.*, 2013). Peak algicidal efficacy was found after an additional day (2 days following hydrogen peroxide administration), where the percentage of intact cells decreased to 16%, 31% and 7% in the 10.2, 51 and 102 mg/L hydrogen peroxide, respectively (Fan *et al.*, 2013).

Hydrogen peroxide has been approved by the US FDA for control of bacterial gill disease (*Flavobacterium branchiophilum*) in freshwater-reared salmonids and external columnaris (*Flavobacterium columnare*) disease in freshwater-reared coolwater finfish and channel catfish (Yanong, 2014). Immersion of freshwater ornamental finfish in 3.1mg/L hydrogen peroxide for 1 hour has been shown effective for control of external bacteria (Russo, Curtis and Yanong, 2007).

Hydrogen peroxide has been recommended at a concentration of 240 mg/L as a general disinfection preventative measure for treating water and surface tanks to reduce risk of marine bacteria, *Tenacibaculum maritimum* (Avendaño-Herrera *et al.*, 2006).

In Thailand prawn (*Penaeus monodon)* farming, *Vibrio harveyi*, is considered one of the most important pathogens, with high levels causing serious health problems to production animals and severe economic losses (Saeed Ganjoor, 2017). To assist management and disinfection, the chemical product, Sanosil-25®, was developed. This contains active constituents of 48% hydrogen peroxide and 0.05% silver ion (Ag+) as a stabilising agent (Saeed Ganjoor, 2017). At 30°C for 48 hour exposure the Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) for Sanosil-25® against *Vibrio harveyi* was determined to be 15 mg/L and 20 Mg/L, respectively (Saeed Ganjoor, 2017). This is equivalent to 7.2 mg/L and 9.6 mg/L hydrogen peroxide.

The minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of human oral *Steptococcus* sp. was found to range between 2.3-14.1 mg/L and 3.5-28.2 mg/L, respectively (García-Mendoza *et al.*, 1993).

The bacteria, *Vibrio* sp., are a particular pathogen of concern for Australian prawn farming. A study by Srisapoom et al., 1999 (In:Schmidt, Gaikowski and Gingerich, 2006), determined the minimum inhibitory concentration (MIC) of hydrogen peroxide for a range *Vibrio spp*, ranging from 9.57-38.27 mg/L and 0.6-2.39 mg/L in the presence of 1.5% NaCl (see also Appendix 5). The growth of some bacteria may be adversely affected by concentrations as low as 0.0034 mg/L (Schmidt, Gaikowski and Gingerich, 2006).

Hydrogen peroxide is reported to have bacterial sporicidal properties at 0.88 mol/l (30mg/L) (Baldry, 1983).

Fungi and Moulds

Hydrogen peroxide has been approved by the US FDA for control of *Saprolegniasis* (a common water mould) on freshwater-reared finfish eggs (Yanong, 2014). US FDA approved treatment regime for *Saprolegniasis* is between 500 and 1000 mg/L hydrogen peroxide immersion for 15 minutes on finfish eggs (Yanong, 2014).

Parasites

Hydrogen peroxide has been shown to be efficacious to eliminate infestation of monogenean skin fluke (*Benedenia seriola* and *Neobenedenia girellae*) and gill fluke (*Zeuxapta seriola*) on kingfish and amberjack (Seriola sp.) (Mansell *et al.*, 2005; Hirazawa, Tsubone and Takano, 2016; Fensham *et al.*, 2018). Hydrogen peroxide dosages used are varied and dependent on water temperature and scenario (Hirazawa, Tsubone and Takano, 2016; Fensham *et al.*, 2018). However at similar water temperature to that expected in an Australian prawn pond of 30°C 100% efficacy can be achieved with 75 mg/L hydrogen peroxide for 30 minutes duration (Hirazawa, Tsubone and Takano, 2016).

Flagellates parasites, including the parasite *Ichthyobodo* sp have been shown to be effectively controlled using an immersion dosage of 6.5 mg/L or greater of hydrogen peroxide for one hour, in swordtails (*Xiphophorus hellerii*) (Russo, Curtis and Yanong, 2007).

Dose determination and confirmation studies field studies (Australian Perspective)

In 2017/2018, hydrogen peroxide (50%) was used in an Australian prawn farm, under emergency-use permit PER85506, to control bacterial pathogens (*Vibrio harveyi* sp.) responsible for Penaeus monodon mortality syndrome (PMMS) in black tiger prawns (Penaeus monodon) in pond water and sediment on an Australian prawn farm.

Vibrio sp. selective TCBS (Thiosulphate-citrate-bile salts-sucrose) and non-selective TSA (Trypticase soy agar) were used to assess bactericidal efficacy.

Efficacy data was collected from this farm to contribute to the data package for minor-use permit application. See Appendices 1-3.

Bactericidal efficacy – 100 mg/L Hydrogen peroxide

Administration of approximately 100 mg/L hydrogen peroxide into a post-production earthen aquaculture pond resulted in a significant bactericidal effect (reduction in bacterial count CFU/mL on TBCS media), relative to the negative control (0 mg/L hydrogen peroxide) and pre-treatment levels, at 1 minute, 3 hours, 6 hours and 12 hours post hydrogen peroxide treatment (See appendix 1, Figure 1 and Table 1).

Administration of an approximately 100 mg/L hydrogen peroxide into a post-production earthen aquaculture pond resulted in a significant bactericidal effect (reduction in bacterial count CFU/mL on TSA media), relative to the negative control (0 mg/L hydrogen peroxide) and pre-treatment levels, at 3 hours, 6 hours and 12 hours post hydrogen peroxide treatment (See appendix 1, Figure 2 and Table 1).

No significant reduction in culturable bacterial colonies was observed on TCBS or TSA in untreated negative control ponds for the duration of the assessment. Achieved hydrogen peroxide concentration in the pond was tested and remained above 100 mg/L for 12 hours post administration of the chemical. At the pre-treatment timepoint there was no significant difference in the abundance of culturable bacterial colonies between treatment and control groups.

Bactericidal efficacy – 50 mg/L Hydrogen peroxide

Administration of an estimated 50 mg/L hydrogen peroxide into a post-production earthen aquaculture pond resulted in a significant bactericidal effect (reduction in bacterial count CFU/mL on both TSA and TCBS media), relative to pre-treatment levels, at 3 hours, 6 hours and 12 hours post hydrogen peroxide treatment (See appendix 2, Figure 1/Table 1).

No significant reduction in culturable bacterial colonies was observed on TCBS or TSA in untreated negative control ponds for the duration of the assessment. At the pre-treatment timepoint there was no significant difference in the abundance of culturable bacterial colonies between treatment and control groups.

Bactericidal efficacy – 30 mg/L Hydrogen peroxide

Administration of an estimated 30 mg/L hydrogen peroxide into a post-production earthen aquaculture pond resulted in a bactericidal effect (reduction in bacterial count CFU/mL on TSA media), relative to pre-treatment levels, at 1-hour post hydrogen peroxide treatment in the water of 2 of 4 and sediment of 4 of 4, treated ponds (See appendix 3, Table 1). The magnitude of the bactericidal effect was less than in the 50 and 100 mg/L hydrogen peroxide treatments (see above). The culturable bacteria reduction (on TSA) varied between 59 to 100,000 times pre-treatment levels and began to rise from 3-6 hours post administration of the hydrogen peroxide treatment.

Administration of an estimated 30 mg/L hydrogen peroxide into a post-production earthen aquaculture pond resulted in a bactericidal effect (reduction in bacterial count CFU/mL on TCBS media), relative to pre-treatment levels, at 1-hour post hydrogen peroxide treatment in the water of all 4 and sediment of 3 of 4 treated ponds (See appendix 3, Table 1). The culturable bacteria reduction (on TCBS) varied between 1.5 to >100,000 times pre-treatment levels and began to rise again from 6-12 hours post administration of the hydrogen peroxide treatment.

Hydrogen peroxide as an emergency source of oxygen

Hydrogen peroxide can be used as an emergency source of oxygen in shrimp culture (Zappi *et al.*, 2000; Boyd and Tucker, 2014).

Molecular oxygen can be supplied to an aquaculture system using hydrogen peroxide, which naturally dissociates in water to produce ½ mol dissolved oxygen/ mol H_2O_2, with a solubility of 40–50 mg/L (Zappi *et al.*, 2000). Beneficial attributes of hydrogen peroxide as an oxygen source include; 1) reasonably inexpensive; 2) non-persistent; 3) stable liquid, which eliminates problems with storage and introduction into water; and 4) generally environmentally benign (Zappi *et al.*, 2000).

Theoretically, the addition of a 4mg/L dose of hydrogen peroxide (e.g. 75L of 50% H_2O_2 in a 15 ML pond) should provide a rapid influx of approximately 2mg/L dissolved oxygen. In an aquaculture setting this action can provide critical relief to a low dissolved oxygen situation in a production pond that could subject cultured stock to hypoxia and subsequent mortality (Zappi *et al.*, 2000; Boyd and Tucker, 2014). Presence of organic material in the water body serves as a catalyst to accelerate the decomposition of hydrogen peroxide and the release of dissolved oxygen (Boyd and Tucker, 2014).

Treatment of pond anoxia with hydrogen peroxide has been found effective from 15 minutes to 24 hours and beyond (Waheed, 2017). At summer temperatures (32°C), as would be expected on an Australian prawn farms, the addition of hydrogen peroxide significantly increased water dissolved oxygen levels (Waheed, 2017). The maximum hydrogen peroxide addition (16mL 6% solution in 40L water, or 24 mg/L) increased dissolved oxygen levels from 2.4 mg/L at 0 minutes to 9.9mg/L (313% increase) at 24 hours post addition (Waheed, 2017). At the lower addition end of the test spectrum, but closer to the proposed use pattern level, 3 mg/L hydrogen peroxide (or 2mL 6% solution in 40L water) increased dissolved oxygen levels from 2.4 mg/L at 0 minutes to est. 3.2 mg/L (33% increase) at 24 hours post

addition (Waheed, 2017). Although much lower increase this can still have a dramatic effect in an aquaculture emergency situation and potentially prevent significant mortalities.

Table 1. Effect of hydrogen peroxide (6%) addition to freshwater at 32°C on dissolved oxygen. Source: (Waheed, 2017).

Hydrogen Peroxide Addition		**Dissolved Oxygen level**			
mL of 6% H_2O_2 in 40L water	**mg/L H_2O_2**	**0 min (mg/L)**	**0 min (% inc.)**	**15 min (mg/L)**	**15 min (% inc.)**
0	0	2.4	0	3.5	4
2	3			2.5	17
4	6			2.8	25
6	9			3.0	38
8	12			3.3	46
10	15			3.5	63
12	18			3.9	67
14	21			4.0	75
16	24			4.2	4

Field studies (Australian Prawn Farm Perspective)

Field data collected under PER 85506 found that all ponds that received a hydrogen peroxide treatment experienced a rapid increase in dissolved oxygen levels in the treated pond (See appendix 4). Relative to pre-treatment levels the dissolved oxygen level in treated ponds increased by 7-54%, 20-94%, 28-84%, 27-104% at 1, 3, 6, and 12 hours post treatment, respectively. The greatest average percentage increase in was seen in the first 3 hours following hydrogen peroxide addition. The dramatic increase in secchi disc reading and transparency of water colour/appearance in all four treated ponds at 1-hour post treatment suggests that the phytoplankton community has been eliminated from suspension (See appendix 4). Therefore, it is considered the rise in water dissolved oxygen level is due to the hydrogen peroxide addition, and not photosynthesis activities of a phytoplankton bloom, which in its elimination would in turn lower dissolved oxygen content.

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Appendix 1. Bactericidal Effect of 100 mg/L Hydrogen Peroxide (H_2O_2)

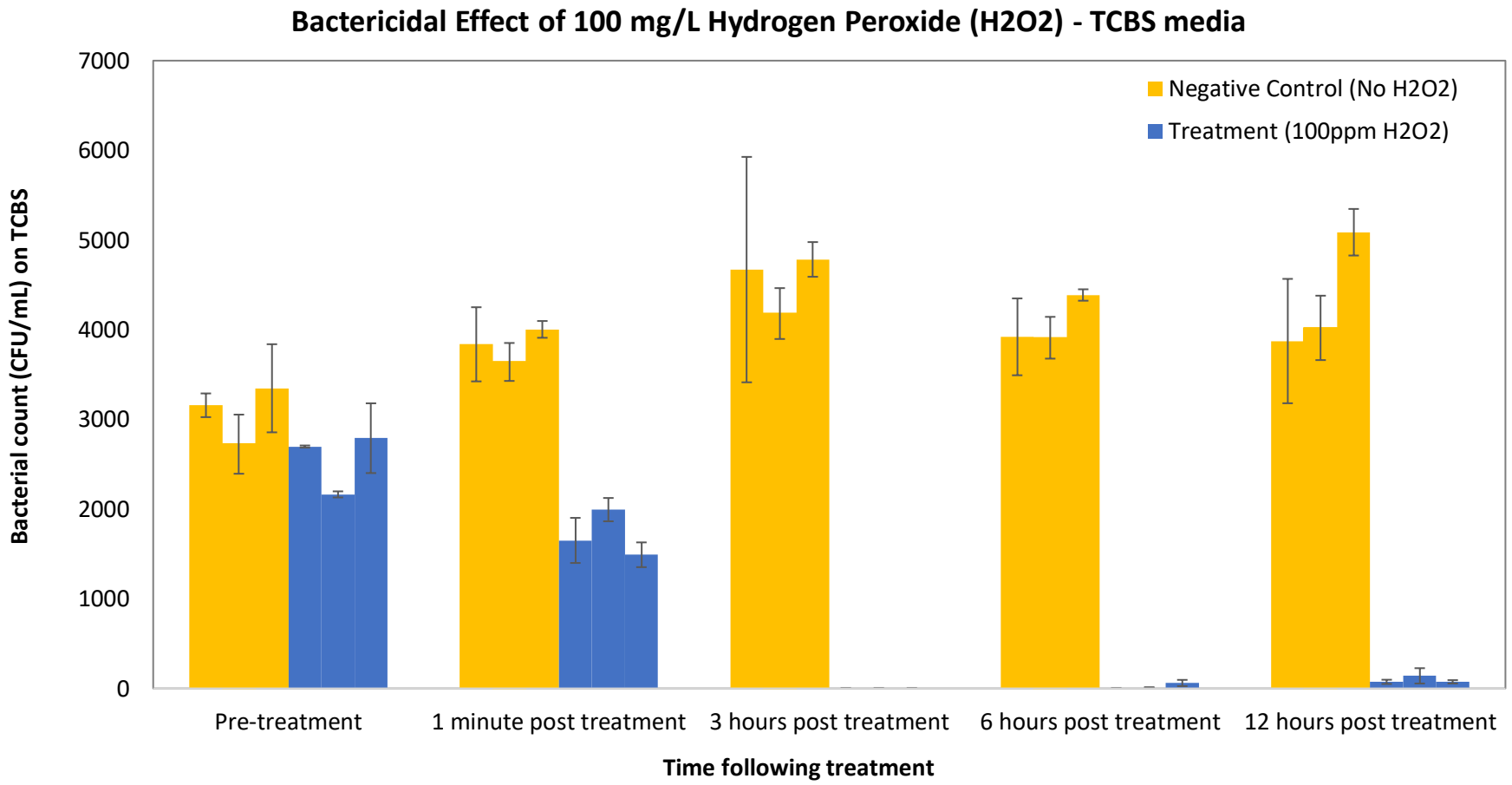

Figure 1. *Bactericidal Effect of 100 mg/L Hydrogen Peroxide (H2O2) of growth of combined green and yellow colonies in three treatment and three negative control prawn pond water samples inoculated on vibrio selective TCBS media, at various time points following treatment. Each bar represents a different pond, for each time point, with 3 bacterial samples from each time point assessed. Error bars represent standard error. Australian Prawn farm field data collected under PER 85506. No prawns present in treated water. Data Commercial in Confidence.*

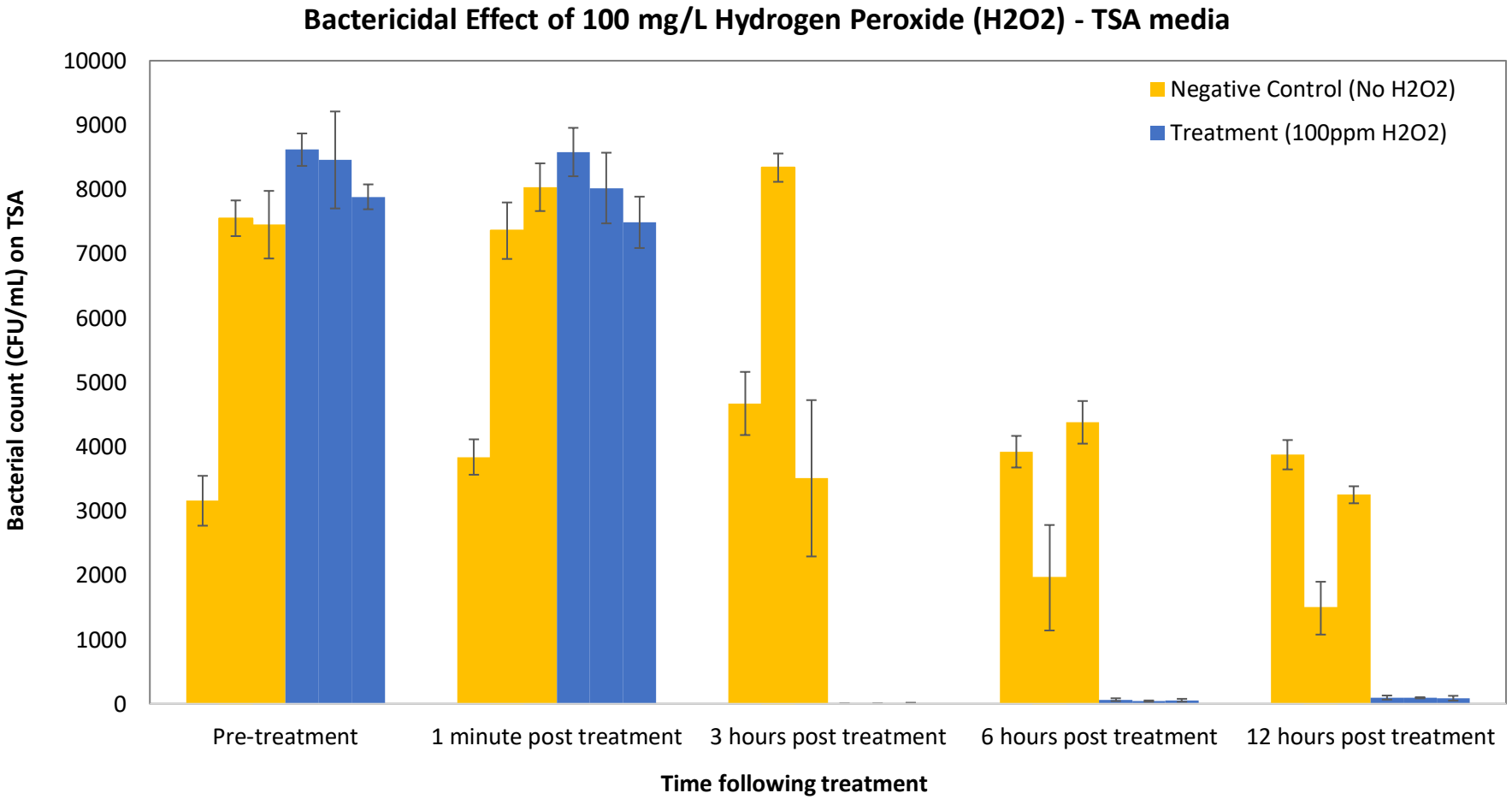

Figure 2. *Bactericidal Effect of 100 mg/L Hydrogen Peroxide (H2O2) of growth of bacterial colonies in three treatment and three negative control prawn pond water samples inoculated on non-selective TSA media, at various time points following treatment. Each bar represents a different pond, for each time point, with 3 bacterial samples from each time point assessed. Error bars represent standard error. Australian Prawn farm field data collected under PER 85506. No prawns present in treated water. Data Commercial in Confidence.*

Table 1. *Bactericidal efficacy of 100ppm Hydrogen peroxide (H_2O_2) in water treatment of prawn pond water samples, inoculated onto either vibrio selective TCBS media or non-selective TSA media, at various time points following treatment. Average counts and standard error also shown. TCBS colony counts reflect the sum of yellow colonies and green colonies. Australian Prawn farm field data collected under PER 85506. No prawns present in treated water. Data Commercial in Confidence.*

Sample time relative to 100ppm H_2O_2 – Treatment Pond	Dissolved Oxygen (mg/L)			pH			Temperature (°C)				Average Bacterial Plate Count (CFU/mL) from 3 sample locations ± standard error (SE)					
											TCBS			TSA		
Before treatment	6	5.4	5.9	7.8	7.8	7.9	29.2	29	28.9	CFU/mL	2697	2163	2790	8617	8457	7883
										SE	±12	±34	±389	±252	±754	±193
1 min post treatment	7.9	8.2	8.3	7.6	7.6	7.6	29	29.2	29	CFU/mL	1650	1993	1490	8580	8020	7487
										SE	±251	±130	±138	±376	±549	±399
3 hours post treatment	6.4	6.6	6.5	7.8	7.8	7.8	33.7	31.9	32.9	CFU/mL	0	0	0	0	0	10
										SE	±0	±0	±0	±0	±0	±6
6 hours post treatment	6.8	6.9	6.8	7.9	7.9	7.9	30.5	30.2	30.5	CFU/mL	0	7	60	63	40	53
										SE	±0	±7	±35	±22	±12	±23
12 hours post treatment	7	7	7	7.8	7.9	7.8	28.8	28.9	28.7	CFU/mL	73	140	73	97	93	87
										SE	±23	±85	±19	±32	±9	±37
Sample time relative to 0ppm H_2O_2 - Negative Control Pond	Dissolved Oxygen (mg/L)			pH			Temperature (°C)				Average Bacterial Plate Count (CFU/mL) from 3 sample locations ± standard error (SE)					
											TCBS			TSA		
Before treatment	6	6.4	6.1	7.7	7.8	7.9	29	29	29.2	CFU/mL	3037	2723	3347	8813	7550	7450
										SE	±132	±330	±491	±388	±278	±526
1 min post treatment	6.8	6.8	6.9	7.6	7.7	7.7	28.9	28.8	28.9	CFU/mL	2733	3640	4003	9150	7357	8033
										SE	±413	±212	±94	±275	±439	±371
3 hours post treatment	6.3	6.4	6.4	7.9	7.9	7.9	33.2	32.7	32.3	CFU/mL	2973	4180	4783	7167	8337	3507
										SE	±1257	±284	±193	±491	±220	±1215
6 hours post treatment	6.7	6.7	6.8	7.9	7.8	7.9	30.7	30	30	CFU/mL	3583	3910	4387	2910	1960	4377
										SE	±428	±233	±64	±246	±820	±331
12 hours post treatment	6.9	6.9	6.9	7.7	7.8	7.8	28.6	28.2	28	CFU/mL	4363	4020	5087	1710	1487	3250
										SE	±693	±359	±260	±228	±411	±132

Appendix 2. Bactericidal Effect of 50 mg/L Hydrogen Peroxide (H_2O_2)

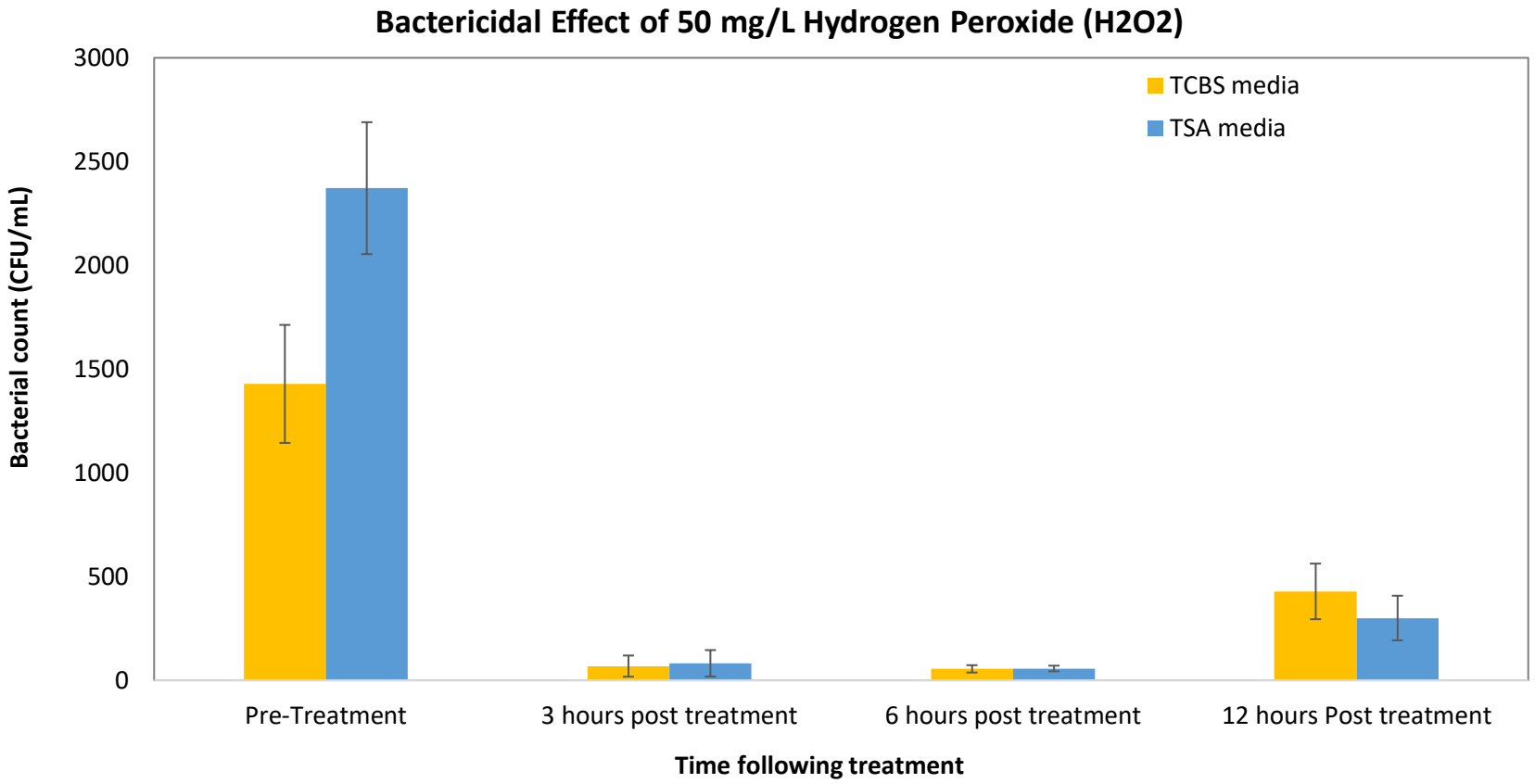

Figure 1. *Bactericidal Effect of 50 mg/L Hydrogen Peroxide (H2O2) on growth of bacterial colonies in six prawn pond water samples, inoculated on either vibrio selective TCBS media or non-selective TSA media, at various time points following treatment. Each bar represents the average of the six prawn pond samples, for each time point*

assessed. Error bars represent standard error. No negative control. Australian Prawn farm field data collected under PER 85506. No prawns present in treated water. Data Commercial in Confidence.

Table 1. *Bactericidal efficacy of 50 ppm Hydrogen peroxide (H_2O_2) in water treatment of six prawn pond water samples, inoculated onto either vibrio selective TCBS media or non-selective TSA media, at various time points following treatment. Average counts and standard error also shown. TCBS colony counts reflect the sum of yellow colonies and green colonies. Data collected under PER 85506. Australian Prawn farm field data. No prawns present in treated water. Commercial in Confidence.*

Sample time relative to 50 ppm H_2O_2 – Treatment Ponds		Average Bacterial Plate Count (CFU/mL) from 6 prawn ponds ± standard error (SE)		Bacterial Plate Count (CFU/mL) from 6 prawn ponds											
				Pond 224		Pond 255		Pond 233		Pond 103		Pond 105		Pond 101	
		TCBS	TSA	TCBS	TSA	TCBS	TSA	TCBS	TSA	TCBS	TSA	TCBS	TSA	TCBS	TSA
Before treatment	CFU/mL	1428	2372	1700	1750	1500	2600	2000	2780	550	1100	2200	2900	620	3100
	SE	284	318												
3 hours post treatment	CFU/mL	68	82	0	30	0	10	30	30	320	400	50	20	10	0
	SE	51	64												
6 hours post treatment	CFU/mL	55	57	0	10	20	50	100	110	50	40	110	60	50	70
	SE	18	14												
12 hours post treatment	CFU/mL	428	300	20	70	500	700	960	560	270	210	580	150	240	110
	SE	134	108												

Appendix 3. Bactericidal Effect of 30 mg/L Hydrogen Peroxide (H_2O_2)

Table 1. *30ppm H2O2 efficacy in water and soil. Bacterial colony counts (yellow colonies and green colonies) sampled at different locations in pond. Values in tables represent averages taken from North/South/East/West sub-samples. Australian Prawn farm field data. No prawns present in treated water. Data Commercial in Confidence.*

30 PPM Hydrogen Peroxide Treatment				
Pond	Sampling hours	TCBS CFU/mL		TSA CFU/mL
		Yellow	Green	
Pond 236 (water)	1 hours Pre treatment	2.29E+03	3.00E+02	3.24E+05
	1 hours post treatment	0.00E+00	0.00E+00	3.00E+02
	3 hours post treatment	0.00E+00	0.00E+00	1.17E+03
	6 hours post treatment	0.00E+00	0.00E+00	2.40E+02
	12 hours Post treatment	0.00E+00	0.00E+00	2.50E+01
Pond 236 (pond sediment)	1 hours Pre treatment	2.50E+00	0.00E+00	1.13E+09
	1 hours post treatment	2.50E+01	0.00E+00	1.32E+04
	3 hours post treatment	0.00E+00	0.00E+00	4.91E+06
	6 hours post treatment	1.80E+02	0.00E+00	7.15E+06
	12 hours Post treatment	1.05E+02	0.00E+00	8.81E+05
Pond 237 (water)	1 hours Pre treatment	1.55E+02	7.50E+00	1.70E+04
	1 hours post treatment	0.00E+00	0.00E+00	2.88E+02
	3 hours post treatment	0.00E+00	0.00E+00	6.78E+01
	6 hours post treatment	0.00E+00	0.00E+00	8.50E+02
	12 hours Post treatment	0.00E+00	0.00E+00	4.80E+02
Pond 237 (pond sediment)	1 hours Pre treatment	7.50E+01	0.00E+00	1.32E+09
	1 hours post treatment	3.50E+01	0.00E+00	6.64E+04
	3 hours post treatment	1.50E+01	0.00E+00	1.21E+07
	6 hours post treatment	1.50E+01	0.00E+00	7.12E+06
	12 hours Post treatment	1.00E+01	0.00E+00	6.39E+06
Pond 234 (water)	1 hours Pre treatment	5.13E+02	1.00E+02	2.60E+02
	1 hours post treatment	0.00E+00	0.00E+00	2.08E+03
	3 hours post treatment	0.00E+00	0.00E+00	1.65E+03
	6 hours post treatment	0.00E+00	0.00E+00	8.03E+03
	12 hours Post treatment	0.00E+00	0.00E+00	3.75E+02
Pond 234 (pond sediment)	1 hours Pre treatment	7.50E+01	0.00E+00	1.32E+09
	1 hours post treatment	6.00E+01	0.00E+00	9.10E+06
	3 hours post treatment	1.50E+01	0.00E+00	4.44E+06
	6 hours post treatment	1.50E+01	0.00E+00	8.16E+07
	12 hours Post treatment	1.50E+01	0.00E+00	6.12E+07
Pond 233 (water)	1 hours Pre treatment	3.75E+02	0.00E+00	4.05E+02
	1 hours post treatment	0.00E+00	0.00E+00	5.25E+02
	3 hours post treatment	0.00E+00	0.00E+00	8.00E+02
	6 hours post treatment	0.00E+00	0.00E+00	3.00E+02
	12 hours Post treatment	0.00E+00	0.00E+00	3.75E+02
Pond 233 (pond sediment)	1 hours Pre treatment	3.75E+01	0.00E+00	1.32E+09
	1 hours post treatment	2.50E+01	0.00E+00	1.32E+04
	3 hours post treatment	0.00E+00	0.00E+00	6.16E+06
	6 hours post treatment	0.00E+00	0.00E+00	8.16E+07
	12 hours Post treatment	0.00E+00	0.00E+00	1.89E+06

Appendix 4. Dissolved Oxygen Effect of 100 mg/L Hydrogen Peroxide (H_2O_2)

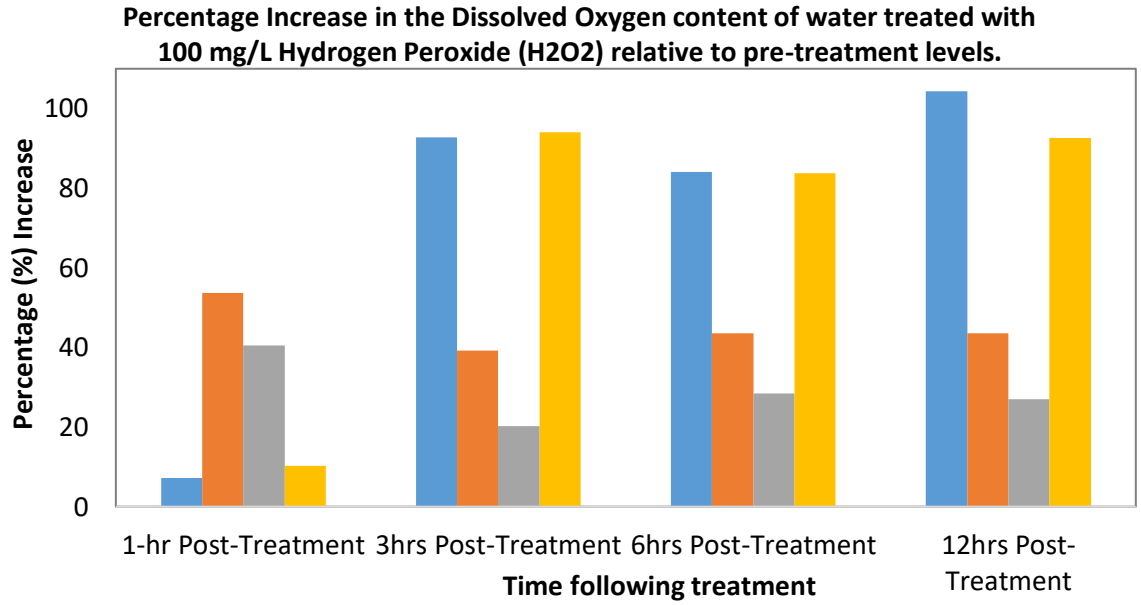

Figure 1. *Water dissolved oxygen effect of 100 mg/L Hydrogen Peroxide (H2O2) addition to four prawn ponds, at various time points, relative to pre-treatment levels for each pond, following treatment. No negative control. See Appendix 4, table 1 for raw data. Data collected under PER 85506. Australian Prawn farm field data. No prawns present in treated water. Commercial in Confidence.*

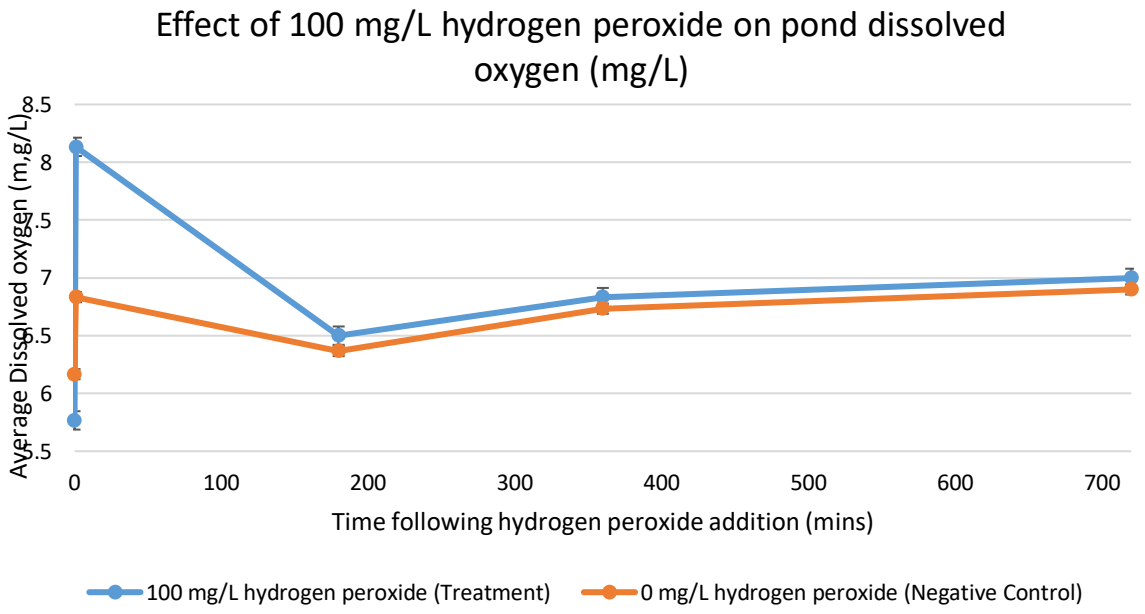

Figure 2. *Effect of 100 mg/L Hydrogen Peroxide (H2O2) on average pond dissolved oxygen content (mg/L) in water samples of three treatment and three negative control prawn ponds, at various timepoints following treatment. Error bars represent average standard error for each treatment type. See Appendix 1, table 1 for raw data. Data collected under PER 85506. Australian Prawn farm field data. No prawns present in treated water. Commercial in Confidence.*

Table 1. *100ppm H2O2 treatment impact on water quality parameters. Data collected under PER 85506. Australian Prawn farm field data. No prawns present in treated water. Commercial in Confidence.*

Pond	Target H2O2 Dose (ppm)	Parameters	Pre-Treatment	Post-Treatment			
				1-hr	3hrs	6hrs	12hrs
236	100	**Dissolved Oxygen, mg/l**	6.8	7.5	13.2	12.5	13.1
		pH	8.5	8.4	8.4	8.3	8.4
		Salinity, ppt	30	30	30	30	30
		Temperature, °C	30.0	30.0	30.9	31.3	33.0
		Water Depth, cm	150	150	150	150	150
		Secchi Reading, cm	85	Clear	Clear	Clear	Clear
		Water Color	Green	Clear	Clear	Clear	Clear
		ORP, mV	172	190	191	188	189
		H_2O_2 Lvl (Strip), ppm	0	>100	>100	>100	>100
237	100	**Dissolved Oxygen, mg/l**	6.9	7.4	13.3	12.7	14.1
		pH	8.4	8.3	8.3	8.2	8.2
		Salinity, ppt	30	30	30	30	30
		Temperature, °C	30.0	30.0	30.9	31.9	33.1
		Water Depth, cm	150	150	150	150	150
		Secchi Reading, cm	92	Clear	Clear	Clear	Clear
		Water Color	Green	Clear	Clear	Clear	Clear
		ORP, mV	168	192	188	189	190
		H_2O_2 Lvl (Strip), ppm	0	>100	>100	>100	>100
233	100	**Dissolved Oxygen, mg/l**	7.4	10.4	8.9	9.5	9.4
		pH	8.3	8.2	8.2	8.1	7.9
		Salinity, ppt	29.2	29.2	29.2	29.2	29.2
		Temperature, °C	31.0	31.3	31.8	33.3	33.3
		Water Depth, cm	150	150	150	150	150
		Secchi Reading, cm	58	Clear	Clear	Clear	Clear
		Water Color	Green	Clear	Clear	Clear	Clear
		ORP, mV	174	192	189	190	195
		H_2O_2 Lvl (Strip), ppm	0	>100	>100	>100	>100
234	100	**Dissolved Oxygen, mg/l**	6.9	10.6	9.6	9.9	9.9
		pH	8.4	8.4	8.4	8.3	8.2
		Salinity, ppt	30.2	30.2	30.2	30.2	30.2
		Temperature, °C	30.5	30.6	31.4	33.3	32.9
		Water Depth, cm	150	150	150	150	150
		Secchi Reading, cm	70	Clear	Clear	Clear	Clear
		Water Color	Green	Clear	Clear	Clear	Clear
		ORP, mV	170	192	189	190	179
		H_2O_2 Lvl (Strip), ppm	0	>100	>100	>100	>100

Appendix 5. Target animal efficacy data

Table 1. *Target animal efficacy data to demonstrated efficacy of Method 1 and 2 - Hydrogen peroxide as a general disinfectant to kill micro-organisms. Proposed dosage – up to 4-100mg/L (ppm) active hydrogen peroxide (HP).*

Species Group	**Species Scientific Name**	**Species Common Name**	**Endpoint**	**Effect**	**Temp (°C)**	**Exposure Duration (hours)**	**H_2O_2 (mg L^{-1}) Concentration**	**Reference**
Algae	*Pseudokirchneriella subcapitata*	Green Algae	EC50	Phys. Tox. Growth rate inhibition	24 +/- 2	96	5.38	(Gregor and Janc, 2008)
	Pseudokirchneriella subcapitata	Green Algae	EC50	Phys. Tox. Photosynthesis inhibition	27	3 (irradiance: 500 $\mu^{-2}s^{-1}$)	4.15	(Drábková, Admiraal and Maršálek, 2007)
						3 (irradiance: 0 $\mu^{-2}s^{-1}$)	21.26	
	Navicula seminulum	Diatom				3 (irradiance: 500 $\mu^{-2}s^{-1}$)	15.78	
						3 (irradiance: 0 $\mu^{-2}s^{-1}$)	71.26	
	Pseudokirchneriella subcapitata	Green Algae	EC50	Phys. Tox. Cell density	27	72	5.74 +/- 0.53	(Drábková, Maršálek and Admiraal, 2007)
	Scenedesmus quadricauda						5.81 +/- 0.43	
	Chlorella kessleri						5.53 +/- 1.51	
		Dinoflagellate	EC80		n/a	2	30	(Burson *et al.*, 2014)

	Alexandrium ostenfeldii		EC95	Phys. Tox. Photosynthesis inhibition			40	
			LC80	Cell rupture		12	40	
	Gyrodinium spp.	Dinoflagellate	EC100	Phys. Tox. No cysts germinated	n/a	48	6.0	(Montani et al., 1995 In: Schmidt, Gaikowski and Gingerich, 2006)
	Chattonella spp.	Raphidophyte					90	
	Alexandrium spp.	Dinoflagellate					150	
	Scrippsiella spp.	Dinoflagellate					150	
	Gymnodinium spp.	Dinoflagellate					150	
	Protoperidinium spp.	Dinoflagellate					150	
	Nitzschia spp.	Diatom	EC50	Phys Tox. Growth decrease		72	0.85	(Florence et al., 1986 In: Schmidt, Gaikowski and Gingerich, 2006)
			NOEC	Growth		72	<0.68	
	Polykrikos spp.	Dinoflagellate	EC100	Phys. Tox. No cysts germinated		48	100	(Ichikawa et al., 1993 In: Schmidt, Gaikowski and Gingerich, 2006)
	Alexandrium spp.	Dinoflagellate	LC100	Mortality		48	30	
Cyanobacteria	*Aphanothece clathrata*	Cyanobacteria	EC50	Phys. Tox. Growth rate inhibition	24 +/- 2	96	2.27	(Gregor and Janc, 2008)

	Aphanizomenon flos-aquae	Cyanobacteria	EC90	Phys. Tox. membrane damage	n/a	1.5	7	(Peterson *et al.*, 1995)
			EC50	Phys. Tox. Inhibition of Nitrogen fixation		22	0.98	
	Microcystis aeruginosa	Cyanobacteria	EC50	Phys. Tox. Photosynthe-sis inhibition	27	3 (irradiance: 500 $\mu^{-2}s^{-1}$)	0.27	(Drábková, Admiraal and Maršálek, 2007)
						3 (irradiance: 0 $\mu^{-2}s^{-1}$)	6.63	
	Synechococcus nidulans	Cyanobacteria	EC50	Phys. Tox. Cell density	27	72	0.69 +/- 0.064	(Drábková, Maršálek and Admiraal, 2007)
	Microcystis incerta						0.71 +/- 0.047	
	Anaaena sp.						0.81 +/- 0.156	
	Planktothrix agardhii	Cyanobacteria	EC50	Phys. Tox. Decay of chlorophyll-a	24 +/-1	14	0.33	(Bauza *et al.*, 2014)
				Phys. Tox. Chemical Oxygen demand		36	3.33	
	Planktothrix agardhii	Cyanobacteria	LC99	Cell abundance	19.5	216	2	(Matthijs *et al.*, 2011)

	Oscillatoria rubescens	Cyanobacteria	LC100	Cell destruction	20	24	1.75	(Barroin and Feuillade, 1986)
			EC90	Phys. Tox. destroyed biliproteins, carotenoids		29	7	
			EC50	Phys. Tox. Destroyed carotenoids		29		
Bacteria	*Tenacibaculum maritimum*	Bacteria	LC100	Bactericidal, no CFU	20	0.5	30	(Avendaño-Herrera *et al.*, 2006)
						0.5	60	
						0.25	120	
	Pseudomonas aeruginosa	Bacteria	MIC	Bacteriostatic	37	120	51	(Baldry, 1983)
	Klebsiella pneumoniae	Bacteria					25.5	
	Streptococcus faecalis	Bacteria					25.5	
	Flavobacterium branchiophilum	Bacteria	EC98	Phys. Tox. Impaired colony formation	n/a	1	100	(Derksen, Ostland and Ferguson, 1999)
			EC100			0.5	100	
	Streptococcus oralis	Bacteria	MIC	Colony forming units (CFU/mL) on	36+/-1	24	7.0	(García-Mendoza *et al.*, 1993)
			MBC				14.1	

	Streptococcus mitis	Bacteria	MIC	Trypticase soy broth (TSB)			2.3, 7.0, 14.1	
			MBC				3.5, 14.1, 28.2	
	Streptococcus sanguis	Bacteria	MIC				7.0	
			MBC				14.1	
	Streptococcus sobrinus	Bacteria	MIC				7.0, 14.1	
			MBC				14.1, 28.2	
	Vibrio alginolyticus	Bacteria	MIC	Probably growth inhibition	n/a	n/a	19.41	(Srisapoom et al., 1999 In: Schmidt, Gaikowski and Gingerich, 2006)
						n/a (1.5% NaCl)	0.6	
	Vibrio harveyi	Bacteria				n/a	9.57	
						n/a (1.5% NaCl)	0.6	
	Vibrio parahaemolyticus	Bacteria				n/a	38.27	
						n/a (1.5% NaCl)	2.39	
	Vibrio vulnificus	Bacteria				n/a	38.27	
						n/a (1.5% NaCl)	2.39	
Invertebrates	*Daphnia and Diaphanosoma spp.*	Zooplankton	LC99	Mean Abundance	19.5	216	2	(Matthijs *et al.*, 2011)
Fungi and Moulds	*Saprolengia parasitica*	Water mould	EC30	Phys. Tox. Lowered mortality to infected	12 +/- 2	1.75 (15 min every 2nd day for 2 weeks)	175	(Schreier, Rach and Howe, 1996)

				rainbow trout eggs				
Parasites	*Benedenia seriola*	Monogenean skin fluke	EC100	Dislodgement from host	30	0.5	75	(Hirazawa, Tsubone and Takano, 2016)
	Neobenedenia girellae	Monogenean skin fluke						
	Zeuxapta japonica	Monogenean gill fluke						
	Caecognathia coralliophila	Parasitic isopod	EC100	Dislodgement from host	24	24	100	(Thing, Ransangan and Hatai, 2016)`
			EC50			1	1000	
	Gyrodactylus salmonis	Monogenean gill fluke	EC99	Dislodgement from host	n/a	1 (total exposure)	50	(Bowker, Carty and Dotso, 2012)

Appendix 4: Hydrogen Peroxide Safety Data Assessment

Abbreviations

CFU – Colony Forming Units
APFA – Australian Prawn Farmers Association
WSD – White Spot Disease
WSSV – White Spot Syndrome Virus
HP / H_2O_2 – Hydrogen Peroxide
LC50 – Lethal dose that kills 50 percent of a test sample.
EC50 – Half maximal effective concentration
LCLo - lowest lethal concentration
NOEC - No observed effect concentration
LOEC - Lowest observed effect concentration
MAC - Maximum allowable concentration
Phys. Tox. – Physiological Toxicity
PL – Post larvae
TCBS - Thiosulphate-citrate-bile salts-sucrose agar
TSA - Trypticase soy agar
MIC – Minimum Inhibitory Concentration
MBC - Minimum Bactericidal Concentration

Chemistry

Common Name: Hydrogen Peroxide

Chemical Name: Hydrogen Peroxide

Other Names: Hydrogen Peroxide 20-60%, INTEROX® EG-ST Hydrogen Peroxide 50%, INTEROX® ST-50, INTEROX® ST-60

Tradenames: Various

CAS Number: 7722-84-1

Molecular Formula: H_2O_2

Molecular Weight: 34.014 g mol^{-1}

Structural Formula: HO-OH

Purity of the active constituent: 50-60% hydrogen peroxide, 40-50% water.

HYDROGEN PEROXIDE 50% (unregistered)
Containing: 598 g L^{-1} HYDROGEN PEROXIDE as its only active constituent.

HYDROGEN PEROXIDE 60% (unregistered)
Containing: 742 g L^{-1} HYDROGEN PEROXIDE as its only active constituent.

Detailed information on the chemistry of hydrogen peroxide is considered to already be held by APVMA. Where data gaps exist please refer to the complete data package including further references (Goor, Glenneber and Jacobi, 2007).

Application Overview

In late 2016, an exotic viral disease, White Spot Disease (WSD), was diagnosed in multiple Australian prawn farms on the Logan River catchment, Queensland. The subsequent disease response program resulted in the complete eradication of the seven infected farms (approximately 25% of the Australian prawn farming industry). Following this exotic disease outbreak an intensive review of farm-level biosecurity practices on Australian prawn farms was performed to safeguard against future endemic and exotic disease incursions. The prawn farming industry are now taking a proactive step to improve the disease preventative measures on farm to reduce the likelihood and impact of disease entry, spread and exit from their farms. In order to improve on-farm biosecurity practices the industry is seeking APVMA permits for several chemical products. Trichlorfon, Hydrogen Peroxide, Calcium and Sodium Hypochlorite, Benzalkonium Chloride and Copper Sulphate which have been highlighted as the most important.

One of the major drivers for disease within a prawn farm is via proliferation of bacteria in culture water and the water conditions that culture animals are subject to. Disease outbreaks usually stem from within the index culture unit. Maintaining optimal culture conditions, monitoring pathogen presence and preventing of pathogen build-up in these systems can help prevent major disease outbreaks from occurring. This risk can be reduced through disinfection and killing micro-organisms, generally in these systems, to safe and manageable levels.

Currently there are no APVMA registered products that provide safe and efficacious control of micro-organisms, generally on Australian prawn (*Penaeus monodon* and *Fenneropenaeus merguiensis*) production ponds and tank systems.

Hydrogen peroxide provides is an effective general disinfectant that can rapidly knockdown micro-organisms in prawn production ponds and tanks. The active constituent is a reserved chemical under Schedule 3C—Reserved Schedule of the Agricultural and Veterinary Chemicals Code (AgVet Code) Regulations 1995. However, this only permits a chemical product of 6% Hydrogen peroxide to be used for this purpose, which is impractical for use on Australian prawn farms.

Future Fisheries Veterinary Service (FFVS) are applying for an item 21 minor-use permit (MUP) for use of unregistered hydrogen peroxide 50-60% as a disinfectant to kill micro-organisms generally responsible for disease in farmed giant black tiger prawns (*Penaeus monodon*) and banana prawns *(Fenneropenaeus merguiensis*).

This unregistered chemical product has a current minor-use permit for treatment of parasitic and fungal infections in saltwater and freshwater finfish and eggs (PER83276); expired emergency-use permits for control of viral infections in prawns (PER83658); expired emergency-use permits for control of bacterial infections in prawns (PER85506). This wide range of uses demonstrates the diverse disinfection properties of this chemical.

The ability of this industry to remain viable and expand will in part depend on its ability to strengthen on-farm biosecurity measures. An essential element of these measures is the safe, efficacious and legal access and use of hydrogen peroxide to assist farm disinfection procedures and control and prevention of future disease outbreaks.

Overseas Regulatory Activity

United States of America

In 2007, 35 % PEROX-AID® (Eka Chemicals, Marietta, Georgia) was approved by the US Food and Drug Administration (FDA) for use in aquaculture. This product is supplied in 5 and 55 gallon containers, over the counter, and is permitted as an immersion treatment for control of fungal and bacteria diseases in freshwater-reared finfish eggs; and freshwater-reared salmonids, coolwater finfish and channel catfish. Hydrogen peroxide is classified as a low regulatory compound by the Food and Drug Administration of the United States.

Indications for use:

The control of mortality in freshwater-reared finfish eggs due to saprolegniasis, for the control of mortality in freshwater-reared salmonids due to bacterial gill disease associated with Flavobacterium branchiophilum, and for the control of mortality in freshwater-reared coolwater finfish and channel catfish due to external columnaris disease associated with Flavobacterium columnare (Flexibacter columnaris).

Application and dose rate:

"Freshwater-reared finfish eggs: 500 to 1000 mg L^{-1} for 15 minutes in a continuous flow system once per day on consecutive or alternate days until hatch for all coldwater and coolwater species of freshwater-reared finfish eggs or 750 to 1000 mg L^{-1} for 15 minutes in a continuous flow system once per day on consecutive or alternate days until hatch for all warmwater species of freshwater-reared finfish eggs."
"Freshwater-reared salmonids: 100 mg L^{-1} for 30 minutes or 50 to 100 mg L^{-1} for 60 minutes once per day on alternate days for three treatments in a continuous flow water supply or as a static bath."
"Coolwater species of freshwater-reared finfish (except northern pike & paddlefish) and channel catfish: 50 to 75 mg L^{-1} for 60 minutes once per day on alternate days for three treatments in a continuous flow water supply or as a static bath. Coolwater species of freshwater-reared finfish fry (except northern pike, pallid sturgeon & paddlefish) and channel catfish fry: 50 mg L^{-1} for 60 minutes once per day on alternate days for three treatments in continuous flow water supply or as a static bath."*

Canada

In 2016, Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the Pest Control Products Act and Regulations, granted full registration for the sale and use of Interox M-70 Hydrogen Peroxide, Interox CPMC-50 and Interox Paramove 50, containing the technical grade active ingredient hydrogen peroxide, for the treatment of sea lice on Atlantic salmon reared in marine aquaculture sites (Health Canada Pest Management Regulatory Agency (PMRA), 2016). The registration assessment stated: *"The evaluation found that, under the approved conditions of use, the products have value and do not present an unacceptable risk to human health or the environment."*

In Canada hydrogen peroxide is used as an algaecide, bactericide, fungicide, slimicide, sanitizer and acaricide and has a broad use pattern including use in aquaculture, agriculture, industry and as a hard and soft surface sanitizer (Health Canada Pest Management Regulatory Agency (PMRA), 2018). A recent review of all the above registered hydrogen peroxide products in Canada was performed by the PMRA. The PMRA has determined that continued registration of products containing hydrogen peroxide is acceptable (Health Canada Pest Management Regulatory Agency (PMRA), 2018).

For a full list of Registered Hydrogen Peroxide Products in Canada See attachment (Health Canada Pest Management Regulatory Agency (PMRA), 2018).

Asian

In Asian prawn farms hydrogen peroxide is used as an effective disinfectant agent, to alter the growth of some primary producer species and affect the structure of microbial communities in the pond environment (Ali *et al.*, 2018).

Europe

Hydrogen peroxide is a generally recognised as safe (GRAS) veterinary medicinal product in as declared by the European Agency for the Evaluation of Medicinal Products Veterinary Medicines Evaluation Unit 1996.

Proposed Permit Details

Efficacy Claims

Hydrogen peroxide has proven disinfectant properties against micro-organisms generally (including but not limited to bacteria, cyanobacteria, fungi, algae and parasites).

Hydrogen peroxide has properties that yield rapid emergency oxygen delivery to a body of water when added directly to the water (including saltwater).

Use pattern

Proposed Directions for Use:

For use as a disinfectant to kill micro-organisms, generally, and as for emergency oxygen supplementation of production prawns in farmed giant black tiger prawns (*Penaeus monodon*) and banana prawns *(Fenneropenaeus merguiensis*) in aquaculture earthen pond sediment, water, tanks, equipment and surfaces.

Situation	**Purpose**	**Rate**
Aquaculture earthen pond sediment, water, tanks, equipment and surfaces.	Method 1: General disinfectant to kill micro-organisms in a situation where culture animals are absent.	Up to 100 mg L^{-1} (ppm) active hydrogen peroxide
	Method 2: General disinfectant to kill micro-organisms, OR, as a source of emergency oxygen in a situation where culture animals are present.	Up to 4 mg L^{-1} (ppm) active hydrogen peroxide

Methods of use

Restraint

DO NOT USE in environmental waters. Only for use in waters where the release of treated water can be controlled, following treatment.

The hydrogen peroxide product is not to be used in conjunction or mixed with any other chemical.

For disinfection purposes apply product during daytime.

For emergency oxygen supplementation apply product during day-time or night-time.

Critical Use Comments

Observe all label safety directions relevant to handling of hydrogen peroxide. Ensure all personnel handing the product are wearing correct PPE (refer to product label and MSDS).

Only apply product when the potential for undiluted product drift is minimal (e.g. sub-surface application via leaky hoses). Minimise bystander exposure from any spray drift.

DO NOT discharge or re-use treated water until the active hydrogen peroxide level is below detectable limits (<0.5 mg L^{-1} active hydrogen peroxide).

DO NOT stock post larvae into a treated production grow-out ponds until hydrogen peroxide level is safe levels (less than 4 mg L^{-1} active hydrogen peroxide).

Treatment:

Calculate the volume of concentrated hydrogen peroxide required (See Attachment 1).

Introduce the hydrogen peroxide product directly into the water body, ideally behind the water paddlewheel, to aid mixing and distribution throughout the water column. Ensure the entire water column is treated. Pre-dilution of the product will assist even mixing and distribution of the product throughout the water column.

Following the initial hydrogen peroxide addition test the active concentration from multiple locations around the water body to ensure target concentration is achieved. Where a lower than target dose is achieved perform a secondary hydrogen peroxide product addition (See Attachment 1).

Post treatment

Water must be retained to ensure complete breakdown of active hydrogen peroxide actives, prior to release or re-use.

Active hydrogen peroxide will undergo natural degradation to water and oxygen following cessation of product addition. This may vary between hours and days.

Treated water must be retained until the active hydrogen peroxide level is below detectable limits (<0.5 mg L^{-1} active hydrogen peroxide) before discharge or re-use.

Formulation, handling and disposal

Product to be used:

HYDROGEN PEROXIDE 50% (unregistered)
Containing: 598 g L^{-1} HYDROGEN PEROXIDE as its only active constituent.

HYDROGEN PEROXIDE 60% (unregistered)
Containing: 742 g L^{-1} HYDROGEN PEROXIDE as its only active constituent.

Product supplier:
Redox Pty Ltd
4 Holmes Rd
MINTO NSW 2566

Solvay Interox Pty Ltd
20-22 McPherson Street
BANKSMEADOW NSW 2019

Packaging

Concentrated hydrogen peroxide products are required due to the large volume of water required to be disinfected. Product distributed in reusable, multi-use industrial grade intermediate bulk containers (IBC) (approximate liquid volume of 950 L).

Environmental Release and Monitoring

DO NOT discharge treated water until hydrogen peroxide level is below detectable limits (<0.5 mg L^{-1} active hydrogen peroxide).

Data Assessment – Target Animal Safety

Hydrogen peroxide effect on cultured penaeid prawns and other species

A summary of the target animal safety data can be found in Appendix 1. These data aim to demonstrate safety of the proposed treatment dose (Method 2 – up to 4 mg Kg^{-1}) of hydrogen peroxide when used as a general disinfectant to kill micro-organisms, or as an emergency source of oxygen, in a situation where when culture animals (penaeid prawns) are present.

The use of hydrogen peroxide on aquatic animals is quite common in aquaculture in Australia and overseas (see 'Overseas Regulatory Activity' and 'Background' sections above). Hydrogen peroxide doses used in finfish is commonly over 25 times greater and often 250 times greater than the current proposed permit for Method 2, where the product is applied to the water body with production prawns present.

Hydrogen peroxide has been shown to cause significant pathological changes in the gill tissue of olive flounder (*Paralichthys olicaeceus*) treated with 300 and 500 mg/L hydrogen peroxide via a 1 hour immersion (at 12.43°C +/- 0.73°C) (Hwang, Kim and Nam, 2016). When the exposure was replicated at 100 mg/L hydrogen peroxide there was no difference detected (Hwang, Kim and Nam, 2016). In Atlantic salmon at 10.4-16.0°C exposure to 2580 mg L^{-1} for 20 minutes duration caused 100% mortality, whereas exposure to 1370 mg L^{-1} for 20 minutes duration caused 0% mortality and no significant pathological changes to delicate gill tissue (Kiemer and Black, 1997). It is considered in penaeid shrimp that also posses delicate gill tissue that similar physiological and morphological impacts could occur, however the proposed dose (up to 4 mg L^{-1}) is magnitudes lower than the above studies, therefore adverse effects are considered less likely.

Reduced growth, damage to gill and mortality have been reported in finfish subjected to high or lethal concentrations of hydrogen peroxide (Yanong, 2014). Additional blood parameter changes (lactate, osmolality, and pH) have been noted following exposure to high dosages of hydrogen peroxide in kingfish, however changes were considered less significant than untreated animals due to parasite presence (Mansell *et al.*, 2005).

In crustacea, chronic exposure of crab larvae (*Metacarcinus edwardsii)* to hydrogen peroxide concentration of 187.5-1500 mg L^{-1} had a lethal effect on larvae (Gebauer *et al.*, 2017).

Data Assessment - Environmental

Summary

Hydrogen peroxide is exempt from the requirement for approval (i.e. exempted from the operation of section 15(1) (a) (i) of the Agvet Code) and is a reserved chemical product under Schedule 3C of the AgVet Code 1995. The use of this chemical as a general disinfectant is conditionally approved by the APVMA. From an environmental perspective the proposed use pattern does pose an elevated risk to the environment from that which is already conditionally approved, given the above. Therefore, the environmental assessment is not considered necessary.

The exempted active constituent and reserved hydrogen peroxide chemical product only permits up to 6% concentration of hydrogen peroxide, which is not feasible or practical to use in a prawn farm situation and we are seeking to use the product (50-60% concentration). In addition, we are seeking to use hydrogen peroxide at a low dose as a source of emergency oxygen supplementation when production prawns, which differs from the use claims as a reserved chemical.

Hydrogen Peroxide is currently permitted for use in other aquaculture sectors (See PER83276). This approved use pattern also involves use in seawater and of similar environments.

It is considered the proposed use pattern poses a negligible risk in comparison to the currently approved minor-use permit (PER83276). PER83276 involves usages of much higher concentrations of hydrogen peroxide and permits direct release of active hydrogen peroxide into the surrounding environment, whereas this proposed minor-use permit involves holding treated water until active hydrogen peroxide level is below detectable limits (<0.5 mg L^{-1} active hydrogen peroxide). Therefore, it is considered the worst-case environmental assessment has already been performed for PER83276.

The proposed use is for the application of the hydrogen peroxide into an earthen pond, where it is immediately diluted. The risk of concentrated hydrogen peroxide discharging into the environment is considered very low. Hydrogen peroxide. at the maximum concentration on the proposed minor-use permit (100 mg L^{-1}), in ponds with aeration and/or organic matter was undetected after 2-3 days (Russo et al., 2007). The nature of the chemical hydrogen peroxide is that in the proposed conditions rapid breakdown of active hydrogen peroxide is expected, and likelihood of any active constituent being discharged from the farm extremely low. The envrionmental impact of the use of hydrogen peroxide on Australian prawn farms as per the proposed minor-use permit (Section 2.) is considered negligible.

Physico-chemical properties - Literature

Hydrogen Peroxide is a highly reactive, strong oxidizing and bleaching agent that is classified as corrosive at concentrations higher than 20% (Yanong, 2014).

pH

In a freshwater lab trial, hydrogen peroxide addition appeared to result in a reduction of water pH from pre-addition levels of 8.1 to as low as low as 7.4 (Waheed, 2017). The reduction in water pH may be due hydrogen peroxide being a weak acid (Daruti, Rozi and Rahayu, 2018). Product pH information was not available in the (Waheed, 2017) study. However, other hydrogen peroxide products, have a below neutral pH, such as INTEROX ST-50 with a pH between 1-4 (See attached MSDS). This is supported by the Waheed (2017) study, where a linear decrease of pH value in treated water occurred with increasing hydrogen peroxide product addition, with the maximum addition of 16mL (of 6% solution, or 24 mg L^{-1}) in 40L water having the bigger pH decrease. The hydrogen peroxide product in this proposed minor-use permit application is highly concentrated, meaning less product volume of addition is required to achieve the same dose as a less concentrated hydrogen peroxide product (up to 10 times less volume required than the reserved chemical 6% hydrogen peroxide).

Temperature

Toxicity of hydrogen peroxide to finfish has been shown to increase with increasing temperature (Waheed, 2017). However, the hydrogen peroxide concentrations in the proposed minor-use permit, where cultured *Penaeus monodon* are exposed to active chemical are significantly lower than concentrations that have been demonstrated safe to *Penaeus monodon* at a similar temperature (see appendix 1).

Alkalinity

Alkalinity was shown to decrease in water treated with hydrogen peroxide in both summer and winter trials (Waheed, 2017). It is not anticipated that this will cause any health concerns to production *Penaeus monodon,* as sufficient alkalinity is typically present in the pond environment and farms have the ability to exchange water meaning any decrease is likely to be transient and manageable.

Hardness

Hardness was shown to increase in water treated with hydrogen peroxide in both summer and winter trials (Waheed, 2017). It is not anticipated that this will cause any health concerns to production *Penaeus monodon,* as sufficient hardness is typically present in the pond environment and farms have the ability to exchange water meaning any increase is likely to be transient and manageable.

Dissolved Oxygen

Molecular oxygen can be supplied to an aquaculture system using hydrogen peroxide, which naturally dissociates in water to produce ½ mol dissolved oxygen/ mol H2O2, with a solubility of 40–50 mg/L (Zappi et al., 2000). Presence of organic material in the water body serves as a catalyst to accelerate the decomposition of hydrogen peroxide and the release of dissolved oxygen (Boyd and Tucker, 2014). Significant increase in dissolved oxygen has been shown following the addition of hydrogen peroxide to water (Waheed, 2017). This could be an important lifeline for rapid increase in pond dissolved oxygen and prevent significant stock losses, should a rapid decrease in pond dissolved oxygen occur on a prawn farm (e.g. following a phytoplankton bloom crash). In one study the application of a dose of 10 mg/L of hydrogen peroxide increased the dissolved oxygen level from 3.5 mg/L to 13.6 mg/L during winter while it increased from 2.4 mg/L to 9.9 mg/L during summer (Waheed, 2017).

See also:

- "Hydrogen Peroxide Efficacy Data Assessment".

Further information on the Physico-chemical properties of hydrogen peroxide can be found in:

- (European Union, 2015)

Physico-chemical properties - Field studies (Australian Prawn Farm Perspective)

pH

No major changes in pH occurred in any of the four ponds in relation to hydrogen peroxide addition (See appendix 4).

Temperature

Temperature rose slightly in all four ponds. This is in the realms of expected daily temperature fluctuation in an earther prawn pond and was consistently at 2-3°C for surface water samples (Culberson and Piedrahita, 1996). In addition, the dissociation of hydrogen peroxide is an exothermic reaction that

will generate a small amount of heat energy that may contribute to this temperature change of a body of water (Goor, Glenneber and Jacobi, 2007). There was no change in water quality parameter measured that is considered likely to indirectly or directly negatively impact the health of culture prawns.

Nitrogenous wastes

Nitrogenous wastes (Total Ammonia Nitrogen (TAN), Nitrite, and Nitrate) were not measured during this experiment. Total Ammonia Nitrogen (TAN) may rise indirectly following hydrogen peroxide treatment given the algicidal properties of this chemical. Waste nitrogen (TAN) contributes to pond phytoplankton growth in ponds and detoxification of TAN (Boyd and Tucker, 2014). Increasing water exchange and restoration of a functioning phytoplankton bloom following hydrogen peroxide addition provide a means to avoid build-up of nitrogenous wastes in culture systems.

General Environmental Chemistry and Fate

Hydrogen peroxide is highly reactive in natural waters and breaks down rapidly to form water and oxygen (Health Canada Pest Management Regulatory Agency (PMRA), 2014). It is not expected to accumulate over time in either sediment or in the water column (Health Canada Pest Management Regulatory Agency (PMRA), 2014). Hydrogen peroxide dissolves easily in water and is unlikely to move into sediments (Health Canada Pest Management Regulatory Agency (PMRA), 2014).. Hydrogen peroxide is not expected to enter the atmosphere or be subject to long-range transport (Health Canada Pest Management Regulatory Agency (PMRA), 2014). Hydrogen peroxide does not readily bind to organic matter and is not expected to accumulate in animals or plants (Health Canada Pest Management Regulatory Agency (PMRA), 2014).

Given the proposed use pattern hydrogen peroxide is expected to pose a negligible risk to non-target organisms in receiving waters. The rapid break down of hydrogen peroxide and discharge requirements (<0.5mg/L hydrogen peroxide) is considered to pose negligible risk of receiving waters. Water treated with hydrogen peroxide should be held for a minimum of 24 hours in earthen ponds where over 90% degradation of the active constituent is expected to occur (Russo, Curtis and Yanong, 2007). In the presence of aeration and/or organic matter an initial dose of 100mg/L hydrogen peroxide is expected to be undetected after 2-3 days (Russo, Curtis and Yanong, 2007).

Active Breakdown

Hydrogen peroxide appears to degrade relatively rapidly in the presence of organic material and aeration (Yanong, 2014).

Hydrogen peroxide is a natural photochemical formed in water under sunlight (Drábková, Admiraal and Maršálek, 2007). In freshwaters, the natural levels of hydrogen peroxide are usually between 10^{-8} M (0.0034 mg/L) to 10^{-7} M (0.034 mg/L) but can reach up to 10^{-5} M (0.34 mg/L) (Drábková, Admiraal and Maršálek, 2007). In seawaters, the natural levels of hydrogen peroxide hydrogen peroxide can reach 0.0136 mg/L (Schmidt, Gaikowski and Gingerich, 2006).

Following cessation of addition of a 50mg/L dose of active hydrogen peroxide in a small brackish lake in the Netherlands (over 7 hours), water hydrogen peroxide levels the returned to background levels within 48 hours (Burson *et al.*, 2014).

Water treated with hydrogen peroxide held for 24 hours in an earthen pond is expected to degrade rapidly (Yanong, 2014). In the presence of aeration and/or organic matter an initial dose of 100mg/L hydrogen peroxide is expected to be undetected after 2-3 days (Russo et al. 2007 In: Yanong, 2014). Two earthen ponds treated with an initial hydrogen peroxide concentration of 6.46 and 13.60 mg/L, respectively, had concentrations of 1–2 mg/L after 24 hours (Russo et al. 2007 In: Yanong, 2o14)).

The fate of hydrogen peroxide within the soil compartment has been evaluated by reacting the chemical with the various soil specimens using 25% (w/w) soil slurries (See: Zappi *et al.*, 2000).

Breakdown of hydrogen peroxide is temperature dependent (Lyons, Wong and Page, 2014).

At 15°C (~59°F) and 20°C, initial hydrogen peroxide concentrations of 10 and 100 mg L^{-1} in tank culture water were not measurable after 2–3 days in the presence of aeration and/or organic matter (Yanong, 2014). Similar findings occurred in another lake based application of 2.5mg/L hydrogen peroxide (Matthijs *et al.*, 2011).

In two earthen ponds treated with an initial hydrogen peroxide concentrations of 6.46 and 13.60 mg L^{-1}, respectively, had concentrations of 1–2 mg/L after 24 hours (Russo, Curtis and Yanong, 2007).

Further information into the breakdown kinetics of hydrogen peroxide can be found in attached references (Arvin and Pedersen, 2015)(Lyons, Wong and Page, 2014) (Tort *et al.*, 2004)

General Environmental Toxicology

This is considered to have already been reviewed by APVMA under assessment of PER83276.

Prior discussions with the QLD Department of Environment Heritage and Protection (detailed below) regarding the periodic use of hydrogen peroxide in the setting of an Australian prawn farm support the assessment of risk to surrounding environment as low.

> *Hydrogen peroxide could be expected to an order of magnitude less toxic and would behave in a similar way to chlorine in terms of solubility, photo-degradation, volatilisation to atmosphere and expending on organic content in the sediment and water so I don't anticipate any detectable impacts of releases at the low levels likely at discharge or in the mixing zone* (Pers. Comm. EPH 2017).
>
> *Management by confirming that levels are low in the discharge channel and at the point of discharge would be sufficient to show no adverse effects are likely* (Pers. Comm. EPH 2017).
>
> *Given that this (application) is a sporadic discharge and not a continuous discharge related to production a trigger level limit of 1-3 ppm (mg L^{-1}) should provide adequate protection especially outside the mixing zone* (Pers. Comm. EPH 2017).

Further data on environmental toxicity of hydrogen peroxide can be found in: (Schmidt, Gaikowski and Gingerich, 2006).

Potential toxicity to environmental microorganisms from hydrogen peroxide discharged from aquaculture facilities is mitigated by:

1. The holding of hydrogen peroxide treated water for a duration to ensure an efficacious treatment is achieved.
2. The rapid dilution and decay of hydrogen peroxide in an earthen pond.
3. The retention of water treated with hydrogen peroxide until water is tested to be below 0.5 mg L^{-1} active hydrogen peroxide.
4. The ability for microorganisms themselves, should any environmental exposure occur, to quickly rebound or repopulate to ubiquitous sources of microorganisms after exposures cease.

Therefore, no long-term effects on environmental populations or communities of microorganisms are expected to result from hydrogen peroxide use in under this proposed permit.

Effects on terrestrial life are believed to be negligible and are not addressed.

Residues

Hydrogen peroxide is a commonly used chemical in food processing as a disinfectant. According to the Australia New Zealand Food Standards Code - Standard 1.3.3 - Processing Aids, Hydrogen peroxide is a permitted food processing aid used in packaged water and in water used as an ingredient with a maximum permitted level of 5 mg Kg^{-1} in all foods.

Food residue exposure from use of hydrogen peroxide under the proposed use pattern (Method 2) is not expected to appreciably increase levels of hydrogen peroxide over endogenous levels in treated prawns due to the rapid decomposition of hydrogen peroxide to water and oxygen upon contact with moisture and degradation in tissues.

Therefore, the use of hydrogen peroxide is not expected to result in unacceptable dietary risks when the product is used according to permit directions.

Dietary risks from food and water have been determined not of concern of a similar use pattern Canada for the Paramove 50 (50% hydrogen peroxide) product where no maximum residue limit (MRL) (Health Canada Pest Management Regulatory Agency (PMRA), 2014).

A Nil Withholding Period (WHP) is proposed, with stipulation that prawn product harvested following a hydrogen peroxide exposure must be below the Australia New Zealand Food Standards Code (ANZFSC) maximum permitted level of 5 mg Kg^{-1} for all foods.

Human Toxicology

Summary

It is considered that the APVMA already have sufficient data on human health risks of hydrogen peroxide. The proposed products have been used for many years under PER83276, with no known adverse effects. In addition, six of the twelve current APVMA registered hydrogen peroxide products for use in domestic pools and spas are have an active hydrogen peroxide concentration between 50-60%. It is considered the OH&S practices on an aquaculture farm enterprise are at an elevated standard compared to the domestic home, thus a relatively reduced risks to human health.

The toxicity of hydrogen peroxide has been well-characterized in the published scientific literature (Health Canada Pest Management Regulatory Agency (PMRA), 2014). The main mode of action is based on its strong oxidizing and corrosive properties, with its oral, dermal and inhalation toxicities being secondary to corrosivity (Health Canada Pest Management Regulatory Agency (PMRA), 2014). It is considered to be highly acutely toxic by the oral route, slightly acutely toxic by the dermal route, and moderately acutely toxic by the inhalation route. It is corrosive to both skin and eyes, and is not a dermal sensitizer (Health Canada Pest Management Regulatory Agency (PMRA), 2014). Due to the rapid degradation of hydrogen peroxide, the hazard posed by the proposed end-use product is mostly of an acute nature.

It appears unlikely that treatment related effects will result from maternal exposure to hydrogen peroxide (Health Canada Pest Management Regulatory Agency (PMRA), 2014). There is limited evidence of carcinogenicity in experimental animals for the carcinogenicity of hydrogen peroxide (Health Canada Pest Management Regulatory Agency (PMRA), 2014).

Exposure and Risk Assessment

Potential routes of occupational exposure to hydrogen peroxide during the proposed use pattern with the end-use product are dermal, ocular and inhalation.

Workers could be exposed via splashing or vapourization of the hydrogen peroxide if they are in the vicinity when the end-use product is delivered, particularly workers who are sampling the treated water for hydrogen peroxide levels at regular intervals during the delivery and mixing process. However, any such exposures would be very short-term and use of the personal protective equipment (PPE) described on the label for the end-use product (for example, chemical-resistant coveralls, long-sleeved shirt and pants, face shield, socks, and chemical resistant boots and gloves) will mitigate any dermal and ocular exposures and risks.

Potential inhalation exposures and risks are also expected to be minimal because the end-use product will rapidly be diluted with water during the application/mixing process and airborne concentrations of hydrogen peroxide above the paddlewheels are expected to be well below the Safe Work Australia, time-weighted average threshold limit value (TWA-TLV) of 1.4 mg m^{-3} (1ppm) for hydrogen peroxide. Also, there are precautionary statements on the label advising workers not to get the product in the eyes or on the skin, and not to inhale any vapours. Inhalation exposures and risks from hydrogen peroxide vapours released from the storage tanks are expected to be negligible.

Post application exposure could occur from the proposed use pattern. However, the maximum concentration of the hydrogen peroxide treatment (100 mg Kg^{-1}, 0.1% hydrogen peroxide) is considered negligible and well below that of topical antiseptics publicly available from chemists (3000 mg Kg^{-1} 3% hydrogen peroxide). Greater risk may occur from empty IBC storage tanks and when application hoses are rinsed out. Any such exposures would be mitigated by the use of PPE as described above.

Additional assessments and review information can be found in (Health Canada Pest Management Regulatory Agency (PMRA), 2014).

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Appendix 1. Target animal safety data

Table 1. *Target animal safety data to demonstrated efficacy of Method 2 - Hydrogen peroxide as a general disinfectant to kill micro-organisms in a situation where when culture animals are present. Proposed dosage – up to 4mg/L (ppm) active hydrogen peroxide (HP).*

Species Group	**Species Name**	**Life stage**	**Endpoint**	**Temperature**	**Exposure Duration (hours)**	**H_2O_2 concentration (mg L^{-1} or ppm)**	**Reference**
Penaeid crustaceans	Black tiger prawn (*Penaeus monodon*)	Juvenile PL30	LC50	28°C (+/-1°C)	24	39 (95% CI: 7-235)[#]	(Piyatiratitivorakul, Lirdwitayaprasit and Thooithaisong, 2002)
						65 (95% CI: 28-393)*	
		Juvenile PL	LC50	n/a	24	30.6	(Srisapoom et al., 1999 In: (Schmidt, Gaikowski and Gingerich, 2006))
	Banana prawns *(Fenneropenaeus merguiensis*)		No data available				
	White shrimp *(Litopenaeus vannamei)*	Juvenile PL20	Median LC50	28°C	24	68.3 (95% CI: 60.3-77.7)^	(Furtado *et al.*, 2014)
					48	57.7 (95% CI: 49.9-66.4)^	
					72	49.6 (95% CI: 42.3-57.4)^	
					96	41.5 (95% CI: 34.8-49.3)^	
			NOAEC		96	8.41^	
			Safe level		96	4.15^	

	Blue shrimp *Litopenaeus stylirostris*	Juvenile PL12	<LCLo	26.3	6	30	(Cardona *et al.*, 2015)
	Indian white shrimp (*Fenneropenaeus indicus*)	PL15	EC50	n/a	12	132.5^^	(Mehrabi *et al.*, 2010)
					24	67.89^^	
					48	55.56^^	
					72	51.95^^	
					96	48.6^^	
			LC50	n/a	12	239.81^^	
					24	101^^	
					48	74.28^^	
					72	65.72^^	
					96	61.45^^	
		PL45	EC50	n/a	12	147.57^^	
					24	70.83^^	
					48	60.01^^	
					72	54.89^^	
					96	41.19^^	
					12	304.56^^	

					24	160.12^^	
					48	113.1^^	
					72	93.69^^	
					96	79.38^^	
		Sub-adult (12+/-1 grams)	EC50	n/a	12	306.43^^	
					24	174.14^^	
					48	113.62^^	
					72	78.21^^	
					96	61.96^^	
			LC50	n/a	12	712.13^^	
					24	518.44^^	
					48	265.29^^	
					72	145.53^^	
					96	102.76^^	
		Adult (20 +/-2 grams)	EC50	n/a	12	243.25^^	
					24	130.55^^	
					48	75.56^^	
					72	61.18^^	
					96	51.59^^	

			LC50	n/a	12	827.75^^	
					24	508.91^^	
					48	317.2^^	
					72	139.44^^	
					96	85.88^^	
		various	NOEC	n/a	96	20^^	
		various	LOEC	n/a	96	40^^	
		various	MAC	n/a	96	28.8^^	
Other crustaceans	Crab (*Metacarcinus edwardsii*)	Zoea 1 larvae	EC50	15°C	72	1130.25 +/- 43.44	(Gebauer *et al.*, 2017)
			EC50		96	1036.25 +/- 43.4	
			LC50		20 m	1642	

[#] Note - *Penaues monodon* cultured with phytoplankton *Chattonella marina*. * Note - *Penaues monodon* cultured with phytoplankton *Heterosigma akashiwo*. ^ Note - HP value units in the table above differ from the source reference as the source reference considered the 29% H_2O_2 product used in the study as 100% Hydrogen peroxide, therefore calculations were necessary to convert units to a mg/L HP value. ^^Bioassay using a product of 50% hydrogen peroxide an 0.05% silver ion (not hydrogen peroxide only)

Appendix 2. Effects of 100 mg L^{-1} Hydrogen Peroxide (H_2O_2) on pond water quality

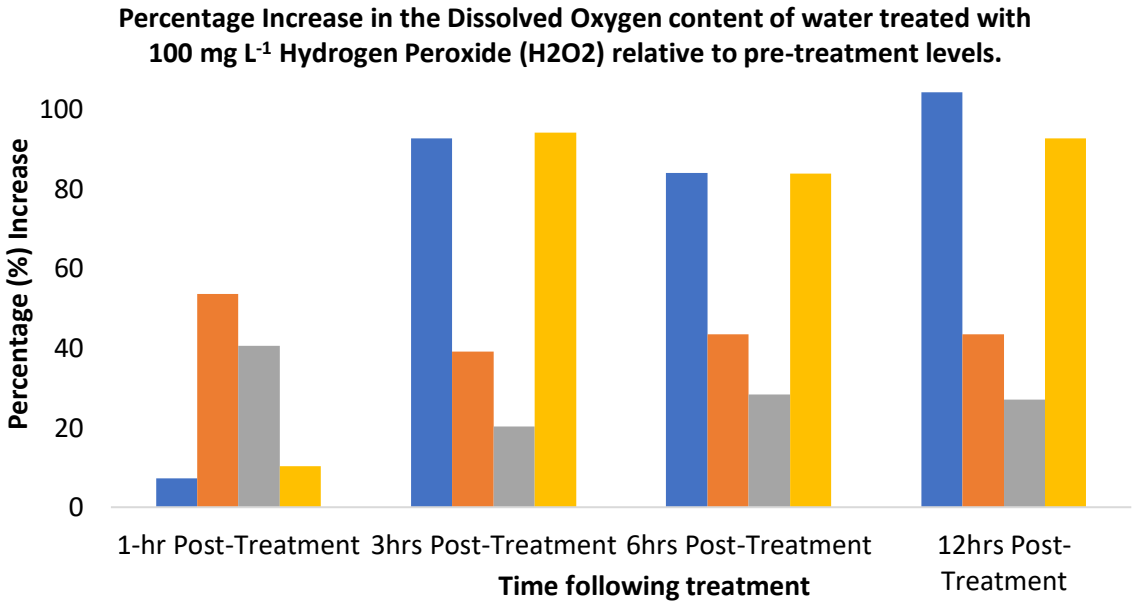


Figure 1. *Water dissolved oxygen effect of 100 mg Kg^{-1} Hydrogen Peroxide (H2O2) addition to four prawn ponds, at various time points, relative to pre-treatment levels for each pond, following treatment. No negative control. Data collected under PER 85506. Australian Prawn farm field data. No prawns present in treated water. Commercial in Confidence.*

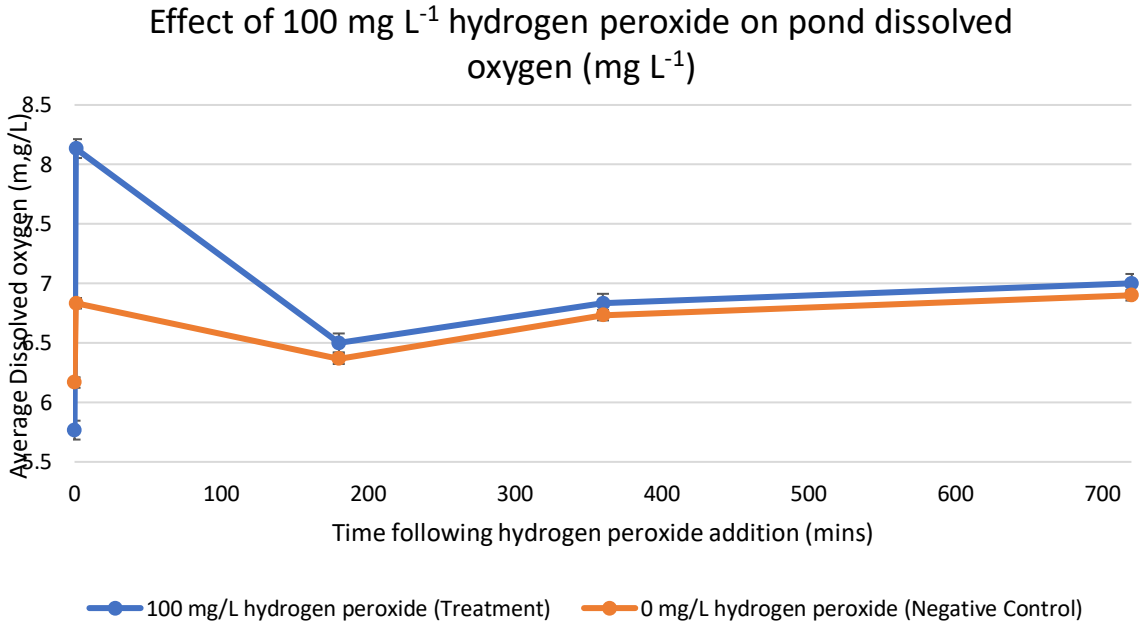


Figure 2. *Effect of 100 mg/L Hydrogen Peroxide (H2O2) on average pond dissolved oxygen content (mg/L) in water samples of three treatment and three negative control prawn ponds, at various timepoints following treatment. Error bars represent average standard error for each treatment type. See Appendix 1, table 1 for raw data. Data collected under PER 85506. Australian Prawn farm field data. No prawns present in treated water. Commercial in Confidence.*

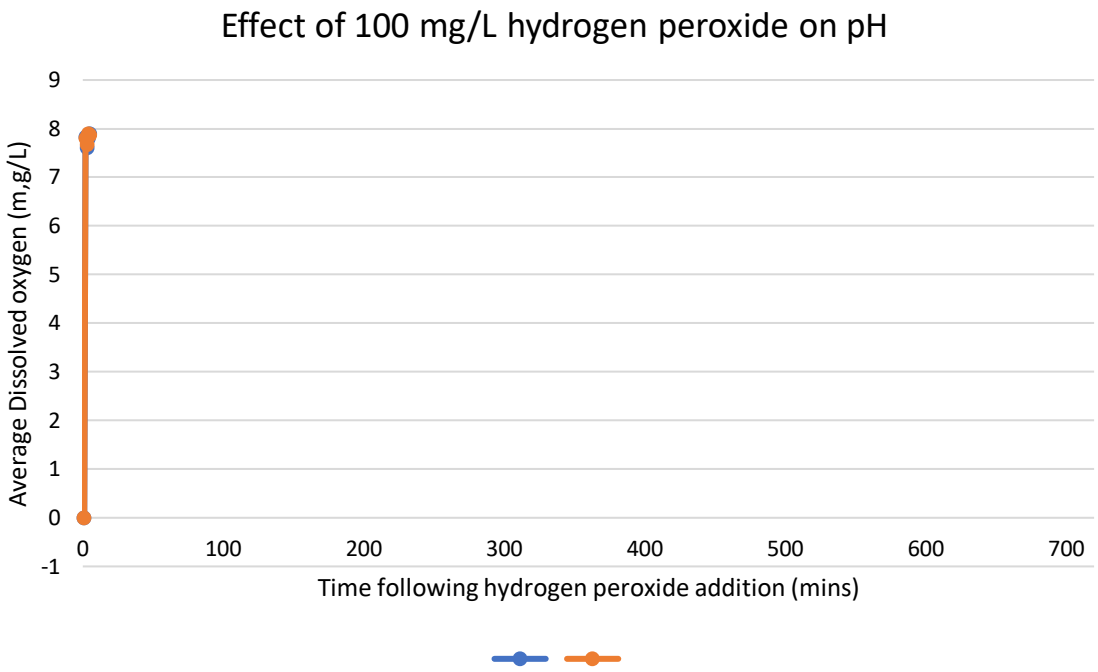


Figure 3. *Effect of 100 mg/L Hydrogen Peroxide (H2O2) on average pond pH in water samples of three treatment and three negative control prawn ponds, at various timepoints following treatment. Error bars represent average standard error for each treatment type. See Appendix 1, table 1 for raw data. Data collected under PER 85506. Australian Prawn farm field data. No prawns present in treated water. Commercial in Confidence.*

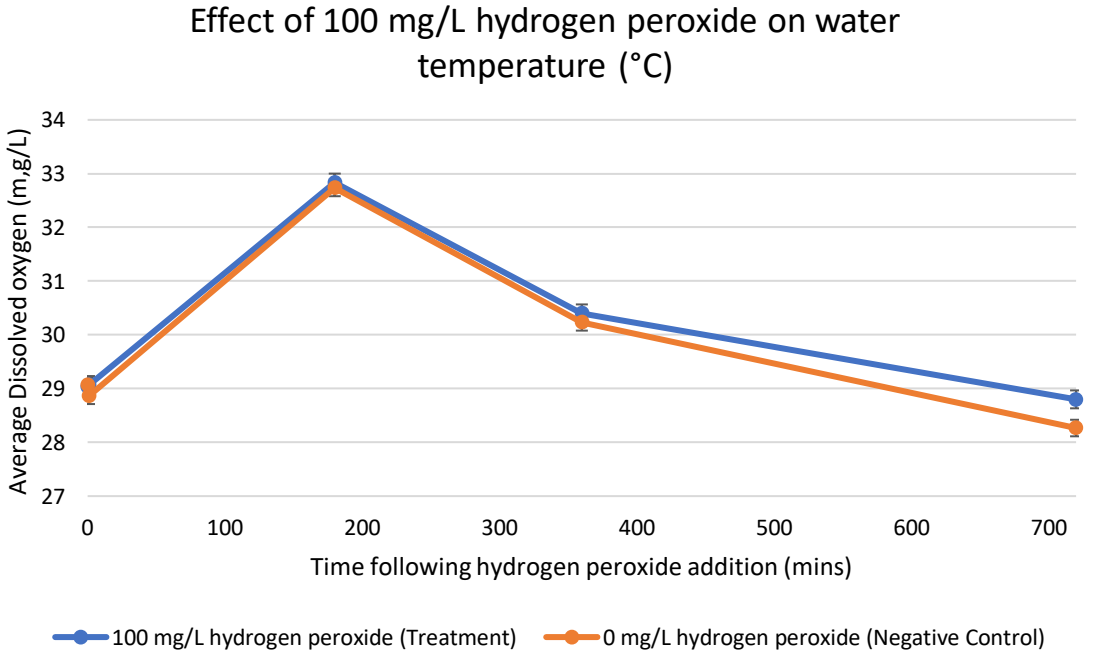


Figure 4. *Effect of 100 mg/L Hydrogen Peroxide (H2O2) on average pond water temperature (°C) in water samples of three treatment and three negative control prawn ponds, at various timepoints following treatment. Error bars represent average standard error for each treatment type. See Appendix 1, Table 1 for raw data. Data collected under PER 85506. Australian Prawn farm field data. No prawns present in treated water. Commercial in Confidence.*

Table 1. *100ppm H2O2 treatment impact on water quality parameters. Data collected under PER 85506. Australian Prawn farm field data. No prawns present in treated water. Commercial in Confidence.*

Pond	Target H2O2 Dose (ppm)	Parameters	Pre-Treatment	Post-Treatment			
				1-hr	3hrs	6hrs	12hrs
236	100	**Dissolved Oxygen, mg L^{-1}**	6.8	7.5	13.2	12.5	13.1
		pH	8.5	8.4	8.4	8.3	8.4
		Salinity, ppt	30	30	30	30	30
		Temperature, °C	30.0	30.0	30.9	31.3	33.0
		Water Depth, cm	150	150	150	150	150
		Secchi Reading, cm	85	Clear	Clear	Clear	Clear
		Water Color	Green	Clear	Clear	Clear	Clear
		ORP, mV	172	190	191	188	189
		H_2O_2 Lvl (Strip), ppm	0	>100	>100	>100	>100
237	100	**Dissolved Oxygen, mg L^{-1}**	6.9	7.4	13.3	12.7	14.1
		pH	8.4	8.3	8.3	8.2	8.2
		Salinity, ppt	30	30	30	30	30
		Temperature, °C	30.0	30.0	30.9	31.9	33.1
		Water Depth, cm	150	150	150	150	150
		Secchi Reading, cm	92	Clear	Clear	Clear	Clear
		Water Color	Green	Clear	Clear	Clear	Clear
		ORP, mV	168	192	188	189	190
		H_2O_2 Lvl (Strip), ppm	0	>100	>100	>100	>100
233	100	**Dissolved Oxygen, mg L^{-1}**	7.4	10.4	8.9	9.5	9.4
		pH	8.3	8.2	8.2	8.1	7.9
		Salinity, ppt	29.2	29.2	29.2	29.2	29.2
		Temperature, °C	31.0	31.3	31.8	33.3	33.3
		Water Depth, cm	150	150	150	150	150
		Secchi Reading, cm	58	Clear	Clear	Clear	Clear
		Water Color	Green	Clear	Clear	Clear	Clear
		ORP, mV	174	192	189	190	195
		H_2O_2 Lvl (Strip), ppm	0	>100	>100	>100	>100
234	100	**Dissolved Oxygen, mg L^{-1}**	6.9	10.6	9.6	9.9	9.9
		pH	8.4	8.4	8.4	8.3	8.2
		Salinity, ppt	30.2	30.2	30.2	30.2	30.2
		Temperature, °C	30.5	30.6	31.4	33.3	32.9
		Water Depth, cm	150	150	150	150	150
		Secchi Reading, cm	70	Clear	Clear	Clear	Clear
		Water Color	Green	Clear	Clear	Clear	Clear
		ORP, mV	170	192	189	190	179
		H_2O_2 Lvl (Strip), ppm	0	>100	>100	>100	>100

Table 2. *Bactericidal efficacy of 100ppm Hydrogen peroxide (H_2O_2) in water treatment of prawn pond water samples, inoculated onto either vibrio selective TCBS media or non-selective TSA media, at various time points following treatment. Average counts and standard error also shown. TCBS colony counts reflect the sum of yellow colonies and green colonies. Australian Prawn farm field data collected under PER 85506. No prawns present in treated water. Data Commercial in Confidence.*

Sample time relative to 100ppm H_2O_2 – Treatment Pond	Dissolved Oxygen (mg L^{-1})			pH			Temperature (°C)				Average Bacterial Plate Count (CFU/mL) from 3 sample locations ± standard error (SE)					
											TCBS			TSA		
Before treatment	6	5.4	5.9	7.8	7.8	7.9	29.2	29	28.9	CFU/mL	2697	2163	2790	8617	8457	7883
										SE	±12	±34	±389	±252	±754	±193
1 min post treatment	7.9	8.2	8.3	7.6	7.6	7.6	29	29.2	29	CFU/mL	1650	1993	1490	8580	8020	7487
										SE	±251	±130	±138	±376	±549	±399
3 hours post treatment	6.4	6.6	6.5	7.8	7.8	7.8	33.7	31.9	32.9	CFU/mL	0	0	0	0	0	10
										SE	±0	±0	±0	±0	±0	±6
6 hours post treatment	6.8	6.9	6.8	7.9	7.9	7.9	30.5	30.2	30.5	CFU/mL	0	7	60	63	40	53
										SE	±0	±7	±35	±22	±12	±23
12 hours post treatment	7	7	7	7.8	7.9	7.8	28.8	28.9	28.7	CFU/mL	73	140	73	97	93	87
										SE	±23	±85	±19	±32	±9	±37

Sample time relative to 0ppm H_2O_2 - Negative Control Pond	Dissolved Oxygen (mg L^{-1})			pH			Temperature (°C)				Average Bacterial Plate Count (CFU/mL) from 3 sample locations ± standard error (SE)					
											TCBS			TSA		
Before treatment	6	6.4	6.1	7.7	7.8	7.9	29	29	29.2	CFU/mL	3037	2723	3347	8813	7550	7450
										SE	±132	±330	±491	±388	±278	±526
1 min post treatment	6.8	6.8	6.9	7.6	7.7	7.7	28.9	28.8	28.9	CFU/mL	2733	3640	4003	9150	7357	8033
										SE	±413	±212	±94	±275	±439	±371
3 hours post treatment	6.3	6.4	6.4	7.9	7.9	7.9	33.2	32.7	32.3	CFU/mL	2973	4180	4783	7167	8337	3507
										SE	±1257	±284	±193	±491	±220	±1215
6 hours post treatment	6.7	6.7	6.8	7.9	7.8	7.9	30.7	30	30	CFU/mL	3583	3910	4387	2910	1960	4377
										SE	±428	±233	±64	±246	±820	±331
12 hours post treatment	6.9	6.9	6.9	7.7	7.8	7.8	28.6	28.2	28	CFU/mL	4363	4020	5087	1710	1487	3250
										SE	±693	±359	±260	±228	±411	±132

Appendix 3. Average water pH for an Australian Prawn Farm

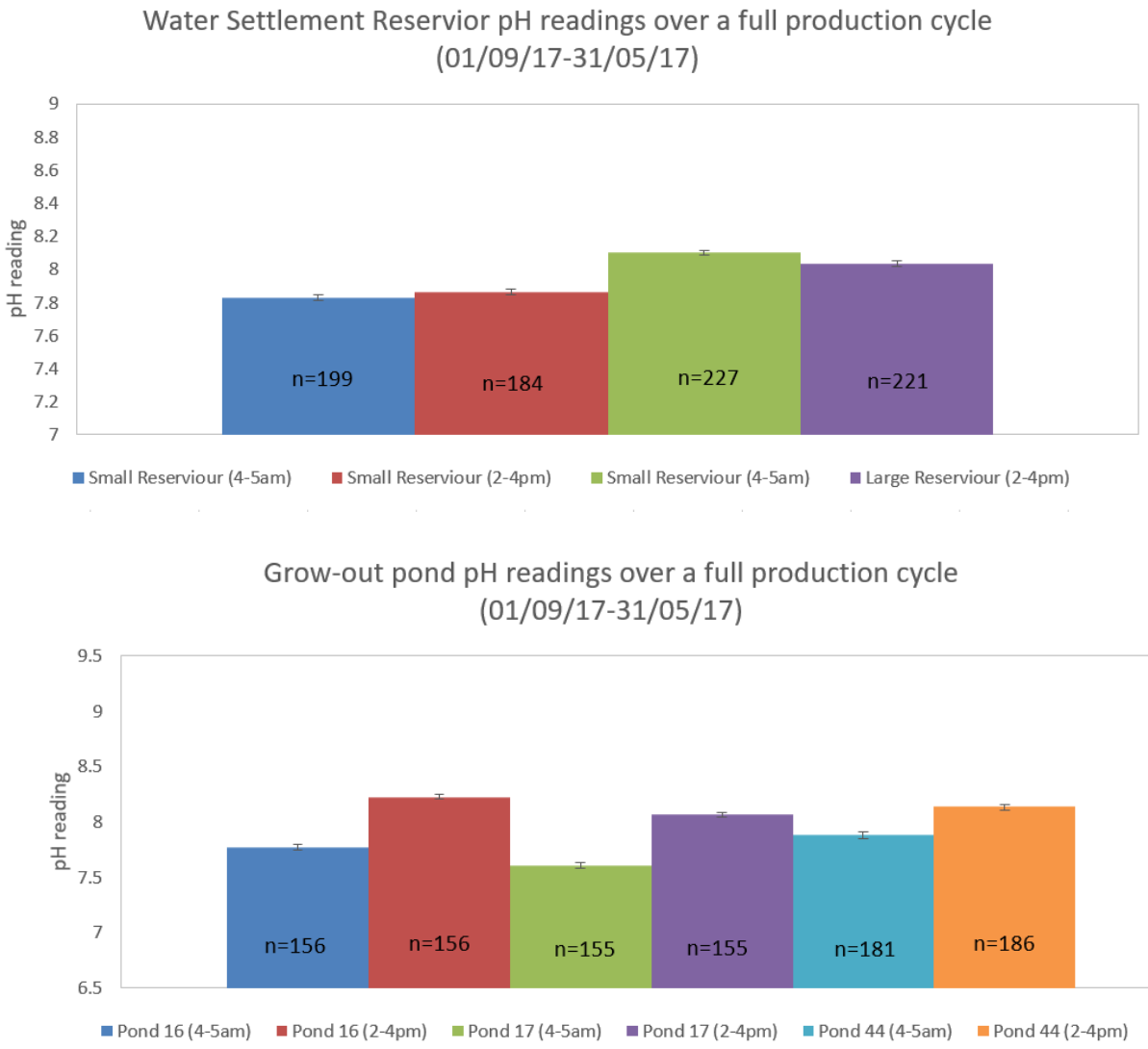


Figure 1. *Water Reservoir and grow-out pond am and pm pH reading during an entire production cycle at an Australian Prawn Farmers Association (APFA) member farm. The number of water samples assessed for each pond and time point are shown in over the coloured column, and error bars are displayed as standard error (standard deviation/square-root of the number of samples). Source data set is available for analysis of additional ponds if required. Australian Prawn farm field data. Data Commercial in Confidence.*

Appendix 5: Sodium and Calcium Hypochlorite Efficacy Data Assessment

Abbreviations

MUP – Minor-use permit

LC50 – Lethal concentration that kills 50 percent of a test population

LC99 – Lethal concentration that kills 99 percent of a test population

EC50 – Effective concentration that causes a biological response in 50 percent of a test population

EC99 – Effective concentration that causes a biological response in 99 percent of a test population

Definitions

Disinfection - The process of eliminating infectious organisms by use of chemical or physical agents (Kahrs, 1995).

Microorganism (or microbe) - microscopic organisms that exists as unicellular, multicellular, or cell clusters. Consisting of bacteria, viruses, fungi, algae, protozoa, archaea.

Proposed Permit Details

Product supplier:
Registrants of swimming pool products as listed on the APVMA's database, PUBCRIS (https://portal.apvma.gov.au/pubcris).

Persons who can use the product under this permit:
Bonafide aquaculturists employed by a licensed Australian prawn farm.

Product to be used:

APVMA registered products, containing; up to 700 g/kg available chlorine as CALCIUM HYPOCHLORITE, OR, up to 200 g/L (20%) available chlorine as SODIUM HYPOCHLORITE, as their only active constituent.

Directions for Use:

For use as a disinfectant to kill micro-organisms, generally, during the production of farmed giant black tiger prawns (*Penaeus monodon*) and banana prawns (*Fenneropenaeus merguiensis*) on licensed Australian prawn farms, in aquaculture earthen pond sediment, water, tanks, equipment and surfaces.

Situation	Purpose	Rate
Clean hard surfaces	General disinfectant to kill micro-organisms	Minimum of 30 mg L^{-1} available chlorine
Water	General disinfectant to kill micro-organisms	Minimum of 30 mg L^{-1} available chlorine. Allow for a minimum of 24 hours contact time. Maintain a minimum of 5 mg L^{-1}residual available chlorine.

Absorbent material such as dip nets, clothing, ropes or absorbent surfaces	General disinfectant to kill micro-organisms	Minimum of 200 mg L^{-1} available chlorine. Allow for a minimum 2 minutes of contact time. Rinse thoroughly with freshwater prior to use.
Floors and walls in culture facilities.	General disinfectant to kill micro-organisms	Minimum of 1,500 mg L^{-1} available chlorine. Allow for a minimum 2 hours of contact time. Rinse thoroughly with freshwater prior to use.
Tanks	General disinfectant to kill micro-organisms	Fill with freshwater and dose with a minimum of 200 mg L^{-1} available chlorine. Allow for a minimum 24 hours of contact time.

Restraint
DO NOT USE in environmental waters. Only for use in waters where the release of treated water can be controlled, following treatment.
The SODIUM HYPOCHLORITE or CALCIUM HYPOCHLORITE products are not to be used in conjunction or mixed with any other chemical.
For general disinfection purposes only.

Critical Use Comments
Observe all label safety directions relevant to handling of SODIUM HYPOCHLORITE or CALCIUM HYPOCHLORITE. Ensure all personnel handing the product are wearing correct PPE (refer to product label and MSDS).
DO NOT discharge treated water unless total chlorine residues meet ANZECC water quality guidelines for ecosystem protection (below 3 µg Cl L^{-1} of total residual chlorine).
DO NOT use treated tanks or equipment until having performed thoroughly rinsing with freshwater to remove chlorine residues.

Treatment:
Calculate the volume of concentrated CALCIUM HYPOCHLORITE or SODIUM HYPOCHLORITE required (See Attachment 1).
Two hours after initial application measure the chlorine concentration and pH, repeat measurements at six hourly intervals to ensure target available chlorine concentration is maintained.
Repeat or expend the treatment if the available chlorine concentration is detected below the target concentration within the target treatment period.

Post treatment
Treated water must be retained to ensure complete breakdown of chlorine residue, prior to release or use of water on farm. This can be achieved via (See attachment 1):

a. Neutralizing with SODIUM THIOSULPHATE, or
b. Exposure to sunlight and /or vigorous aeration for 24 – 48 hours

Consider performing periodic testing of treated water to monitor residual chlorine concentration. Ensure that the concentration of total residual chlorine in treated water meets ANZECC water quality guidelines for ecosystem protection (below 3 µg Cl L^{-1} of total residual chlorine), prior to discharge or use on farm.

Withholding Period:
Meat (prawns) – Nil, when used as directed.
Prawn product harvested following exposure to SODIUM HYPOCHLORITE / CALCIUM HYPOCHLORITE must be below the Australia New Zealand Food Standards Code (ANZFSC) maximum permitted level of 1 mg/kg for all foods.

Water – Concentration of total residual available chlorine in treated water must meet ANZECC water quality guidelines for ecosystem protection (below 3 µg Cl L^{-1} of total residual chlorine), prior to discharge or use on farm.

Jurisdiction:
All states

Supply:
The Suppliers authorised by this permit to supply The Products and make claims must supply The Product in a container that must:

a. be impervious to, and incapable of chemical reaction with, its contents when under conditions of
b. temperature and pressure that are likely to be encountered in normal service; and
c. have sufficient strength and impermeability to prevent leakage of its contents during handling, transport
d. and storage under normal handling conditions; and
e. if it is intended to be opened more than once, be able to be securely and readily closed and reclosed; and
f. have sufficient excess capacity to prevent it from breaking if its contents expand during handling, transport or storage; and
g. enable all or any part of its contents to be removed or discharged in such a way that, with the exercise of no more than reasonable care, the contents cannot:
    i. harm any person; or
    ii. have an unintended effect that is harmful to the environment.

The Permit Holder is to monitor the use of calcium hypochlorite or sodium hypochlorite in aquaculture overseas and inform the APVMA of any change in status of that use, particularly any incidents of environmental contamination. Also, the Permit Holder is to inform the APVMA of any action taken by overseas regulatory authorities in regard to the use of calcium hypochlorite or sodium hypochlorite in aquaculture.

Executive Summary

In late 2016, an exotic viral disease, White Spot Disease (WSD), was diagnosed in multiple Australian prawn farms on the Logan River catchment, Queensland. The subsequent disease response program resulted in the complete eradication of the seven infected farms (approximately 25% of the Australian prawn farming industry). Following this exotic disease outbreak an intensive review of farm-level biosecurity practices on Australian prawn farms was performed to safeguard against future endemic and exotic disease incursions. The prawn farming industry are now taking a proactive step to improve the disease preventative measures on farm to reduce the likelihood and impact of disease entry, spread and exit from their farms. In order to improve on-farm biosecurity practices the industry is seeking APVMA permits for several chemical products. Trichlorfon, Hydrogen Peroxide, Calcium and Sodium Hypochlorite, Benzalkonium Chloride and Copper Sulphate have been highlighted as the most important.

Biosecurity measures offer the prospect of safeguarding against future disease incursions into farms. Extensive investment is currently being allocated into auditing on-farm biosecurity measures to identify weaknesses in bio-exclusion and biocontainment that could allow potential pathogens to enter and spread on farm. Audits being performed consider major routes of transmission, assesses the nature and validity of perceived hazards, rank risks, and provide recommendations for managing identified risks, with the aim of reducing overall risk of pathogen entry and spread within the farm.

Biosecurity audits of Australian prawn farms have identified deficiencies in the tools that are available to control pathogen entry and spread on farms via potentially infected nets, surfaces, clothing, tanks and farm equipment. Equivalent risks are well recognized in other aquaculture sectors and mechanisms have been implemented to control risk through chemical disinfection of nets, surfaces, clothing, tanks and farm equipment using chlorine (Sodium Hypochlorite or Calcium Hypochlorite).

There is a current APVMA minor-use permit (MUP) for disinfection of abalone harvesting equipment for this purpose held by NSW Department of Primary Industries (PER86206 – valid in NSW, SA and WA only); and

an emergency-use permit (PER83695, valid in NSW only) that permits disinfection of aquaculture nets, surfaces, clothing, tanks and farm equipment held by NSW Department of Primary Industries. Additionally, it is considered that sodium hypochlorite is already permitted for the proposed use pattern under schedule 3C – Reserved Schedule of the AgVet code, as a general disinfectant for application to an inanimate object to kill micro-organisms generally.

It is considered that the duplication of the assessment of many data modules is not required, as the proposed use does not differ greatly from current permitted uses and that APVMA have ample data surrounding these active constituents. Sodium hypochlorite and Calcium hypochlorite are both exempted from the requirement for approval (i.e. exempted from the operation of section 15(1)(a)(i) of the Agvet Code), through a formal process involving a gazettal of the active as exempt from approval (https://apvma.gov.au/node/4176). As of 20/11/19, there are currently 85 APVMA registered products containing CHLORINE PRESENT AS CALCIUM HYPOCHLORITE, and 62 APVMA registered products containing CHLORINE PRESENT AS SODIUM HYPOCHLORITE.

The proposed minor-use permit involves both sodium hypochlorite and calcium hypochlorite and is considered equivalent to the current permitted use pattern with these chemicals in wide use throughout Australia and are readily available in supermarkets and retail outlets for use in pools and as general disinfectants in households. We are seeking a new MUP to provide guidance to permit bonafide aquaculturists employed by a licenced Australian prawn farm to use these chemical products as general disinfectants as part of their integrated biosecurity program. The proposed MUP will assist farms to control future disease outbreaks by reducing the risk of pathogen entry and spread onto, within and between farm areas through effective disinfection. The ability for the Australian prawn farming industry to remain viable and expand into the future will depend of the ability to strength on-farm biosecurity measures, for which permitted use of chlorine for nets, surfaces, clothing, tanks and farm equipment disinfection is considered a requirement.

We are seeking to use peer reviewed, published, and publicly available literature to provide additional information where deemed necessary to satisfy safety, efficacy and trade criteria of sodium hypochlorite and calcium hypochlorite for this application.

Chemical Properties

Chemistry and manufacture information is considered to be not required for this minor use permit application as sodium hypochlorite and calcium hypochlorite are both exempted from the requirement for approval (i.e. exempted from the operation of section 15(1)(a)(i) of the Agvet Code), through a formal process involving a gazettal of the active as exempt from approval (https://apvma.gov.au/node/4176). Additionally, there are numerous APVMA approved chemical products, which are considered to contain equivalent chemistry to be used under the proposed minor-use permit. This allows for Australian prawn farms, which are generally in located in remote and regional areas, to have easier access to approved products under the proposed permit.

Some chemical properties are listed in the table below and discussed in sections below of this report as considered relevant to the overall assessment of 'Module 8. Efficacy and target animal safety'.

Table 2. Chemical properties of sodium hypochlorite and calcium hypochlorite

Common Name	Calcium hypochlorite	Sodium hypochlorite
Chemical Name:	Calcium hypochlorite	Sodium hypochlorite
Tradenames:	Various	Various
CAS Number:	7778-54-3	7681-52-9
Molecular Formula:	$Ca(OCl)_2$	NaOCl
Molecular Weight:	142.98 g mol^{-1}	74.442 g mol^{-1}

Structural Formula:	$Cl\text{-}O^{-}$ - Ca^{2+} - $O^{-}\text{-}Cl$	Na^{+}- $(OCl)^{+}$
Purity of the active constituent:	650 g kg^{-1} CALCIUM HYPOCHLORITE as its only active constituent 700 g kg^{-1} CALCIUM HYPOCHLORITE as its only active constituent	5-20% sodium hypochlorite and 80-95% water
Properties of the Substance:	white solid that readily decomposes in water	colourless transparent liquid
Water Solubility (25°C):	2.14E+05 mg L^{-1}	-
Vapour pressure (25°C):	7.22E-13 mmHg	-
Melting Point (°C):	100	18
Boiling point (°C):	-	40
Density (25°C):	2.35	1.209
Reaction products produced in water:	Calcium, hypochlorite ions, and hypochlorous acid	Sodium, hypochlorite ions, and hypochlorous acid

Sources: Ropp (2013), USFDA (2006)

Local Regulatory Activities

Australia

There are currently eighty-five (85) APVMA registered calcium hypochlorite products and sixty-two (62) APVMA registered sodium hypochlorite products. These products are predominantly registered for use in swimming pools and spas (See https://portal.apvma.gov.au/pubcris).

There are currently five (5) calcium hypochlorite and nine (9) sodium hypochlorite APVMA active minor-use permits. These minor-use permits are predominantly for the disinfection of general and/or targeted microorganisms that cause disease in plants, crops, oysters, abalone and livestock (See https://portal.apvma.gov.au/permits).

The current APVMA minor-use permits PER83795 and PER86206 are equivalent to the proposed minor use permit in this application with application in the aquaculture sector. These permits differ in the 'jurisdiction' (we are seeking to extended to multiple states) and 'persons who can use the product' (we are seeking users to include bonafide aquaculturists employed by a licensed Australian prawn farm).

The now expired emergency use permit PER85540 allowed for an equivalent the use pattern and user group to that in the current proposed minor use permit in this application.

Sodium hypochlorite and Calcium hypochlorite are both exempted from the requirement for approval (i.e. exempted from the operation of section 15(1)(a)(i) of the Agvet Code), through a formal process involving a gazettal of the active as exempt from approval (https://apvma.gov.au/node/4176). Sodium hypochlorite and Calcium hypochlorite are both listed as agricultural chemical products that destroy bacteria, viruses and protozoa in Schedule 3B Part 2 (1) of the AgVet code (2007) page 6.

Additionally, sodium hypochlorite (up to 20% active constituent) is a listed a reserved chemical product in Schedule 3C Part 2 (9) of the AgVet code (2007) that is recommended by its manufacturer for application to an inanimate object to kill micro-organisms generally.
(See: https://www.legislation.gov.au/Details/F2019C00270).

Sodium hypochlorite and Calcium hypochlorite are both widely available and permitted for use in Australia as general disinfectants.

The Australian and New Zealand Environment and Conservation Council (ANZECC) Water Quality guidelines provided a freshwater environmental protection trigger value of moderate reliability of 3 µg chlorine per litre measured as total residual chlorine was derived using the statistical distribution method with 95% protection (ANZECC, 2000). This figure was adopted as a marine low reliability trigger value, to be used only as an indicative interim working level (ANZECC, 2000). In the near 20 years since this report was published, to the authors knowledge, a marine environmental trigger value has not yet been established (https://www.waterquality.gov.au/anz-guidelines/guideline-values/default/water-quality-toxicants/toxicants/chlorine-2000).

In some parts of Australia chlorination is used as a method to disinfect drinking water, with known high efficacy against bacteria, with generally a free chlorine residual of between 0.2 and 0.5 mg L^{-1} maintained (Page 66 - NHMRC (National Health and Medical Research Council), 2011). A guideline value of 3mg/L free chlorine is advised (Page 116- NHMRC (National Health and Medical Research Council), 2011).

Overseas Regulatory Activities

The World Organisation for Animal Health (OIE) provides protocol and recommendations for similar proposed use (disinfection of aquaculture earthen pond sediment, water, tanks, equipment and surfaces) as an international standard for disease control and management in aquaculture (World Organisation for Animal Health (OIE), 2009). Further details on overseas uses of chlorine can be found in: (FAO, 2000)

United States

In the Unites States sodium hypochlorite (EPA chemical code: 014703) and calcium hypochlorite (EPA chemical code: 014701) are chlorinated inorganic disinfectants used in laundries, swimming pools, ponds, drinking water, and other water and wastewater systems; on food and non-food contact surfaces; and as a postharvest, seed or soil treatment on various fruit and vegetable crops (Page 1 - EPA, 1991). Sodium hypochlorite and calcium hypochlorite are used to control bacteria, fungi, and slime-forming algae that can cause diseases in people and animals (Page 1 - EPA, 1991).

Sodium hypochlorite and calcium hypochlorite were first registered in 1957 (Page 1 - EPA, 1991). The US EPA issued a registration standard for sodium hypochlorite and calcium hypochlorite in 1986, which can be found in the document (EPA, 1992). The thorough review of the scientific data base and all relevant information supported the reregistration of sodium hypochlorite and calcium hypochlorite (Page 8 - EPA, 1992). The review concluding that no additional scientific data would be necessary to register or reregister products that contain sodium hypochlorite from 5.25% to 12.5%, or calcium hypochlorite from 65% to 70%, as long as the products contain no other active ingredients contain no inert ingredients other than water, and bear Toxicity Category I labeling (EPA, 1991; Page 8 - EPA, 1992). This review concluded that current registered uses of sodium and calcium hypochlorite will not result in unreasonable adverse effects to the environment (Page 8 -EPA, 1992). In USA, sodium hypochlorite is a GRAS (Generally Recognised as Safe) (40 CFR 180.2) chemical and calcium hypochlorite is exempt under section 408 of the FFDCA from the requirements of a tolerance for use pre-harvest and post-harvest on raw agricultural commodities (Page 8 - EPA, 1992). Human health, exposure and toxicity were assessed deemed satisfactory (EPA, 1991).

Aquaculture uses of chlorine (calcium hypochlorite) include for disinfection of (Page 15 - USEPA, 2011):

- Fish pond (with fish removed) using 10 mg/L free chlorine with a residual free chlorine above 1 mg L^{-1} after 5-minute duration.
- Fish pond equipment using 200 mg/L free chlorine, with porous equipment being soaked for 1 hour duration.

- Control of scavengers in fish hatchery ponds suing 200 mg L^{-1} free chlorine, with testing performed to ensure residual chlorine has dropped to 0 mg/L before fish are added.

The United States Environmental Protection Agency (USEPA) has established Water Quality Criteria for 'total residual chlorine' (TRC) concentrations permissible in receiving waters, to protect aquatic life and water quality (Tikkanen *et al.*, 2001). These concentrations are based on acute and chronic toxicity effects for aquatic life (Tikkanen *et al.*, 2001). Under the acute toxicity criterion, the 1-hour average chlorine concentration of the stream should not exceed 19 µg L^{-1} more than once every three years, on the average (Tikkanen *et al.*, 2001). Under the chronic toxicity criterion, the 4-day average concentrations should not exceed 11 µg L^{-1} more than once every three years, on the average (Tikkanen *et al.*, 2001). Many states within the USA require no general permits that may or may not require individual permits for select discharges of chlorinated water.

Canada

Canadian Environmental Quality Guidelines (1987) propose a water quality criterion of 2 µg L^{-1} of total residual chlorine for receiving streams. Many provincial regulatory agencies have adopted this chlorine concentration as the Water Quality Criteria (Tikkanen *et al.*, 2001). The provinces of British Columbia and Ontario require all water releases to contain less than 2

µg L^{-1} of chlorine Tikkanen *et al.*, 2001). In British Columbia, the regulatory discharge limit for intermittent flows is a function of the duration of the release as given by the following equation:

$$Total\ Residual\ Chlorine\ (\mu g\ L-1) = [1074(duration\ in\ minutes)^{-0.74}]$$

The maximum concentration of total residual chlorine should not exceed 100 $\mu g\ L-1$ regardless of the exposure period (Tikkanen *et al.*, 2001).

Nova Scotia requires all new facilities to dechlorinate completely prior to discharge into receiving streams (Tikkanen *et al.*, 2001).

Europe

The European Union performed an environmental and human health risk assessment on sodium hypochlorite in 2007 (See Binetti and Attias, 2007). This assessment concluded that at present there was no need for further information or testing or for risk reduction measures beyond those which being applied already

The use of an initial concentration of greater than 50 mg L^{-1} free chlorine and greater than 5 mg L^{-1} residual free chlorine for 24 hours duration is a method approved by the Norwegian veterinary authority for disinfecting waste water (Page 430 - Torgersen and Håstein, 1995).

Asia

Chlorine in forms such as calcium hypochlorite and sodium hypochlorite are used to disinfect the water supplies in fish and shrimp hatcheries and for water and sediment disinfection between production cycles throughout Asia (Rico *et al.*, 2012).

Efficacy and target animal safety

Contents

We are seeking to use peer reviewed, published, and publicly available literature to provide any necessary additional information to satisfy the efficacy and target animal safety criteria for the proposed calcium hypochlorite and sodium hypochlorite minor-use permit.

Additionally, we are seeking draw upon the information provided alongside the current APVMA registered sodium hypochlorite and calcium hypochlorite products and use pattern of current APVMA minor use permits, where there does not appear to be a significant difference in the efficacy claims.

This data package is formatted as per APVMA guidelines and details a collated brief summary of relevant data and provision of links and further resources to the extensive literature that supports the efficacy claims made in this proposed minor use permit.

In order to limit duplication of already existing data we have endeavoured to keep this report concise. Where additional data is required, this can be obtained and made available for APVMA to consider.

Data summary

The proposed minor-use permit does not involve the direct contact of sodium hypochlorite and calcium hypochlorite products within or onto the farmed giant black tiger prawns (*Penaeus monodon*) and banana prawns (*Fenneropenaeus merguiensis*).

Therefore, we consider:

- *Target animal:* General microorganisms (e.g. bacteria, viruses, fungi and protozoa) of the marine environment, which we are seeking demonstrate efficacy of disinfection.

- *Non-target animal*: Farmed giant black tiger prawns (*Penaeus monodon*) and banana prawns (*Fenneropenaeus merguiensis*), which we are seeking to demonstrate safety of the above disinfection.

Giant black tiger prawns (*Penaeus monodon*) and banana prawns (*Fenneropenaeus merguiensis*) are farmed in marine and estuarine water. Australian prawn farms operate in both indoor/outdoor tank-based systems and outdoor earthen/lined pond-based systems which may be exchange water via partial to complete recirculation or single directional flow through. There are a range of different system types that vary in their design and operation. Majority of Australian prawn farms used marine or estuarine water from ocean or river sources for their systems and discharge effluent water back natural waterways.

The environmental source water naturally contains a whole host of microorganisms. Some of these microorganisms can be harmful to farmed Giant black tiger prawns (*Penaeus monodon*) and banana prawns (*Fenneropenaeus merguiensis*) directly or indirectly through acting as vectors for harmful pathogens, therefore posing a biosecurity risk to the farms. A range of physical and chemical methods can be used to eliminate the microorganisms. Chlorination is the most common way of disinfecting drinking water and wastewater (Page 287- Boyd and Tucker, 2014). Chlorination has also been used to disinfect indoor aquaculture hatcheries and in intensive culture ponds before introducing animals (Page 289- Boyd and Tucker, 2014). Sodium hypochlorite and calcium hypochlorite are the two most common sources of chlorine, with calcium hypochlorite the usual chlorination agent used in aquaculture (Page 287- Boyd and Tucker, 2014).

The efficacy of Sodium hypochlorite and calcium hypochlorite as disinfectants of organisms, generally, are demonstrated below to support the Efficacy and target animal safety of the proposed minor-use permit.

Pharmacological data/studies

Summary

When Sodium hypochlorite and calcium hypochlorite are dissolved in water, four chlorine species can result, including; (Page 287 - Boyd and Tucker, 2014)

- Chlorine gas (Cl_2)
- Hypochlorous acid (HOCl)
- Hypochlorite ion (OCl^-)
- Chloride ion (Cl^-)

$$Cl_2 + H_2O = HOCl + H^+ + Cl^-$$

$$HOCl = OCl^- + H^+$$

$$Cl_2 + H_2O = HOCl + H^+ + Cl^-$$

Page 287- Boyd and Tucker, 2014

Chloride (Cl^-) has no appreciable disinfecting power, whereas all three other chlorine species (Cl_2, HOCl, OCl^-), known as free (or available) chlorine residuals, vary in their relative proportion with pH (Page 288 - Boyd and Tucker, 2014). Cl_2 and HOCl have approximately 100 times greater disinfection power than OCl^- (Page 288 - Boyd and Tucker, 2014). In an aquaculture setting the pH will usually be above pH 5 so only HOCl and OCl^- will be in measurable concentrations (Page 288 - Boyd and Tucker, 2014). (see figure below). Therefore, optimal disinfection potential of Chlorine residuals will be between pH 7-8, when the mole fraction of HOCl is between 0.5-1 (see figure below).

minimum concentration of free chlorine residual for effective disinfection at pH 7 is about 1 mg/L, and the amounts of calcium hypochlorite (65% active ingredient) required to provide the equivalent of this dose at different pH values are provided in Table 21.2.

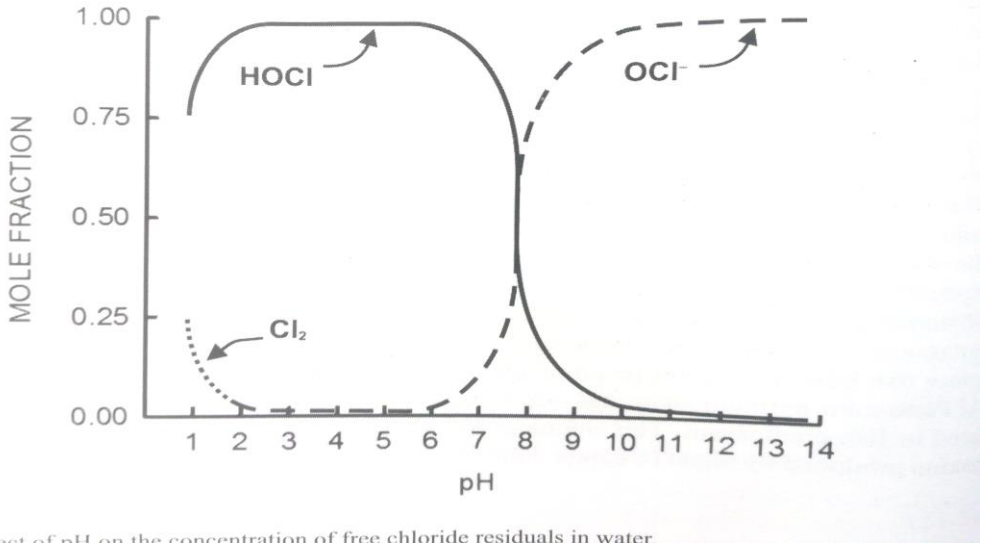


Figure 1. Effect of pH on the concentration of free chloride residuals in water. Source: Page 287- Boyd and Tucker, 2014

Within an aquaculture pond or tank setting there may be particulate organic matter and ammonia present which can react with the chlorine residuals which can lessen the disinfecting power and form chloramines (Page 289 - Boyd and Tucker, 2014). Chloramine products include NH_2Cl, $NHCl_2$ and NCl_3 (Page 289 - Boyd and Tucker, 2014). Chlorine reacts readily with nitrogenous substances (e.g. ammonia) to form N-chlorinated compounds which constitute the combined chlorine (ANZECC, 2000). These compounds are more persistent than the free chlorine (ANZECC, 2000). Among these N-chlorinated compounds is monochloramine (NH_2Cl) which contributes significantly to the combined available chlorine in water. After water treatment, the sum of free chlorine and combined chlorine is referred to as total residual chlorine (TRC) (ANZECC, 2000).

Due to extraneous reactions the calcium hypochlorite (or sodium hypochlorite) dose necessary for effective disinfection is often greater than determined via prediction of resulting chlorine residual species (Page 289 - Boyd and Tucker, 2014). Appropriate chlorination dosing can be established by determining the concentration of free chlorine residual in treated water alongside consideration of pH on disinfecting power (Page 289 - Boyd and Tucker, 2014).

The concentrated liquid solutions of sodium hypochlorite degrades over time, with open containers losing up to 50% of their original concentration within one month (Quinn and Markey 2001, In: Page 69 Department of Agriculture Fisheries and Forestry, 2008). Calcium hypochlorite is significantly more stable, however can react with moisture if open to the air (Page 69 - Department of Agriculture Fisheries and Forestry, 2008). It is considered that active constituent degradation information has been assessed by APVMA in the product registration stage of each registered product available on the market.

A range of advantages and disadvantages of hypochlorite products as disinfectants can be found in Page 69-70 (Department of Agriculture Fisheries and Forestry, (2008).

The relative amounts of the different chlorine forms in equilibrium are governed by pH, temperature and ionic strength (ANZECC, 2000). Between pH 2 and 7, HOCl is the dominant form while at pH 7.4 and 20°C, there is equimolar contribution of HOCl and OCl^- (ANZECC, 2000; See also Fig. 1).

Pharmacokinetics and Pharmacodynamics

Chlorine residues cause derangement and disruption of cell membranes (Piyatiratitivorakul, Lirdwitayaprasit and Thooithaisong, 2002). Sodium and calcium hypochlorite acts as an organic and fat solvent degrading fatty acids, transforming them into fatty acid salts (soap) and glycerol (alcohol), that reduces the surface tension of the remaining solution (saponification) (Estrela, Barbin and Pécora, 2002). Sodium and calcium hypochlorite neutralizes amino acids forming water and salt (Estrela, Barbin and Pécora, 2002). With the exit of hydroxyl ions, there is a reduction of pH (Estrela, Barbin and Pécora, 2002).. Hypochlorous acid, a substance present in sodium hypochlorite solution, when in contact with organic tissue acts as a solvent, releases chlorine that, combined with the protein amino group, forms chloramines (Chloramination) (Estrela, Barbin and Pécora, 2002). Hypochlorous acid (HOCl-) and hypochlorite ions (OCl-) lead to amino acid degradation and hydrolysis (Estrela, Barbin and Pécora, 2002).

Chlorine (strong oxidant) presents antimicrobial action inhibiting bacterial enzymes leading to an irreversible oxidation of SH groups (sulphydryl group) of essential bacterial enzymes (Estrela, Barbin and Pécora, 2002). The antimicrobial effectiveness of sodium hypochlorite, based in its high pH (hydroxyl ions action), is similar to the mechanism of action of calcium hydroxide (Estrela, Barbin and Pécora, 2002).

Efficacy studies

Summary

Sodium hypochlorite and calcium hypochlorite have a demonstrated as efficacious disinfectant products to reduce the number of microorganisms (such as bacteria, fungus and viruses), generally. We are seeking to utilise the disinfectant properties of these products of Australian prawn farms as part to assist farm biosecurity. We have collated the publicly available efficacy data to support the efficacy claims listed on the proposed minor-use permit (and below).

Efficacy Claims:

1. A minimum of 30 mg L^{-1} available chlorine is an efficacious general disinfectant to kill micro-organisms on clean hard surfaces.
2. A minimum of 30 mg L^{-1} available chlorine for a minimum of 24-hour contact time of exposure (minimum of 5 mg L^{-1} available residual chlorine) is an efficacious general disinfectant to kill micro-organisms in water.
3. A minimum of 200 mg L^{-1} available chlorine for a minimum of 2 minutes contact time of exposure is an efficacious general disinfectant to kill micro-organisms on absorbent material such as dip nets, clothing, ropes or absorbent surfaces.
4. A minimum of 1500 mg L^{-1} available chlorine for a minimum of 2 hours contact time of exposure is an efficacious general disinfectant to kill micro-organisms on floors and walls in culture facilities.
5. A minimum of 200 mg L^{-1} available chlorine for a minimum of 24-hour contact time of exposure (minimum of 5 mg L^{-1} available residual chlorine) is an efficacious general disinfectant to kill micro-organisms in tanks refilled with freshwater.

The above efficacy claims align with the recommendations of the AQUAVETPLAN Operational Procedures Manual: Decontamination Version 1.0, 2008 (Department of Agriculture Fisheries and Forestry, 2008); and other technical reports referencing the AQUAVETPLAN document (Lees and McDonald, 2016).

Laboratory model efficacy studies

See throughout the document.

Target animal efficacy studies

The efficacy of free (or available) chlorine as a general disinfectant is demonstrated in the tables below for a range of general microorganisms. The tables below demonstrate that the sodium hypochlorite and calcium

hypochlorite dose rates in the proposed minor-use permit are appropriate to provide efficacious disinfectant activity against a range of different microorganism.

Table 3. Studies demonstrating efficacy of free chlorine to the target animals (Bacteria).

Study Type	**Species**	**Free Chlorine concentration**	**End Point**	**Efficacy**	**pH**	**Temp (°C)**	**Additional variables**	**Reference**
Lab	*Vibrio harveyi; Vibrio splendidus*	1 mg/L	1-minute post addition	LC99 (CFU/mL)	8	30 ± 0.1	Salinity 35 ppt, clean water	(page 56 - Abraham, Palaniappan and Dhevendaran, 2002)
Lab	*Vibrio harveyi; Vibrio splendidus*	50 mg/L	30-minute post addition	LC99 (CFU/mL)	8	30 ± 0.1	Salinity 35 ppt, Dirty (+0.1% peptone)	(Page 57- Abraham, Palaniappan and Dhevendaran, 2002)
Lab	*Vibrio harveyi; Vibrio splendidus*	100 mg/L	1-minute post addition	LC99 (CFU/mL)	8	30 ± 0.1	Salinity 35 ppt, Dirty (+0.1% peptone)	(Page 57- Abraham, Palaniappan and Dhevendaran, 2002)
Lab	*Vibrio para-haemolyticus*	25 mg/L	1 minutes	EC-99 (loss of viability)	n/a	n/a		(Chaiyakosa *et al.*, 2007)
Field	*Vibrio para-haemolyticus)*	50 mg/L	30 minutes	EC-90 (loss of viability)	n/a	n/a	naturally contaminated	(Chaiyakosa *et al.*, 2007)

Table 4. Studies demonstrating efficacy of free chlorine to the target animals (Viruses).

Study Type	**Species**	**Free Chlorine concentration**	**End Point**	**Efficacy**	**pH**	**Temp (°C)**	**Additional variables**	**Reference**
Lab	*infectious hematopoietic necrosis virus (IHNV)*	10 mg/L	30 minutes	LC99 (no viral CPE)	n/a	12	-	(Amend and Pietsch, 1972) P
Lab	*yellow-head baculovirus (YHD)*	20 mg/L	24 hours	EC (reduced viral activity)	n/a	n/a	Exposed virus suspension injected into *Penaeus monodon* adult	(Bunyaratphalin, 2001)
n/a	*Yellow head virus (YHV)*	30 mg/L	n/a	EC (effective disinfection)	n/a	n/a	No supportive primary data	(Flegel, Boonyaratpalin and Withyachumnarnkul, 1997)
Lab	*Baculovirus penaei*	25 mg/L	24 hours	EC-99 (virus loss of infectivity)	n/a	28	5 mg/L residual chlorine at 24 hours.	(Leblanc and Overstreet, 1991)

							Assessed at 46 hours.	
Lab	*Baculovirus penaei*	200 mg/L	60 seconds	EC-99 (virus loss of infectivity)	n/a	28	150 mg/L residual chlorine at 60 seconds. Assessed at 46 hours.	(Leblanc and Overstreet, 1991)
Lab	*Baculovirus penaei*	1,600 mg/L	20 seconds	EC-99 (virus loss of infectivity)	n/a	28	1,200 mg/L residual chlorine at 24 hours. Assessed at 46 hours.	(Leblanc and Overstreet, 1991)
Lab	*White spot syndrome virus (WSSV)*	200 mg/L	10 minutes	EC-99 (virus loss of infectivity)	n/a	28	active principle-concentration only. Not tested free chlorine levels.	(Satheesh Kumar *et al.*, 2013)
Lab	*White spot syndrome virus (WSSV)*	5 mg/L	10 minutes	EC-99 (virus loss of infectivity)	n/a	n/a	active principle-concentration only. Not tested free chlorine levels. IM injection.	(Oseko *et al.*, 2006)
Field	*Channel Catfish Virus*	20 – 50 mg/	n/a single addition	Virus elimination	n/a	n/a	Pond treatment. No fish present	(Page 271- Noga, 2010)
n/a	*Infectious pancreatic necrosis virus (IPNV)*	40 mg/L	30 minutes	Virus inactivation	n/a	n/a	n/a	(Page 274- Noga, 2010)

Table 5. Studies demonstrating efficacy of free chlorine to the target animals (Fungi).

Study Type	**Species**	**Free Chlorine concentration**	**End Point**	**Efficacy**	**pH**	**Temp (°C)**	**Additional variables**	**Reference**
Lab	*Penicillium sp.*	0.045 mg/L	1 minute	inactivation	n/a	n/a	2 mg/L chlorine calculated dose	(Wen *et al.*, 2017)
Lab	*Cladosporium sp.*	0.079 mg/L	1 minute	inactivation	n/a	n/a	2 mg/L chlorine calculated dose	(Wen *et al.*, 2017)
Lab	*Trichoderma sp.*	0.037 mg/L	1 minute	inactivation	n/a	n/a	2 mg/L chlorine calculated dose	(Wen *et al.*, 2017)

Lab	*Aspergillus fumigatus, Aspergillus versicolor, and Penicillium purpurogenum*	48.99-194.7 mg/L	1 minute	Inactivation of 99.9%	n/a	n/a	n/a	(Ma and Bibby, 2017)
Lab	*Cladosporium tenuissimum,*	71	1 minute	Inactivation of 99.9%	7	21	Surface water	(Pereira *et al.*, 2013)
Lab	*Cladosporium cladosporioides,*	139	1 minute	Inactivation of 99.9%	7	21	Surface water	(Pereira *et al.*, 2013)
Lab	*Phoma glomerata,*	152	1 minute	Inactivation of 99.9%	7	21	Surface water	(Pereira *et al.*, 2013)
Lab	*Aspergillus terreus,*	1404	1 minute	Inactivation of 99.9%	7	21	Surface water	(Pereira *et al.*, 2013)
Lab	*Aspergillus fumigatus,*	946	1 minute	Inactivation of 99.9%	7	21	Spring/surface /ground water	(Pereira *et al.*, 2013)
Lab	*Penicillium griseofulvum,*	107	1 minute	Inactivation of 99.9%	7	21	Surface water	(Pereira *et al.*, 2013)
Lab	*Penicillium citrinum*	959	1 minute	Inactivation of 99.9%	7	21	Surface water	(Pereira *et al.*, 2013)

Table 6. Studies demonstrating efficacy of free chlorine to the target animals (Parasites).

Study Type	**Species**	**Free Chlorine concentration**	**End Point**	**Efficacy**	**pH**	**Temp (**C)	**Additional variables**	**Reference**
Lab	*Marteilia sydneyi*	200 mg/L	2 hours post addition	EC-99 (spore inactivation)	n/a	25	Salinity 30 ppt	(Page 224 - Wesche, Adlard and Lester, 1999)
Lab	*Marteilia sydneyi*	200 mg/L	4 hours post addition	EC-99 (spore inactivation)	n/a	25	Salinity 30 ppt	(Page 224 - Wesche, Adlard and Lester, 1999)
Lab	*Marteilia sydneyi*	100 mg/L	4 hours post addition	EC-50 (spore inactivation)	n/a	25	Salinity 30 ppt	(Page 224 - Wesche, Adlard and Lester, 1999)

Lab	*Perkinsus marinus*	300 mg/L	0.5 hours	EC-99 (no dye uptake)	n/a	n/a	Culture media water	(Bushek, Holley and Kelly, 1997)
Lab	*Perkinsus marinus*	52.5 mg/L	4 hours	EC-99 (no dye uptake)	n/a	n/a	Marine water	(Bushek, Holley and Kelly, 1997)
Lab	*Perkinsus marinus*	170 mg/L	18 hours	EC-99 (no dye uptake)	n/a	n/a	Marine water	(Bushek, Holley and Kelly, 1997)
n/a	*Pseudoloma neurophilia*	>100 mg/L	n/a	EC-95 (spore inactivation)	n/a	n/a	n/a	Ferguson et al. 2007 In: Noga, (2010)
n/a	*Glugea anomala*	1,500 mg/L	n/a	EC-95 (spore inactivation)	n/a	n/a	n/a	Ferguson et al. 2007 In: Noga, (2010)

Table 7. Studies demonstrating efficacy of free chlorine to the target animals (Phytoplankton).

Study Type	**Species**	**Free Chlorine concentration**	**End Point**	**Efficacy**	**pH**	**Temp (**C)	**Additional variables**	**Reference**
Lab	*Chattonella marina*	66 mg/L	24 hours	LC-50	7.98-8.28	27-30	Salinity 30-32ppt	(Piyatiratitivorakul, Lirdwitayaprasit and Thooithaisong, 2002)
Lab	*Heterosigma akashiwo*	35 mg/L	24 hours	LC-50	7.98-8.28	27-30	Salinity 30-32ppt	(Piyatiratitivorakul, Lirdwitayaprasit and Thooithaisong, 2002)
Lab	*Chattonella marina*	65.77 mg/L	24 hours	LC-50	n/a	28	Salinity 30ppt	(Thuithaisong, 2001)
Lab	*Heterosigma akashiwo*	34.69 mg/L	24 hours	LC-50	n/a	28	Salinity 30ppt	(Thuithaisong, 2001)

Dose determination studies

The efficacy of free (or available) chlorine as a general disinfectant in a range of different use patterns. Sodium hypochlorite and calcium hypochlorite have been described to be effective at disinfecting against organisms such as bacteria, fungus and viruses. Various concentrations and contact durations have been described. As a general disinfectant for surfaces and water, 30 mg/L concentrations of available chlorine for 3 hours has been described (Torgersen and Håstein, 1995). This concentration of 30 mg/L has also been found to be efficacious against bacteria, fungi, viruses, protozoa and spores on hard surfaces and in water with low organic loading (when in contact for 24 hours with 5 mg/L available chlorine maintained) (Lees and McDonald, 2016). For infrastructure such as pipelines, 50 mg/L available chlorine for 30 minutes has been found to be effective against most microbial agents (World Organisation for Animal Health (OIE), 2009). At 50 mg/L available chlorine, general disinfection of equipment and clothing (footbath or equipment wash) can be achieved (World Organisation for Animal Health (OIE), 2009). When disinfecting equipment such as small hatchery and

broodstock tanks (i.e. tanks for broodstock maturation, mating, spawning, larval rearing and indoor nurseries) and non-expendable equipment, effective general disinfection can be achieved with 200 mg L^{-1} available chlorine for 24-48 hours (World Organisation for Animal Health (OIE), 2009).

Chlorine at a concentration between 100-1000 mg L^{-1} is reported to have a high level of disinfection against a range of bacteria, lipophilic viruses, fungi, hydrophilic viruses and mycobacteria (Scarfe, Lee and O'Bryen, 2006). Higher concentrations at 200-500 mg L^{-1} available chlorine for 10-60 minutes have been described as a general pathogen disinfectant of bacteria, enveloped and non-enveloped viruses and fungi have been used (Yanong and Erlacher-Reid, 2012). Longer durations of 24 hours have been described to disinfect tanks against general pathogens such as bacteria, enveloped and non-enveloped viruses and fungi (Yanong and Erlacher-Reid, 2012). The AquaVetPlan reported that WSSV can be deactivated by 100 mg L^{-1} of free chlorine for 10 minutes and 10 mgL^{-1} for 30 minutes (Department of Agriculture, 2013).

The difference in required chlorine dose in water with high organic levels has been shown by Abraham, Palaniappan and Dhevendaran, (2002), and De Bodt and Defoirdt, (2018). Abraham, Palaniappan and Dhevendaran, (2002) mimicked high organic load water with 0.1% peptone and found with all other conditions equivalent to achieve a 100% efficacy disinfection against the bacteria *Vibrio harveyi* and *Vibrio splendidus* in "dirty" water required 100 times higher concentration of free chlorine and/or a longer duration of exposure contact time.

The variable dose provided in the proposed minor use permit aims to account for the differing water quality settings and provide efficacious exposure (concentration and duration of contact time) to ensure general disinfection against a range of microorganisms.

Table 8. Studies demonstrating efficacy of free chlorine to the target animals (general microorganisms) in different types of water.

Study Type	Species	Free Chlorine concentration	End Point	Efficacy	pH	Temp (°C)	Additional variables	Reference
Lab	*Vibrio harveyi; Vibrio splendidus*	1 mg L^{-1}	1-minute post addition	~100%	8	30 ± 0.1	Salinity 35 ppt, clean water	(Abraham, Palaniappan and Dhevendaran, 2002)
Lab	*Vibrio harveyi; Vibrio splendidus*	50 mg L^{-1}	30-minute post addition	~100%	8	30 ± 0.1	Salinity 35 ppt, Dirty (+0.1% peptone)	(Abraham, Palaniappan and Dhevendaran, 2002)
Lab	*Vibrio harveyi; Vibrio splendidus*	100 mg L^{-1}	1-minute post addition	~100%	8	30 ± 0.1	Salinity 35 ppt, Dirty (+0.1% peptone)	(Abraham, Palaniappan and Dhevendaran, 2002)

Resistance

The proposed minor-use permits includes product dose rates that account for variation in microorganism resistance profiles, ensuring that sufficient concentration of active product is maintained for an appropriate duration. Resistance of some microorganisms to free chlorine residues is suggested, such as is *Mycobacterium* species (Vaerewijck et al., 2005, in Noga, 2010).

Dose confirmation studies

See throughout the document.

Confirmatory clinical/field studies

See throughout the document.

Palatability studies

The proposed minor-use permit does not involve a use pattern where sodium hypochlorite or calcium hypochlorite is used directly the presence of animals that will enter the food chain, therefore 'when used as directed', palatability studies are considered not applicable to this application.

Target and non-target animal safety studies

Summary

Exposure of free (available) chlorine residuals and products from use of sodium hypochlorite and calcium hypochlorite are considered potentially harmful to non-target macro-organisms and micro-organisms. As such the proposed minor-use permit includes conditions in place to reduce the likelihood of exposure of non-target animals in receiving environmental waterways to resulting chemical residues, including details on discharge levels and methods to inactivate and neutralise free chlorine residues (See 'Inactivation of active constituents' below). Ensuring that the concentration of total residual chlorine in treated water meets ANZECC water quality guidelines for ecosystem protection that requires below 3 µg L^{-1} (0.003 mgL^{-1}) of total residual chlorine, prior to discharge or use on farm.

The proposed minor-use permit suggested discharge limit of below 3 µg L^{-1} (0.003 mgL^{-1}) of total residual chlorine to align with (ANZECC, 2000). This value appears to be very conservative with toxicity not apparent in a range of marine aquatic species until significantly higher (>10 times) available chlorine levels occur (see tables below). Australian human drinking water total chlorine residue levels are maintained at greater than 150 times this level. In addition, majority of the studies assessing these toxicity levels were performed in a lab setting where maintenance of residual chlorine levels is significantly easier. It is considered the risk to surrounding aquatic animals from the proposed MUP as extremely low.

Margin of safety studies

The Australian and New Zealand Environment and Conservation Council (ANZECC) Water Quality guidelines provided a freshwater environmental protection trigger value of moderate reliability of 3 μgL^{-1} chlorine measured as total residual chlorine was derived using the statistical distribution method with 95% protection (ANZECC, 2000). This figure was adopted as a marine low reliability trigger value, to be used only as an indicative interim working level (ANZECC, 2000). In the near 20 years since this report was published, to the authors knowledge, a marine environmental trigger value has not yet been established (https://www.waterquality.gov.au/anz-guidelines/guideline-values/default/water-quality-toxicants/toxicants/chlorine-2000).

Table 9. Studies demonstration the margin of safety for exposure of non-target marine water animals to free chlorine.

Study Type	Species	Free Chlorine concentration	End Point	Efficacy	pH	Temp (C)	Additional variables	Reference
Lab	Inland silverside fish *(Menidia beryllina)*	87-186 μgL^{-1}	7-day	NOEC (reproductive impairment)	n/a	n/a	Marine water	(ANZECC, 2000)
Lab	opossum shrimps (*Mysidiopsis bahia*)	20-87 μgL^{-1}	7-day	NOEC (reproductive impairment)	n/a	n/a	Marine water	(ANZECC, 2000)

Dose rate

See throughout the document.

Duration of treatment related studies

See throughout the document.

Compatibility studies

See throughout the document.

Effects on hides and fleeces

Not relevant to this application.

Accidental administration or exposure to non-target animals

Data in relation to the margin of safety to non-target animals, in the event of accidental exposure, is detailed in Table 10 below.

Table 10. Studies demonstration the margin of safety for exposure of non-target marine water animals to free chlorine.

Study Type	Species	Life-stage	Free Chlorine concentration	End Point	Efficacy	pH	Temp (°C)	Additional variables	Reference
Lab	Giant tiger prawn *(Penaeus monodon)*	PL18 - 0.02 grams	6.96 mgL^{-1}	24-hour	LC-50	8.49-8.71	27.9	Residual chlorine of 0.91 mgL^{-1} (at 24 hours)	(Lin, 2002)
Lab	Giant tiger prawn *(Penaeus monodon)*	1.5 months - 2.75 grams	2.05 mgL^{-1}	24-hour	LC-50	8.49-8.71	27.0	Residual chlorine of 1.39 mgL^{-1} (at 24 hours)	(Lin, 2002)
Lab	Giant tiger prawn *(Penaeus monodon)*	3 months - 8.47 grams	11.50 mgL^{-1}	24-hour	LC-50	8.49-8.71	28.4	Residual chlorine of 1.74 mgL^{-1} (at 24 hours)	(Lin, 2002)
Lab	Giant tiger prawn *(Penaeus monodon)*	4.5 months - 23.65 grams	13.34 mgL^{-1}	24-hour	LC-50	8.49-8.71	27.3	Residual chlorine of 1.98 mgL^{-1} (at 24 hours)	(Lin, 2002)
Lab	Giant tiger prawn *(Penaeus monodon)*	PL30	52 mgL^{-1}	24-hour	LC-50	7.98-8.28	27-30	Salinity 30-32ppt; *Chattonella marina* present	(Piyatiratitivorakul, Lirdwitayaprasit and Thooithaisong, 2002)
Lab	Giant tiger prawn *(Penaeus monodon)*	PL30	51 mgL^{-1}	24-hour	LC-50	7.98-8.28	27-30	Salinity 30-32ppt; *Heterosigma akashiwo* present	(Piyatiratitivorakul, Lirdwitayaprasit and Thooithaisong, 2002)
Lab	Giant tiger prawn *(Penaeus monodon)*	PL30	52.26 mgL^{-1}	24-hour	LC-50	n/a	28	Salinity 30 ppt; *Chattonella marina* present	(Thuithaisong, 2001)
Lab	Giant tiger prawn *(Penaeus monodon)*	PL30	50.78 mgL^{-1}	24-hour	LC-50	n/a	28	Salinity 30 ppt; *Heterosigma akashiwo* present	(Thuithaisong, 2001)
Lab	Giant tiger prawn *(Penaeus monodon)*	Nauplii	1.87 mgL^{-1}	24-hour	LC-50	n/a	n/a	n/a	(Chanratchakool and Worasing, 2001)
Lab	Giant tiger prawn *(Penaeus monodon)*	Zoea 2	2.12 mgL^{-1}	24-hour	LC-50	n/a	n/a	n/a	(Chanratchakool and Worasing, 2001)
Lab	Giant tiger prawn *(Penaeus monodon)*	Mysis 2	0.77 mgL^{-1}	24-hour	LC-50	n/a	n/a	n/a	(Chanratchakool and Worasing, 2001)
Lab	Giant tiger prawn *(Penaeus monodon)*	PL6	2.66 mgL^{-1}	24-hour	LC-50	n/a	n/a	n/a	(Chanratchakool and Worasing, 2001)

Lab	Giant tiger prawn *(Penaeus monodon)*	PL15	2.77 mgL^{-1}	24-hour	LC-50	n/a	n/a	n/a	(Chanratchakool and Worasing, 2001)
Lab	Giant tiger prawn *(Penaeus monodon)*	Adult 10-13cm	56.82 mgL^{-1}	96-hour	LC-50 (median)	n/a	n/a	n/a	(Bunyaratphalin, 2001)
Lab	Eastern King Prawn *(Penaeus plebejus)*	n/a	0.180 mg/L^{-1}	24-hour	LC-50	n/a	n/a	n/a	(Manning, Wilson and Chapman, 1996)
Lab	Amphipod (*Hyale barbicornis*)	juvenile	2.5 mgL^{-1}	48-hour	LC-50	7.5	20	Salinity 33.7 ppt	(Anasco *et al.*, 2008)
Lab	Amphipod (*Hyale barbicornis*)	juvenile	2.3 mgL^{-1}	72 hour	LC-50	7.5	20	Salinity 33.7 ppt	(Anasco *et al.*, 2008)
Lab	Amphipod (*Hyale barbicornis*)	juvenile	2.2 mgL^{-1}	96 hour	LC-50	7.5	20	Salinity 33.7 ppt	(Anasco *et al.*, 2008)
Lab	Javanese ricefish (*Oryzias javanicus*)	larvae	0.32 mgL^{-1}	24-hour	LC-50	7.5	26	Salinity 33.7 ppt	(Anasco *et al.*, 2008)
Lab	Javanese ricefish (*Oryzias javanicus*)	larvae	0.29mgL^{-1}	48-hour	LC-50	7.5	26	Salinity 33.7 ppt	(Anasco *et al.*, 2008)
Lab	Javanese ricefish (*Oryzias javanicus*)	larvae	0.20 mgL^{-1}	72 hour	LC-50	7.5	26	Salinity 33.7 ppt	(Anasco *et al.*, 2008)
Lab	Javanese ricefish (*Oryzias javanicus*)	larvae	0.19 mgL^{-1}	96 hour	LC-50	7.5	26	Salinity 33.7 ppt	(Anasco *et al.*, 2008)
Lab	*Angora loach (Nemacheilus angorae)*	n/a	0.5 mgL^{-1}	36 hours	EC (increased erythrocyte fragility)	n/a	n/a	n/a	(Gul *et al.*, 2008)
Lab	*Angora loach (Orthrias angorae)*	n/a	0.551 mgL^{-1}	96 hour	LC-50	n/a	n/a	n/a	(Gul *et al.*, 2008)

Effects on taste or produce (organoleptic effects)

The proposed minor-use permit does not involve the use of sodium hypochlorite or calcium hypochlorite in the presence of animals that will enter the food chain, therefore 'when used as directed', effects on taste or produce (organoleptic effects) are considered not applicable to this application.

Other studies or data

In vivo or in vitro bioequivalence studies

See throughout the document.

Clinical case studies

Considered not relevant as difficult to perform. Laboratory based studies considered sufficient to demonstrate efficacy.

Scientific references or extrapolated scientific argument

See throughout the document.

Topical studies, inhalation studies, tissue irritation studies

Considered not relevant.

Reproductive function studies

Considered not relevant.

Minimum inhibitory concentration studies

Considered less appropriate that the studies included to demonstrate efficacy. The bactericidal activity of sodium and calcium hypochlorite is well known, and chlorine residues of these products can be significantly affected by presence of organic material, so MIC studies considered less relevant.

Inactivation of active constituents

Chlorine residues generated from calcium hypochlorite and sodium hypochlorite are rapidly neutralised by organic matter and therefore any toxic effects tend are short lived (Page 70 - Department of Agriculture Fisheries and Forestry, 2008). Chlorine is a relatively unstable, moderately reactive element (Tikkanen *et al.*, 2001)**.** In the environment, chlorine is neutralized upon reaction with air, sunlight and other contacting surfaces (Tikkanen *et al.*, 2001)**.**

Organic and inorganic impurities in soil and pavements exert a significant amount of chlorine demand and rapidly neutralize chlorine in waters (Tikkanen *et al.*, 2001) **.**Hence, spraying chlorinated waters onto soils or pavements can be a very effective method for disposing of chlorine-containing waters (Tikkanen *et al.*, 2001)**.**

It is considered that the level of organic matter, sunlight, aeration and breakdown time in a commercial production aquaculture farm is highly likely to neutralise any chlorine residues prior to farm discharge. However, to account for the slight potential risk a range of safeguards have been implemented within the proposed minor-use permit to account for the slight potential risk.

Retention, aeration and exposure to sunlight

The chlorine concentration in stored water gradually decreases with time due to aeration, reaction with sunlight/surfaces of holding tanks (Tikkanen *et al.*, 2001).

Since dechlorination of super-chlorinated water can require a large amount of chemicals, so retention of treated water holding tanks can reduce the volume of chlorine residuals, prior to adding dechlorination chemicals (Tikkanen *et al.*, 2001)**.** However, there are several limitations to this method (Tikkanen *et al.*, 2001), including:

- Chlorine decay through natural reactions is extremely slow.
- Decay of chlorine to meet regulatory discharge limits may take several hours to a few days.
- Activities such as reservoir cleaning produce a large volume of chlorinated water, requiring very large tanks for storage creating a logistical challenge.

Exposure to sunlight can transform chlorine residuals to non-toxic chloride (Page 289 - Boyd and Tucker, 2014).

$$2HOCl = 2H^{+}\ 2Cl^{-} + O_2$$

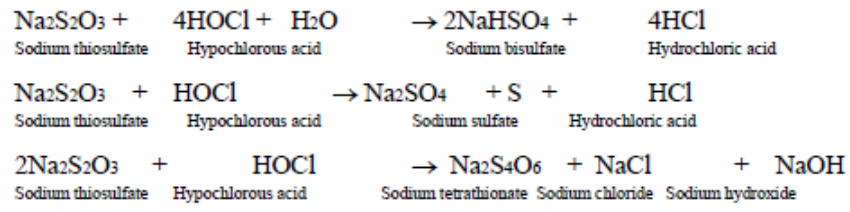
Page 280- Boyd and Tucker, 2014

Exposure of chlorinated water to sunlight and air for approximately 48 hours has been suggested prior to discharge of water treated with free chlorine generating products (Page 3- Bell and Lightner, 1992). Vigorous aeration by bubbling air through chlorine treated water at rate of 9L/min for 6 minutes is reported to speed up this passive breakdown process (Hassan and Edyvean, 2019).

Sodium Thiosulphate ($Na_2S_2O_3$)

Dechlorination can be accomplished by carbon filtration, laboratory water conditioning units, or the use of sodium thiosulphate (Page 22 - EPA, 2002).

Sodium thiosulphate is a colourless, transparent monoclinic crystal widely used by municipalities for dechlorination (Tikkanen *et al.*, 2001)**.** It undergoes multiple reactions with free and combined chlorine, depending on solution pH Reaction with chlorine yields the following:



(Tikkanen *et al.*, 2001)

The use of 3.6 mg (anhydrous) sodium thiosulphate per litre will reduce 1.0 mg free chlorine per litre (Page 22 - EPA, 2002). After dechlorination, total residual chlorine should be non-detectable (Page 22 - EPA, 2002). Another method to dechlorinate water involves the addition of sodium thiosulphate at a rate five (5) molecules of sodium thiosulphate for each 4 (four) molecules of chlorine (or the weight of sodium thiosulphate being 2.85 times the weight of chlorine in the water) (Bell and Lightner, 1992) has been suggested prior to discharge of water treated with free chlorine generating products (Page 3- Bell and Lightner, 1992). Another recommendation 7mg sodium thiosulphate to 1 mg of free chlorine (Yanong and Erlacher-Reid, 2012). The use of sodium thiosulphate in this was is well described (Tikkanen *et al.*, 2001), and has been shown to be both effective and not cause any further harm to organism exposed to dechlorinated water (Hose, King and Stephens, 1984). An EPA toxicity study indicated that sodium thiosulphate is not very toxic to aquatic species (Tikkanen *et al.*, 2001)**.** Sodium thiosulphate may react slowly with chlorine under some conditions (Tikkanen *et al.*, 2001)**.**

Additional data resources

Globally sodium hypochlorite and calcium hypochlorite have been extensively research and used for their efficacy as a disinfectant for the control general microorganisms. As such there is a plethora of available primary literature, review papers and reports relevant to this use pattern. It is not feasible nor beneficial to review this to the level of detail that has been performed numerous times in the past, given the identical chemistries and predictable outcomes. Further information regarding efficacy of sodium hypochlorite and calcium hypochlorite for the purpose of disinfectants against microorganisms, generally, can be found at: https://echa.europa.eu/registration-dossier/-/registered-dossier/16137/1 (accessed 20/11/19).

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Appendix 6: Benzalkonium chloride Data review and gap analysis

Abbreviations

MUP – Minor-use permit

LC50 – Lethal concentration that kills 50 percent of a test population

LC99 – Lethal concentration that kills 99 percent of a test population

EC50 – Effective concentration that causes a biological response in 50 percent of a test population

EC99 – Effective concentration that causes a biological response in 99 percent of a test population

NOEL – No observed effect level

Definitions

Algicide - a biocide used for killing and preventing the growth of algae (phytoplankton)

Proposed Permit Details

Product supplier:
Registrants of products containing 250 g L^{-1} BENZALKONIUM CHLORIDE as listed on the APVMA's database, PUBCRIS (https://portal.apvma.gov.au/pubcris).

Persons who can use the product under this permit:
Bonafide aquaculturists employed by a licensed Australian prawn farm.

Product to be used:

AGRIQUAT DISINFECTANT-SANITIZER-DEODORANT (41450)
PLUS OTHER REGISTERED PRODUCTS
Containing: 250 g L^{-1} BENZALKONIUM CHLORIDE as their only active constituent.

Directions for Use:

For use as an algicide to control heterosigma and other potentially harmful marine phytoplankton species during the production of farmed giant black tiger prawns (*Penaeus monodon*) and banana prawns (*Fenneropenaeus merguiensis*) on licensed Australian prawn farms, in aquaculture earthen ponds.

Dose rate: Further details to be determined

Restraint
DO NOT USE in environmental waters. Only for use in waters where the release of treated water can be controlled, following treatment.
The BENZALKONIUM CHLORIDE product are not to be used in conjunction or mixed with any other chemical.
For use as per directions only.

Critical Use Comments
Observe all label safety directions relevant to handling of BENZALKONIUM CHLORIDE. Ensure all personnel handing the product are wearing correct PPE (refer to product label and MSDS).
Further details to be determined

Treatment:
Further details to be determined

Post treatment
Further details to be determined

Withholding Period:
Meat (prawns) – Further details to be determined
Water – Further details to be determined

Jurisdiction:
All states

Executive Summary

In late 2016, an exotic viral disease, White Spot Disease (WSD), was diagnosed in multiple Australian prawn farms on the Logan River catchment, Queensland. The subsequent disease response program resulted in the complete eradication of the seven infected farms (approximately 25% of the Australian prawn farming industry). Following this exotic disease outbreak an intensive review of farm-level biosecurity practices on Australian prawn farms was performed to safeguard against future endemic and exotic disease incursions. The prawn farming industry are now taking a proactive step to improve the disease preventative measures on farm to reduce the likelihood and impact of disease entry, spread and exit from their farms. In order to improve on-farm biosecurity practices the industry is seeking APVMA permits for several chemical products. Trichlorfon, Hydrogen Peroxide, Calcium and Sodium Hypochlorite, Benzalkonium Chloride and Copper Sulphate have been highlighted as the most important.

Biosecurity measures offer the prospect of safeguarding against future disease incursions into farms. Extensive investment is currently being allocated into auditing on-farm biosecurity measures to identify weaknesses in bio-exclusion and biocontainment that could allow potential pathogens to enter and spread on farm. Audits being performed consider major routes of transmission, assesses the nature and validity of perceived hazards, rank risks, and provide recommendations for managing identified risks, with the aim of reducing overall risk of pathogen entry and spread within the farm.

Biosecurity audits of Australian prawn farms identified a major risk pathway for white spot syndrome virus (WSSV) pathogens to enter prawn farms through incoming source water, which is brought onto farms from local rivers and waterways to fill semi-closed ponds for stocking prawn larvae (Landos, 2017) (Landos, 2017). The ability for phytoplankton (algae) to act as vehicles for WSSV dispersion in water, and thus onto a prawn farm, has been demonstrated (Esparza-Leal *et al.*, 2009). Phytoplankton such as *Isochrysis galbana*, *Skeletonema costatum*, *Chlorella sp.*, *Scrippsiella trochoidea*, *Dunaliella salina* have been demonstrated abilities to carry and transmit WSSV (Liu *et al.*, 2011). Additionally, some phytoplankton species (e.g. *Chattonella marina* and *Heterosigma akashiwo*) can themselves be harmful to farmed prawn species and the ability to control these organisms can offer further health benefits to cultured stock.

To strengthening of Australian prawn farms biosecurity, we are seeking a minor-use permit for the use of benzalkonium chloride (APVMA registered products containing: up to 250 g/kg benzalkonium chloride as their only active constituent) by bonafide aquaculturist employed by a licensed Australian prawn farm to assist control of future disease outbreaks and allow treating water to control potential phytoplankton (algae) vectors and direct drivers of disease. The ability for this industry to remain viable and grow into the future will depend of the ability to strengthen on-farm biosecurity, which permitted minor-use of benzalkonium chloride is considered essential.

Benzalkonium chloride (BKC) is exempt from the requirement for approval (i.e. exempted from the operation of section 15(1)(a)(i) of the Agvet Code), through a formal process involving a gazettal of the active as exempt from approval (https://apvma.gov.au/node/4176). There are currently five (5) APVMA minor-use permits (MUPs) for the use of BKC as a disinfectants. The use of BKC as an algicide is well established through the numerous APVMA registered products for this use pattern. We are seeking to demonstrate its efficacy against marine phytoplankton (algae) species common to Australian prawn farming and safety for the use of this product in the presence of farmed giant black tiger prawns (*Penaeus monodon*) and banana prawns (*Fenneropenaeus merguiensis*).

We are seeking a new MUP to provide guidance to permit bonafide aquaculturists employed by a licenced Australian prawn farm to use BKC as an algicide as part of their integrated biosecurity and disease management program. The proposed MUP will assist farms to control future disease outbreaks by reducing the risk of pathogen entry and spread onto, and impact within farm areas. The ability for the Australian prawn farming industry to remain viable and expand into the future will depend of the ability to strength on-farm biosecurity measures, for which permitted use of BKC as an algicide is considered a requirement.

We are seeking to use peer reviewed, published, and publicly available literature to provide additional information where deemed necessary to satisfy safety, efficacy and trade criteria of BKC for this application.

Gap Analysis

A review of publicly available literature to support the use of benzalkonium chloride (BKC) as an algicide on Australian Prawn farms was performed to assemble the data package and create a MUP application.

We have identified a few subject areas that we consider further data may be required in order to fulfill the data package and application.

These include:

- Data to demonstrate safety of BKC to farmed giant black tiger prawns (*Penaeus monodon*) and banana prawns (*Fenneropenaeus merguiensis*), *including:*
    - Acute (24 hour) and chronic (96 hour)
    - LC50 and NOEL
    - Various prawn sizes (e.g PL15, 10g, 20g)
    - Range of water quality conditions (temperature, pH, salinity)
    - Both lab and field setting

- Data to demonstrate efficacy of BKC to kill phytoplankton (algae)
    - *Heterosigma* sp.; Other algal species.
    - Both lab and field setting

- Data to breakdown of BKC following application and to demonstrate zero environmental

- Data to demonstrate no or minimal BKC residues on harvested prawn product.
    - Further information may need to be sought from Food standards Australia in relation to acceptable levels of BKC residues on food products in Australia.

It is recommended that this report be drafted into a PAA for submission to determine if the APVMA agrees with the conclusions and/or have additional requirements that need to be addressed.

Chemical Properties

Chemistry and manufacture information is considered to be not required for this minor use permit application as benzalkonium chloride is exempt from the requirement for approval (i.e. exempted from the operation of section 15(1)(a)(i) of the Agvet Code), through a formal process involving a gazettal of the active as exempt from approval (https://apvma.gov.au/node/4176). Additionally, there are numerous APVMA approved chemical products, which contain equivalent chemistry to be used under the proposed minor-use permit.

Some chemical properties are listed in the table below and discussed in sections below of this report as considered relevant to the overall assessment of 'Module 8. Efficacy and target animal safety'.

Table 11. Chemical properties of benzalkonium chloride

Common Name	Benzalkonium chloride

Chemical Name:	Quaternary ammonium compounds, alkylbenzyldimethyl, chlorides
Other names:	benzalkonium chloride benzyl-C12-18-alkyldimethylammonium chlorides
CAS Number:	CAS 8001-54-5
Molecular Formula:	$C_{21}H_{38}ClN$
Molecular Weight:	340.00 g mol^{-1}
Purity of the active constituent:	250 g kg^{-1} BENZALKONIUM CHLORIDE as its only active constituent

Sources: (NICNAS, 2016a) (USEPA, 2006).

Environmental Fate

Benzalkonium chloride is a quaternary ammonium compounds (QACs). These chemical products are the major class of cationic surfactants used as the ingredients in fabric softeners, antistatics, disinfectants, biocides, detergents, phase transfer agent and numerous personal care products, such as hair care products (Zhang *et al.*, 2015). Benzalkonium chloride contains at least one hydrophobic hydrocarbon chain linked to a positively charged nitrogen atom, and other alkyl groups which are mostly short-chain substituents such as methyl or benzyl groups (Zhang *et al.*, 2015). Further information can be found in: USEPA, (2006); NICNAS, (2016a); Zhang et al., (2015).

One environmental risk assessment on quaternary ammonium compounds concluded that the pollution sources could be related to two single source polluters, hospitals and laundries, although surface water could not be excluded as an environmental risk (Kreuzinger *et al.*, 2007).

Dissolution, Speciation and Partitioning

Benzalkonium chlorides (BACs) are a type of cationic surfactant and are highly adsorptive to negatively charged surfaces during the wastewater treatment process (Khan, 2016). They can, therefore, enter the aquatic environment via the suspended organic matter in wastewater effluents, and the terrestrial environment through the application of biosolids as a soil amendment for crop production or by the use of reclaimed wastewater for irrigation (Khan, 2016).

Benzalkonium chloride have a soil adsorption coefficient (Koc) range of 640,389 to 6,171,657 indicating that benzalkonium chloride is expected to adsorb to suspended solids and sediment (USEPA, 2006). Benzalkonium chloride is a quaternary salt and will exist entirely in the cation form at pH values of 5 to 9 and therefore volatilization from water surfaces is not expected to be an important fate process (USEPA, 2006). Bioconcentration in freshwater fish of benzalkonium chloride is expected to be low as this compound is strongly absorbed to sediment (USEPA, 2006). Benzalkonium chloride is considered to be stable to microbial degradation (USEPA, 2006).

If applied to natural waters, benzalkonium chlorides are expected to dissociate and release their quaternary ammonium cations and chloride anions. The quaternary ammonium cations can adsorb to clays and natural organic materials, such as humic substances (NICNAS, 2016a). Adsorption coefficient values reported for the cationic surfactants in this group indicate strong adsorption and immobility in soil (NICNAS, 2016a).

Removal rates higher than 90% have been obtained for QACs in waste water treatment plants, however, adsorption usually outcompetes biodegradation (Zhang *et al.*, 2015).

Degradation

Benzalkonium chloride cations are biodegradable (NICNAS, 2016a). The degradation pathway for Benzalkonium chloride cations is considered to occur through N-dealkylation, followed by N-debenzylation and N-demethylation (Zhang *et al.*, 2015).

Benzalkyl quaternary ammonium compounds with longer alkyl chains are relatively more persistent (NICNAS, 2016a). Although benzalkyl chain quaternary ammonium compounds have decreased biodegradability compared to their mono- and di-alkyl chain analogues (Zhang, et al., 2015), they are nevertheless expected to be biodegradable in water (NICNAS, 2016a).

Aerobic Biodegradation

Benzalkonium chloride is considered to be stable to microbial degradation (USEPA, 2006). Benzalkonium chloride, present at 50 ppm, was degraded using an acclimated activated sludge in a 2L laboratory-scale submerged fixed-film reactor; however, the compound inhibited activated sludge before acclimation with cresol and phenol were identified as degradation products (Toxicology Data Network, 2019)

Anaerobic Biodegradation

Benzalkonium chloride, present at 20 and 40 mg L^{-1} was 5 and 13% inhibitory, respectively, towards biogas production using municipal digester solids as the source of anaerobic bacteria (Toxicology Data Network, 2019). High concentrations of QACs would adversely impact the anaerobic digestion process by inhibiting methanogenesis, resulting in methane inhibition and volatile fatty acid (VFA) accumulation (Zhang *et al.*, 2015).

Bioaccumulation

Benzalkonium chloride have low bioaccumulation potential in aquatic organisms (NICNAS, 2016a).

Local Regulatory Activities

Australia

Benzalkonium chloride (BKC) is exempt from the requirement for approval (i.e. exempted from the operation of section 15(1)(a)(i) of the Agvet Code), through a formal process involving a gazettal of the active as exempt from approval (https://apvma.gov.au/node/4176). There are currently five (5) APVMA minor-use permits (MUPs) for the use of BKC as a disinfectants. The use of BKC as an algicide is well described/approved through the numerous APVMA registered products for this use pattern.

The Australian and New Zealand Environment and Conservation Council (ANZECC) Water Quality guidelines consider quaternary ammonium compounds (including Benzalkonium chloride) as cationic surfactants within the detergent groups of chemicals (ANZECC, 2000). However, there is no established protection guidelines on 95% protection levels available. No Australian environmental monitoring data were located for Benzalkonium chloride (NICNAS, 2016a).

The Predicted Environmental Concentration (PEC) in receiving waters is calculated to be 8.5 µg L^{-1} for chemicals with introduction volumes of 100 tonnes or less per annum (NICNAS, 2016a). A recent review of environmental monitoring studies for quaternary ammonium compounds has reported the concentration of these compounds in sewage, sludge, surface water and sediment across a number of countries including China, Taiwan, Poland, Austria, Spain, England and the USA (Zhang *et al.*, 2015). The total concentration of benzalkyl chain quaternary ammonium compounds measured in a Taiwanese river was up to 65 µg L^{-1}. The measured concentration in other regions ranged from mainly below 1 µgL^{-1} (Austria) to 342 µg L^{-1} (Poland) (Zhang *et al.*, 2015).

Overseas Regulatory Activities

United States

In 1947 the first product containing benzalkonium chloride (BAC) was registered with the EPA in the US (Pereira and Tagkopoulos, 2019). The toxicological and environmental effects of benzalkonium chloride has been review in detail in the 2006 US EPA Re-registration Eligibility Decision (RED) report (USEPA, 2006). The US EPA recognized the toxicity of BACs to the aquatic environment and its inhabitants, such as fish, oysters, shrimp, and invertebrates, advising against the release of benzalkonium chloride into lakes, oceans, or other waters (USEPA, 2006). Despite this BACs are commonly found in wastewater treatment plants (Pereira and Tagkopoulos, 2019); Zhang *et al.*, 2015) suggesting that aquatic applications are unlikely a major contributor to the presence of BACs or QACs in the aquatic environment. The chemical properties or BACs and QACs (as described in the environmental fate section above) likely result in a significant run-off effect to application of these chemicals from the myriad of uses in society today.

Aquatic area uses in the US include Golf courses, recreational parks, amusement parks, universities, cemeteries, and greenhouse/nurseries, targeting Slime-forming bacteria, odor causing/staining bacteria, Gram-negative and Gram-positive bacteria, Pseudomonas aeruginosa, pathogenic fungi (Trichophyton mentagrophytes), envelope and non-envelope viruses, mold/mildew, algae (USEPA, 2006).

Europe

Benzalkonium chloride is fully registered in Europe with an estimated total usage of 1,000-10,000 tonnes per annum (EC/List no. 939-350-2). The chemical is used in washing & cleaning products, air care products, polishes and waxes, biocides (e.g. disinfectants, pest control products), cosmetics and personal care products and lubricants and greases.

In October 2012 the Standing Committee on the Food Chain and Animal Health (SCoFCAH) endorsed Guidelines on measures to be taken as regards the presence of Benzalkonium chloride in or on food and feed (EU Reference Laboratories for Residues of Pesticides, 2016). These guidelines contained an agreed temporary enforcement level of 0.5 mg kg^{-1} for food and feed. A flat MRL level of 0.1 mg/kg was established in 2014 for benzalkonium chloride in all food products (Reg. 1119/2014/EU). A revision of this MRLs is planned to take place by 31 December 2019 and if necessary, they will be amended modified based on newer residue data. Before the agreement on temporary MRLs (0.5 mg kg^{-1}) in 2012 and the implementation of specific MRLs in 2014 (0.1 mg kg^{-1}), the default MRL of 0.01 mg kg^{-1} laid down in Reg. 396/2005/EC used to apply (EU Reference Laboratories for Residues of Pesticides, 2016).

Efficacy and target animal safety

Contents

We are seeking to use peer reviewed, published, and publicly available literature to provide any necessary additional information to satisfy the efficacy and target animal safety criteria for the proposed benzalkonium chloride (BKC) minor-use permit.

Additionally, we are seeking draw upon the information provided alongside the current APVMA registered benzalkonium chloride (BKC) products and current APVMA minor use permits, to support this proposed MUP application.

This data package is formatted as per APVMA guidelines as best possible and details a collated brief summary of relevant data and provision of links and further resources to the extensive literature that supports the efficacy claims made in this proposed minor use permit.

In order to limit duplication of already existing data we have endeavoured to keep this report concise. Where additional data is required, this can be obtained and made available for APVMA to consider.

Data summary

The proposed minor-use permit does involve the direct contact of benzalkonium chloride (BKC) products to the pond environment containing farmed giant black tiger prawns (*Penaeus monodon*) and banana prawns (*Fenneropenaeus merguiensis*).

Therefore, we consider:

- *Target animal:* Phytoplankton (microalgae) of the marine environment, which we are seeking demonstrate algicidal efficacy.

- *Non-target animal*: Farmed giant black tiger prawns (*Penaeus monodon*) and banana prawns (*Fenneropenaeus merguiensis*), which we are directly exposing to treatment and general marine organisms.

Giant black tiger prawns (*Penaeus monodon*) and banana prawns (*Fenneropenaeus merguiensis*) are farmed in marine and estuarine water. Australian prawn farms operate in both indoor/outdoor tank-based systems and outdoor earthen/lined pond-based systems which may be exchange water via partial to complete recirculation or single directional flow through. There are a range of different system types that vary in their design and operation. Majority of Australian prawn farms used marine or estuarine water from ocean or river sources for their systems and discharge effluent water back natural waterways.

The environmental source water naturally contains a whole host of microorganisms. Some of these microorganisms can be harmful to farmed Giant black tiger prawns (*Penaeus monodon*) and banana prawns (*Fenneropenaeus merguiensis*) directly or indirectly through acting as vectors for harmful pathogens, therefore posing a biosecurity risk to the farms. A range of physical and chemical methods can be used to eliminate the phytoplankton. Benzalkonium chloride has widespread applications due to their broad spectrum antimicrobial properties (Pereira and Tagkopoulos, 2019).

Pharmacological data/studies

Summary

Benzalkonium chlorides (BACs), also known as alkyl dimethyl benzyl ammonium chlorides, alkyl dimethyl (phenylmethyl) quaternary ammonium chlorides, ammonium alkyl dimethyl (phenylmethyl) chlorides or ammonium alkyl dimethyl benzyl chlorides, are a class of quaternary ammonium compounds (QACs) (Pereira and Tagkopoulos, 2019).

Toxicity of benzalkonium chloride to aquatic organisms is not due to bioaccumulation but due to disruptions to the membrane surface by alkyl chains of cationic surfactants (NICNAS, 2016b). This results in a loss of membrane integrity, resulting in toxicity (NICNAS, 2016b). Laboratory derived toxicity values are generally many times lower than actual field toxicity (NICNAS, 2016b).

Efficacy studies

Summary

Benzalkonium chloride is a demonstrated as algicide product to control phytoplankton. We are seeking to utilise the algicidal properties of this product in Australian prawn farms to assist farm biosecurity and disease management. We have collated the publicly available efficacy data to support the efficacy claims listed on the proposed minor-use permit (and below).

Laboratory model efficacy studies

See throughout the document.

Target animal efficacy studies

The efficacy of benzalkonium chloride as an algicide for a range of phytoplankton species is demonstrated in Table 12.

Table 12. Studies demonstrating efficacy of benzalkonium chloride to the target animals (phytoplankton).

Study Type	Species	BKC concentration	End Point	Effect	pH	Temp (˚C)	Additional variables	Reference
Lab	*Heterosigma akashiwo*	0.1 mg L^{-1}	24 hours	LC-50	7.98-8.28	28 ±1	Marine water. Cultured with 40,000 cells mL^{-1}	(Piyatiratitivorakul, Lirdwitayaprasit and Thooithaisong, 2002)
Lab	*Chattonella marina*	0.3 mg L^{-1}	24 hours	LC-50	7.98-8.28	28 ±1	Marine water. Cultured with 40,000 cells per^{-1}	(Piyatiratitivorakul, Lirdwitayaprasit and Thooithaisong, 2002)
Lab	*Heterosigma akashiwo*	1.3 mg L^{-1}	3 hours	100% cells ruptured	7.8-8.2	24.5-28.5	Marine water (33-36 ppt). Cultured with ~150,000 cells mL^{-1}	(Mann, 2017)
Lab	*Heterosigma akashiwo*	0.5 mg L^{-1}	0.5 hours	0% cells ruptured	7.8-8.2	24.5-28.5	Culture medium	(Mann, 2017)
Lab	*Heterosigma akashiwo*	0.5 mg L^{-1}	24 hours	100% cells ruptured	7.8-8.2	24.5-28.5	Culture medium	(Mann, 2017)
Lab	*Heterosigma akashiwo*	1 mg L^{-1}	45 minutes	~50% cells ruptured	7.8-8.2	24.5-28.5	Culture medium	(Mann, 2017)
Lab	*Heterosigma akashiwo*	0.12 mg L^{-1}	24 hours	LC-50	n/a	28	Marine water (30 ppt).	(Thuithaisong, 1998)
Lab	*Chattonella marina*	0.17 mg L^{-1}	24 hours	LC-50	n/a	28	Marine water (30 ppt)	(Thuithaisong, 1998)
Lab	*Chlorella pyrenoidosa*	0.67 mg L^{-1}	96 hours	EC-50	n/a	n/a	n/a	(NICNAS, 2016b)
Lab	*Chaetoceros gracilis*	>120 µg L^{-1}	48 hours	EC-50	n/a	n/a	n/a	(Pérez, Fernández and Beiras, 2009)
Lab	*Chaetoceros gracilis*	106.0 µg L^{-1}	72 hours	EC-50	n/a	n/a	n/a	(Pérez, Fernández and Beiras, 2009)
Lab	*Chaetoceros gracilis*	87.3 µg L^{-1}	96 hours	EC-50	n/a	n/a	n/a	(Pérez, Fernández and Beiras, 2009)
Lab	*Chaetoceros gracilis*	73.9 µg/L	120 hours	EC-50	n/a	n/a	n/a	(Pérez, Fernández and Beiras, 2009)
Lab	*Isochrysis galbana*	111.5 µg L^{-1}	48 hours	EC-50	n/a	n/a	n/a	(Pérez, Fernández and Beiras, 2009)
Lab	*Isochrysis galbana*	75.2 µg/L	72 hours	EC-50	n/a	n/a	n/a	(Pérez, Fernández and Beiras, 2009)
Lab	*Isochrysis galbana*	66.4 µg L^{-1}	96 hours	EC-50	n/a	n/a	n/a	(Pérez, Fernández and Beiras, 2009)

Lab	*Isochrysis galbana*	43.7 µg L^{-1}	120 hours	EC-50	n/a	n/a	n/a	(Pérez, Fernández and Beiras, 2009)
Lab	*Pseudokirchneriella subcapitata*	0.255 mgL^{-1}	72 hours	EC-50 (growth inhibition)	n/a	n/a	n/a	(Elersek, Ženko and Filipič, 2018)
Field	phytoplankton	1-2 mgL^{-1}	n/a	Reduce densities	n/a	n/a	With shrimp present. Mortality not discussed	(Browdy *et al.*, 2001)

Dose determination studies

Further data required

Resistance

No data was found on resistance of phytoplankton to benzalkonium chloride.

The use of BKC has been suggested to promote antibiotic resistance (Kim. *et al*., 2018)

Dose confirmation studies

See throughout the document.

Confirmatory clinical/field studies

See throughout the document.

Palatability studies

Considered not relevant. European Union guidelines detail a flat MRL level of 0.1 mg/kg established in 2014 for benzalkonium chloride in all food products (EU Reference Laboratories for Residues of Pesticides, 2016). This would unlikely be exceeded with the proposed MUP application.

Non-target animal safety studies

Summary

Exposure of Benzalkonium chloride residuals and metabolites are considered potentially harmful to non-target organisms. As such the proposed minor-use permit will need to include conditions in place to reduce the likelihood of exposure of non-target animals in receiving environmental waterways to chemical residues.

Further research may be required to demonstrate that the proposed use pattern does not result in any detectable levels of BKC, beyond background environmental levels. Current farm practice to remove sludge following harvest may reduce the likelihood to BKC and related metabolites entering the natural waterway, given a significant proportion binds to sediment and organic material (see section above). Ensuring treated water is discharged through a settlement system may further reduce the risk of environmental discharge and exposure to non-target organisms.

Given the farmed prawns (*Penaeus* sp.) appear sensitive to low levels of BKC, it is unlikely that significant impact to non-target environmental organisms would occur at the determined safe levels on the proposed MUP. Further data is required for this determination.

Margin of safety studies

The Australian and New Zealand Environment and Conservation Council (ANZECC) Water Quality guidelines consider quaternary ammonium compounds (including Benzalkonium chloride) as cationic surfactants within the detergent groups of chemicals (ANZECC, 2000). However, there is no established

protection guidelines on 95% protection levels available. No Australian environmental monitoring data were located for Benzalkonium chloride (NICNAS, 2016a). The Predicted Environmental Concentration (PEC) in receiving waters is calculated to **be 8.5 µg/L for** chemicals with introduction volumes of 100 tonnes or less per annum (NICNAS, 2016a).

Dose rate

Still to be determined. Further data required.

Duration of treatment related studies

See throughout the document.

Compatibility studies

See throughout the document.

Effects on hides and fleeces

Not relevant to this application.

Accidental administration or exposure to non-target animals

Data in relation to the margin of safety to non-target marine water animals, in the event of accidental exposure are presented in Table 14.

Table 13. Studies demonstration the margin of safety for exposure of non-target culture animals to benzalkonium chloride.

Study Type	Species	Life stage	BKC concentration	End Point	Effect	pH	Temp (°C)	Additional variables	Reference
Lab	Giant tiger prawn *(Penaeus monodon)*	PL-30	0.2 mg L^{-1}	24 hours	LC-50	7.98-8.28	28 ±1	Marine water. Cultured with 40,000 cells mL^{-1} of *Heterosigma akashiwo*	(Piyatiratitivorakul, Lirdwitayaprasit, Thooithaisong, 2002)
Lab	Giant tiger prawn *(Penaeus monodon)*	PL-30	0.2 mg L^{-1}	24 hours	LC-50	7.98-8.28	28 ±1	Marine water. Cultured with *Chattonella marina* 40,000 cells mL^{-1}	(Piyatiratitivorakul, Lirdwitayaprasit and Thooithaisong, 2002)
Lab	Giant tiger prawn *(Penaeus monodon)*	*PL-30*	0.16 mg L^{-1}	24 hours	LC-50	n/a	28	Marine water (30 ppt).	(Thuithaisong, 1998)
Lab	Giant tiger prawn *(Penaeus monodon)*	*Juvenile (12.1g)*	1.3 mg L^{-1}	3 hours	0% mortality	7.8-8.2	24.5-28.5	Marine water (33-36ppt) Cultured with *Heterosigma akashiwo* ~150,000 cells mL^{-1}.	(Mann, 2017)

Table 14. Studies demonstration the margin of safety for exposure of non-target marine water animals to benzalkonium chloride.

Study Type	Species	Life-stage	BKC concentration	End Point	Effect	pH	Temp (C)	Additional variables	Reference
Lab	Japanese oyster (*Crassostrea gigas*)	embryo	0.138 mgL^{-1}	24 hours	EC50	n/a	24	Marine water (30 ppt)	His et al., 1996 In: (Graslund and Bengtsson, 2001)
Lab	Diatoms (*Melosira nummuloides, Amphora coffeaeformis, Nitzschia incrustans, Navicula hanseni, Cylindrotheca closterium, Achnanthes sp., Opephora sp., Navicula sp., Amphora sp.*)	n/a	10 mgL^{-1}	7 days	100% Non-viable	n/a	n/a	n/a	(Beveridge *et al.*, 1998)
Lab	*Artemia franciscana*	24 hours	0.033 mgL^{-1}	24 hours	LC-50	8.6	25	Salinity 35 ppt	(Bartolomé and Sánchez-Fortún, 2005)
Lab	*Artemia franciscana*	48 hours	0.009 mgL^{-1}	24 hours	LC-50	8.6	25	Salinity 35 ppt	(Bartolomé and Sánchez-Fortún, 2005)
Lab	*Artemia franciscana*	72 hours	<0.001 mgL^{-1}	24 hours	LC-50	8.6	25	Salinity 35 ppt	(Bartolomé and Sánchez-Fortún, 2005)
n/a	Eastern oyster (Crassostrea virginica)	embryo	0.055 mgL^{-1}	48 hours	LC-50	n/a	n/a	n/a	(Toxicology Data Network, 2019)
n/a	Copepod (Nit*ocra spinipes*)	adult	0.9 mgL^{-1}	96 hours	LC-50	7.8	n/a	Salinity 7 ppt, alkalinity 75 mgL^{-1} CaCO3	(Toxicology Data Network, 2019)
n/a	Mysis shrimp (*Mysidopsis bahia*)	n/a	0.092 mgL^{-1}	96 hours	LC-50	n/a	n/a	n/a	Toxicology Data Network, 2019)

There is extensive data on the toxicity of BKC to freshwater organisms available. However, it was considered less relevant for this proposed use as these animals are unlikely to be exposure given the proposed use pattern.

Effects on taste or produce (organoleptic effects)

No MRL information could be found from Australia.

European Union guidelines detail a flat MRL level of 0.1 mg/kg established in 2014 for benzalkonium chloride in all food products (EU Reference Laboratories for Residues of Pesticides, 2016). This would unlikely be exceeded with the proposed MUP application. However, additional testing data may be required.

Other studies or data

In vivo or in vitro bioequivalence studies

See throughout the document.

Clinical case studies

Limited information available.

Scientific references or extrapolated scientific argument

See throughout the document.

Topical studies, inhalation studies, tissue irritation studies

Considered not relevant.

Reproductive function studies

Considered not relevant.

Minimum inhibitory concentration studies

Considered less relevant to the proposed MUP as we are seeking to demonstrate efficacy of algicidal activity not bactericidal efficacy.

Table 15. Minimum Inhibitory Concentration (MIC) of various microorganisms of to benzalkonium chloride. (Toxicology Data Network, 2019).

Microorganism	**MIC (ug/mL)**
Aerobacter aerogenes	64
Clostridium histolyticum	5
Clostridium oedematiens	5
Clostridium tetani	5
Clostridium welchii	5
Eschericia coli	16
Pneumococcus II	5
Proteus vulgaris	64
Pseudomonas aeruginosa	30
Salmonella enteritidis	30
Salmonella paratyphi	16
Salmonella typhosa	4
Shigella dysenteriae	2
Staphylococcus aureus	1.25
Streptococcus pyrogenes	1.25
Vibrio cholerae	2

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Appendix 7: Copper sulphate Data review and gap analysis

Abbreviations

MUP – Minor-use permit
LC50 – Lethal concentration that kills 50 percent of a test population
LC99 – Lethal concentration that kills 99 percent of a test population
EC50 – Effective concentration that causes a biological response in 50 percent of a test population
EC99 – Effective concentration that causes a biological response in 99 percent of a test population
TLm - median tolerance limit

Definitions

Algicide - a biocide used for killing and preventing the growth of algae (phytoplankton)

Proposed Permit Details

Product to be used:
COPSUL4400
Containing: 250 g kg^{-1} available copper as COPPER SULPHATE PENTAHYDRATE as their only active constituent, and other equivalent APVMA registered products.
Directions for Use:

1. Algicide to kill carrier phytoplankton (microalgae) in semi-closed grow-out ponds and effluent channels adjacent to ponds when White Spot Syndrome Virus (WSSV) is suspected in a population of farmed giant black tiger prawns (*Penaeus monodon*) and banana prawns (*Fenneropenaeus merguiensis*) on licensed Australian prawn farms.
2. Algicide to kill WSSV carrier phytoplankton (microalgae) in influent water, prior to spelling, then for use or discharge in prawn production ponds in influent and effluent waters of farmed giant black tiger prawns (*Penaeus monodon*) and banana prawns (*Fenneropenaeus merguiensis*) on licensed Australian prawn farms.

Dose rate: Further details to be determined

Restraint

DO NOT USE in environmental waters. Only for use in waters where the release of water can be controlled, following treatment.

The COPPER SULPHATE PENTAHYDRATE products are not to be used in conjunction or mixed with any other chemical.

For use as per directions only.

Critical Use Comments
Observe all label safety directions relevant to handling of COPPER SULPHATE PENTAHYDRATE. Ensure all personnel handing the product are wearing correct PPE (refer to product label and MSDS).

DO NOT discharge treated water unless free copper meet ANZECC water quality guidelines for ecosystem protection (below 1.3 µg Cu L^{-1}).

Treatment:

Calculate the volume of concentrated COPPER SULPHATE PENTAHYDRATE required (See below)

Calculating the COPPER SULPHATE PENTAHYDRATE requirement (Target Concentration):
The following equations can be used to assist in calculating the quantity of COPPER SULPHATE PENTAHYDRATE required to achieve a target concentration of free copper in a volume of water, to ensure attaining the target concentrations.

a. **COPPER SULPHATE (kg)**

$$\boldsymbol{Required\ Copper\ sulphate\ (kg) = \frac{1mg\ L-1}{250g\ -1\ \times 1000} \times volume\ of\ water\ (L)}$$

For example: To obtain a target concentration of 0.4 mg L^{-1} of free copper in a 1ML or 1,000,000L body of water, we would require 1.6 Kg of COPPER SULPHATE (250 g Kg^{-1})

$$\boldsymbol{Required\ Copper\ sulphate\ (kg) = \frac{1mg\ L-1}{250g\ kg-1\times 1000} \times 1000000} = 4\ kg$$

Post treatment

Treated water must be retained to ensure complete breakdown of copper residue, prior to release or use of water on farm.

Treated water must be monitored for free copper concentration to ensure that the concentration of free copper in treated water meets ANZECC water quality guidelines for ecosystem protection (below 1.3 µg Cu L^{-1}), prior to discharge or use on farm.

Withholding Period:

Meat (prawns) – Nil, when used as directed.

Any prawn product harvested must be below the Food Standards Australia New Zealand (FSANZ) 90th percentile generally expected levels of copper expected in crustacea of 20 mg kg^{-1}.

Water – Concentration of total residual free copper in treated water must meet ANZECC water quality guidelines for ecosystem protection (below 1.3 µg Cu L^{-1}), prior to discharge or use on farm.

Jurisdiction:

All states

Executive Summary

In late 2016, an exotic viral disease, White Spot Disease (WSD), was diagnosed in multiple Australian prawn farms on the Logan River catchment, Queensland. The subsequent disease response program resulted in the complete eradication of the seven infected farms (approximately 25% of the Australian prawn farming industry). Following this exotic disease outbreak an intensive review of farm-level biosecurity practices on Australian prawn farms was performed to safeguard against future endemic and exotic disease incursions. The prawn farming industry are now taking a proactive step to improve the disease preventative measures on farm to reduce the likelihood and impact of disease entry, spread and exit from their farms. In order to improve on-farm biosecurity practices the industry is seeking APVMA permits for several chemical products. Trichlorfon, Hydrogen Peroxide, Calcium and Sodium Hypochlorite, Benzalkonium Chloride and Copper Sulphate have been highlighted as the most important.

Biosecurity measures offer the prospect of safeguarding against future disease incursions into farms. Extensive investment is currently being allocated into auditing on-farm biosecurity measures to identify weaknesses in bio-exclusion and biocontainment that could allow potential pathogens to enter and spread on farm. Audits being performed consider major routes of transmission, assesses the nature and validity of perceived hazards, rank risks, and provide recommendations for managing identified risks, with the aim of reducing overall risk of pathogen entry and spread within the farm.

Biosecurity audits of Australian prawn farms identified a major risk pathway for white spot syndrome virus (WSSV) pathogens to enter prawn farms through incoming source water, which is brought onto farms from local rivers and waterways to fill semi-closed ponds for stocking prawn larvae (Landos, 2017) (Landos, 2017) (Diggles, 2017). The ability for phytoplankton (microalgae) and zooplankton (rotifers) to act as vehicles for

WSSV dispersion in water, and thus onto a prawn farm, has been demonstrated (Esparza-Leal et al., 2009). Microalgae such as *Isochrysis galbana*, *Skeletonema costatum*, *Chlorella sp.*, *Scrippsiella trochoidea*, *Dunaliella salina* have been demonstrated abilities to carry and transmit WSSV (B. Liu et al., 2011). Additionally, some phytoplankton species (e.g. *Chattonella marina* and *Heterosigma akashiwo*) can themselves be harmful to farmed prawn species and the ability to control these organisms can offer further health benefits to cultured stock (Piyatiratitivorakul et al., 2002).

To strengthen Australian prawn farm biosecurity, we are seeking a minor-use permit for the use of copper sulphate (APVMA registered products containing: 250 g/kg copper as copper sulphate pentahydrate, or equivalent) by bonafide aquaculturists employed by a licensed Australian prawn farm to assist control of future disease outbreaks and allow treating incoming water to control potential algal vectors of disease. The ability for this industry to remain viable and grow into the future will depend of the ability to strengthen on-farm biosecurity, which permitted minor-use of copper sulphate for water treatment and control of future disease outbreaks is considered essential.

Copper sulphate is exempt from the requirement for approval (i.e. exempted from the operation of section 15(1)(a)(i) of the Agvet Code), through a formal process involving a gazettal of the active as exempt from approval (https://apvma.gov.au/node/4176). There are currently twelve (12) APVMA minor-use permit (MUP) for the use of copper sulphate and similar products. These permits are primarily for the control of fungal disease in the horticultural sector. However, there are a few quite relevant to the current proposed MUP application within aquatic systems directly. These include PER88742 for the use of copper sulphate (250g/Kg) as an algicide to control cyanobacteria in freshwater reservoir systems; and PER14748 for the use of copper sulphate (250g/Kg) to control a range of pathogens and diseases in freshwater aquaculture systems. The proposed chemical product is unregistered (CAS 7758-99-8).

We are seeking a new MUP to provide guidance to permit bonafide aquaculturists employed by a licenced Australian prawn farm to use copper sulphate as an algicide as part of their integrated biosecurity and disease management program. The proposed MUP will assist farms to control future disease outbreaks by reducing the risk of pathogen entry and spread onto, and impact within, farm areas. The ability for the Australian prawn farming industry to remain viable and expand into the future will depend of the ability to strength on-farm biosecurity measures, for which permitted use of copper sulphate as an algicide is considered a requirement.

We are seeking to use peer reviewed, published, and publicly available literature to provide additional information where deemed necessary to satisfy safety, efficacy and trade criteria of copper sulphate for this application.

Gap Analysis

A review of publicly available literature to support the use of copper sulphate an algicide on Australian Prawn farms was performed to assemble the data package and create a MUP application.

We have identified a few subject areas that we consider further data may be required in order to fulfill the data package and application.

These include:

- Data to demonstrate safety of copper sulphate on farmed giant black tiger prawns (*Penaeus monodon*) and banana prawns (*Fenneropenaeus merguiensis*), *including:*
    - Acute (24 hour) and chronic (96 hour)
    - LC50 and NOEL
    - Various prawn sizes (e.g PL15, 10g, 20g)
    - Range of water quality conditions (temperature, pH, salinity)
    - Both lab and field setting

- Data to demonstrate efficacy of copper sulphate to kill phytoplankton (algae) typical of Australian prawn farms - field setting.

- Data to breakdown of copper sulphate following application and to demonstrate nil environmental discharge.

- Research into methods to neutralise or absorb copper sulphate and metabolites/derivatives from treated pond and sediment

- Validation data for impurities may be required for each listed chemical product, where not already held by APVMA.

It is recommended that this report be drafted into a PAA for submission to determine if the APVMA agrees with the conclusions and/or have additional requirements that need to be addressed.

Chemical Properties

Chemistry and manufacture information is considered to be not required for this minor use permit application as copper sulphate is exempt from the requirement for approval (i.e. exempted from the operation of section 15(1)(a)(i) of the Agvet Code), through a formal process involving a gazettal of the active as exempt from approval (https://apvma.gov.au/node/4176). Additionally, there are numerous APVMA approved chemical products, which contain equivalent chemistry to be used under the proposed minor-use permit.

Some chemical properties are listed in the table below and discussed in sections below of this report as considered relevant to the overall assessment of 'Module 8. Efficacy and target animal safety'.

Common Name	Copper Sulphate
Chemical Name:	Copper(II) sulphate pentahydrate
CAS	7758-99-8
Molecular Formula:	$CuSO_4.5H_2O$ or $CuSO_4 \cdot 5H_2O$ or $CuH_{10}O_9S$
Molecular Weight:	249.69 g mol^{-1}
Form:	Large, blue or ultramarine, triclinic crystals or blue granules or light-blue powder
Melting point:	Decomposition above 110°C
Density/Specific Gravity:	2.286 @ 15.6 deg C/4 deg C
pH:	pH of 0.2 molar aq soln: 4.0
Solubilities:	31.6 g/100 cc water @ 0 deg C, 203.3 g/100 cc @ 100 deg C In water: 148 g/kg @ 0 deg C; 230.5 gkg @ 25 deg C; 335 g/kg @ 50 deg C; 736 g/kg @ 100 deg C
Stability	Indefinite when kept dry; stable to heat, cold, & light.

Source: (Toxicology Data Network., 2019)

Analytical methods for copper sulphate pentahydrate are detailed in the report "Standard – Copper sulphate pentahydrate" published by the APVMA (APVMA, 2016).

The APVMA guidelines on validation of analytical methods state that 'Analytical methods described in CIPAC handbooks and AOAC International Manual, and in recognized pharmacopoeias [BP, BP (Vet), Ph Eur and USP] for a particular active constituent or formulation are regarded as validated and do not require revalidation (APVMA, 2016). This includes copper sulphate pentahydrate.

The suitability of these methods must be verified under actual conditions of use i.e., the selectivity and accuracy of the method should be demonstrated for the published method when applied to the relevant sample matrix and laboratory conditions (APVMA, 2016).

Local Regulatory Activities

Australia

Copper sulphate is exempt from the requirement for approval (i.e. exempted from the operation of section 15(1)(a)(i) of the Agvet Code), through a formal process involving a gazettal of the active as exempt from approval (https://apvma.gov.au/node/4176). There are currently twelve (12) APVMA minor-use permit (MUP) for the use of copper sulphate and similar products. These permits are primarily for the control of fungal disease in the horticultural sector. However, there are a few quite relevant to the current proposed MUP application within aquatic systems directly. These include PER88742 for the use of copper sulphate (250g/Kg) as an algicide to control cyanobacteria in freshwater reservoir systems; and PER14748 for the use of copper sulphate (250g/Kg) to control a range of pathogens and diseases in freshwater aquaculture systems. The proposed chemical product is unregistered (CAS 7758-99-8).

Background copper levels of Australian marine waters is considered 0.003 – 0.38 µg/L and 0.06-1.3 µg/L in estuarine water (ANZECC, 2000). No data could be found on the time required for active copper to fall below the ANZECC guidelines for copper in marine waters.

Overseas Regulatory Activities

United States

Copper sulphate is approved by the EPA as a general use material (algaecide, fungicide, insecticide, water treatment, molluscicide); it is not a restricted-use pesticide. Further details on label use patterns can be found in (United States Environmental Protection Agency, 2012). Copper sulphate is not approved by the FDA for use on food fish, with regulatory action deferred pending ongoing research. Additional information can be found in (United States Environmental Protection Agency, 2009)

Europe

Detailed information on the re-registration status of copper sulphate can be found in (Arena et al., 2018).

Toxicology

Brief summary of copper sulphate toxicological information can be found in table 1 below.

Table 16 Detailed descriptions of copper sulphate of the ANZECC & ARMCANZ (2000) guidelines

Acceptable daily intakes	0.2mg/kg/bw/d (page 26) (APVMA, 2017)
Acute reference doses	Acute Oral LD50 (mg/kg): M= 790; F= 450; Tox Cat II 43396201 (page 29) (USEPA, 2009) Acute dermal LD50 (mg/kg): >2000 Tox Cat IV 43452201 (page 29) (USEPA, 2009) Acute inhalation (mg/L): none available Primary Eye irritation: Severe eye irritation day 1 to day 21 Tox Cat I 43396201 (page 29) (USEPA, 2009) Dermal irritation: Non-irritating Tox Cat IV 43396201 (page 29) (USEPA, 2009)
Poison scheduling	Schedule 6

First aid instructions	If poisoning occurs, contact a doctor or Poisons Information Centre. Phone Australia 13 11 26, New Zealand 0800 764 766. If swallowed, do NOT induce vomiting. * If skin contact occurs, remove contaminated clothing and wash skin thoroughly. If in eyes, hold eyes open, flood with water for at least 15 minutes and see a doctor.
Safety directions	APVMA FAISD Hazard codes:129 133 161 162 163 164 210 211 220 221 222 279 283 290 312 351 (APVMA, 2019)
Warning statements	Not available (APVMA, 2019)
Other limitations on use (for example, restraints, restrictions).	Persons who can use the product under this permit: Bonafide aquaculturists employed by a licensed Australian prawn farm.

Acute toxicity studies

Shrimp/ prawns/ lobsters/ crustacea

- *Penaeus monodon*
  - 24 hours TLm (median tolerance limit), post larvae: 436 mg L^{-1}
  - 96hr LC50, post larvae and juveniles range: 0.66mg/L to 7.73 mg L^{-1} (Jiann-chu Chen et al., 2001; Rajkumar et al., 2011)
- *Callianassa australiensis*
  - 96h LC50, 35ppt seawater at 19°C: 1030 µg Cu L^{-1} (Ahsanullah et al., 1981)
  - 168h LC50, 35ppt seawater at 19 °C: 340 µg Cu L^{-1} (Ahsanullah et al., 1981)
  - 240h LC50, 35ppt seawater at 19°C: 220 µg Cu L^{-1} (Ahsanullah et al., 1981)
- *Crangon crangon*
  - 48h LC50 at 15°C: 2950 µg Cu L^{-1} (Portmann, 1971)
  - 96h LC50 at 15°C: 1900 µg Cu L^{-1} (Portmann, 1971)
- Homarus americanus
  - 22h LC50, 30ppt seawater at 13°C: 1000 µg Cu L^{-1} (McLeese, 1974)
  - 70h LC50 30ppt seawater at 5°C: 560 µg Cu L^{-1} (McLeese, 1974)
  - 105h LC50 30ppt seawater at 13°C: 80 µg Cu L^{-1} (McLeese, 1974)
- *Litopenaeus vannamei*
  - 24h LC50 in juveniles: 47.23 mg/L (Frías-espericueta et al., 2008)
  - 48h LC50 in juveniles: 39.15 mg/L (Frías-espericueta et al., 2008)
  - 72h LC50 in juveniles: 39.15 mg/L (Frías-espericueta et al., 2008)
  - 96h LC50 in juveniles: 35.12 mg/L (Frías-espericueta et al., 2008)
- *Penaeus californiensis*
  - 96h LC50 after 1h exposure to CuSO4 at 27 °C, 24ppt salinity: 250 mg/L (Hanks, 1976)
- *Penaeus japonicus*
  - 48h LC50 for nauplii at 25 °C: 1 µg Cu L^{-1} (Barnbang et al., 1995)
  - 48h LC50 for zoeae at 25°C: 3-46 µg Cu L^{-1} (Barnbang et al., 1995)
  - 48h LC50 37ppt seawater at 25°C: 2500 µg Cu L^{-1} L (Barnbang et al., 1995)
  - 48h LC50 17ppt seawater at 25°C: 1700 µg Cu L^{-1} (Barnbang et al., 1995)
  - 96h LC50 post-larvae in 37ppt seawater at 25°C: 2050 µg Cu L^{-1} (Barnbang et al., 1995)
  - 96h LC50 post-larvae in dilute seawater 17ppt at 25°C: 1200 µg Cu L^{-1} (Barnbang et al., 1995)
- *Penaeus merguiensis*
  - 96h LC50 36ppt seawater at 35°C: 350 µg Cu L^{-1} (Denton et al., 1982)
  - 96h LC50 20ppt seawater at 35°C: 210 µg Cu L^{-1} (Denton et al., 1982)
  - 96h LC50 36ppt seawater at 30°C: 900 µg Cu L^{-1} (Denton et al., 1982)
  - 96h LC50 36ppt seawater at 20°C: 6100 µg Cu L^{-1} (Denton et al., 1982)
  - 96h LC50 20ppt seawater at 20°C: 720 µg Cu L^{-1} (Denton et al., 1982)

- *Penaeus monodon*
    - 96h LC50 in juveniles in 15ppt seawater: 3.13 mg L^{-1} (Jiann-chu Chen et al., 2001)
    - 96h LC50 in juveniles in 25ppt seawater: 7.73 mg L^{-1} (Jiann-chu Chen et al., 2001)
    - 30d EC50 in juveniles: 2.82 mg L^{-1} (Jiann-chu Chen et al., 2001)
    - 60d EC50 in juveniles: 1.89 mg L^{-1} (Jiann-chu Chen et al., 2001)
    - 96h LC50 in post larvae in 28ppt seawater at 28°C: 0.66 mg L^{-1} (Rajkumar et al., 2011)

Fish

- *Pimephales promelas*
    - 6h LC50: 0.90 mg L^{-1} (Closson et al., 2014)
    - 24h LC50: 0.60 mg L^{-1} (Closson et al., 2014)
    - 48h LC50: 0.44 mg L^{-1} (Closson et al., 2014)
    - 72h LC50: 0.34 mg L^{-1} (Closson et al., 2014)
    - 96h LC50: 0.28 mg L^{-1} (Closson et al., 2014)
    - 6h EC50: 0.71 mg L^{-1} (Closson et al., 2014)
    - 24h EC50: 0.52 mg L^{-1} (Closson et al., 2014)
    - 48h EC50: 0.35 mg L^{-1} (Closson et al., 2014)
    - 72h EC50: 0.25 mg L^{-1} (Closson et al., 2014)
    - 96h EC50: 0.20 mg L^{-1} (Closson et al., 2014)
- *Salvelinus fontinalis*
    - 8h LC50 in smaller fish: >1.00 mg L^{-1} (Closson et al., 2014)
    - 24h LC50 in smaller fish: 0.71 mg L^{-1} (Closson et al., 2014)
    - 48h LC50 in smaller fish: 0.39 mg L^{-1} (Closson et al., 2014)
    - 72h LC50 in smaller fish: 0.36 mg L^{-1} (Closson et al., 2014)
    - 96h LC50 in smaller fish: 0.36 mg L^{-1} (Closson et al., 2014)
    - 8h LC50 in larger fish: 1.15 mg L^{-1} (Closson et al., 2014)
    - 24h LC50 in larger fish: 0.53 mg L^{-1} (Closson et al., 2014)
    - 48h LC50 in larger fish: 0.48 mg L^{-1} (Closson et al., 2014)
    - 72h LC50 in larger fish: 0.48 mg L^{-1} (Closson et al., 2014)
    - 96h LC50 in larger fish: 0.48 mg L^{-1} (Closson et al., 2014)
    - 8h EC50 in smaller fish: 0.58 mg L^{-1} (Closson et al., 2014)
    - 24h EC50 in smaller fish: 0.27 mg L^{-1} (Closson et al., 2014)
    - 48h EC50 in smaller fish: 0.22 mg L^{-1} (Closson et al., 2014)
    - 72h EC50 in smaller fish: 0.21 mg L^{-1} (Closson et al., 2014)
    - 96h EC50 in smaller fish: 0.20 mg L^{-1} (Closson et al., 2014)
    - 8h EC50 in larger fish: 0.37 mg L^{-1} (Closson et al., 2014)
    - 24h EC50 in larger fish: 0.28 mg L^{-1} (Closson et al., 2014)
    - 48h EC50 in larger fish: 0.27 mg L^{-1} (Closson et al., 2014)
    - 72h EC50 in larger fish: 0.26 mg L^{-1} (Closson et al., 2014)
    - 96h EC50 in larger fish: 0.24 mg L^{-1} (Closson et al., 2014)
- *Rutilus rutilus caspicus*
    - 96h LC50: 0.228 mg L^{-1} (Farhangi et al., 2014)
- *Danio rerio*
    - 48h LC50: 15.71–68.69 µg L^{-1} (Bui et al., 2016)
- *Centropomus parallelus*
    - 96h LC50 in juveniles: 1.88 mg L^{-1} (Oliveira et al., 2014)
- *Menidia menidia*
    - LC50 or EC50
        - Larvae: 0.0666 – 0.2165 mg L^{-1} (USEPA, 1984)
- *Trachinotus carolinus*
    - LC50 or EC50
        - 0.360 – 0.510 mg L^{-1} (USEPA, 1984)

Birds and mammals

- Bobwhite
    - Acute oral toxicity: LC50 384 mg kg^{-1} (USEPA, 2009)
    - Acute dietary toxicity: LC50 1892 mg kg^{-1}(USEPA, 2009)
    - Reproductive toxicity: LOAEL: 500 mg kg^{-1} NOAEL 100 mg kg^{-1} (USEPA, 2009)

- Rats:
    - Acute oral toxicity: LD50 790 mg kg^{-1} BW (male); 450 mg kg^{-1} BW (female)

Bees

- Honeybee
    - Acute LD50 >0.1mg/bee (USEPA, 2009)
    - Copper is practically non-toxic to honeybees (USEPA, 2009)

Human

- Acute oral LD50
    - Male: 790 mg kg^{-1} (USEPA, 2009)
    - Female: 450 mg kg^{-1} (USEPA, 2009)
- Acute dermal toxicity LD50
    - >2000 mg kg^{-1} (USEPA, 2009)

Saltwater species LC50 or EC50

- Polychaete worm
    - *Phyllodoce maculate*
        - 0.120 mg kg^{-1} (USEPA, 1984)
    - *Nerels diversicolor*
        - 0.200 – 0.480 mg kg^{-1} (USEPA, 1984)
- Abalone
    - *Hallotis cracherodii*
        - 0.050 mg kg^{-1} (USEPA, 1984)
    - *Hallotis rufescens*
        - 0.065 – 0.114 mg kg^{-1} (USEPA, 1984)
- Mussel
    - *Mytilus edulis* (embryo)
        - 0.0058 mg kg^{-1} (USEPA, 1984)
- Oyster
    - *Crassostrea gigas* (embryo)
        - 0.0053 – 0.0115 mg kg^{-1} (USEPA, 1984)
    - *Crassostrea gigas* (adult)
        - 0.560 mg kg^{-1} (USEPA, 1984)
- Lobster
    - *Homarus americanus* (larvae)
        - 0.048 mg kg^{-1} (USEPA, 1984)
    - *Homarus americanus* (adult)
        - 0.100 mg kg^{-1} (USEPA, 1984)
- Crab
    - *Cancer magister* (larvae)
        - 0.600 mg kg^{-1} (USEPA, 1984)

Directions for safe use

Safety directions have been published by the APVMA in the "FAISD Handbook". The APVMA FAISD Hazard codes for copper sulphate pentahydrate are as followed: 129 133 161 162 163 164 210 211 220 221 222 279 283 290 312 351 (APVMA, 2019).

Lowest-observed adverse effect level

The trigger value for copper in marine water in Australia is 1.3 µg L^{-1} (ANZECC, 2000). The predicted no effect concentration of copper in saltwater is 5.6 µg L^{-1} (Hall et al., 1999).

Acceptable daily intake for humans

The acceptable daily intake for copper for humans is detailed by the APVMA. The acceptable daily intake for copper is 0.2 mg kg^{-1} bw^{-1} d^{-1} (page 26) (APVMA, 2017).

First aid directions

First aid directions have been published by the APVMA in the "FAISD Handbook". The APVMA FAISD first aid directions for copper sulphate pentahydrate are as followed: (APVMA, 2019)

- If poisoning occurs, contact a doctor or Poisons Information Centre. Phone Australia 13 11 26, New Zealand 0800 764 766.
- If swallowed, do NOT induce vomiting. *
- If skin contact occurs, remove contaminated clothing and wash skin thoroughly.
- If in eyes, hold eyes open, flood with water for at least 15 minutes and see a doctor.

Toxicology (poison schedule classification)

Copper sulphate pentahydrate is classified as Poisons Schedule S6.

Metabolism and kinetics

Metabolism and pharmacokinetic studies in target animals

The active component of copper sulphate pentahydrate is copper (II) ions.

Phytoplankton

Copper ions affect phytoplankton by inhibiting photosynthesis, cell division, respiration, ATP production, electron transport and cellular ultrastructure (pages 517 to 518 – see page 518 for conclusion) (Stauber et al., 1987). Additionally, copper can decrease concentrations of glucides, proteins, amino-acids, and chlorophyll in algal cells, and decreased alkaline phosphatase activity (see page 79-80) (Prosnier et al., 2015).

Zooplankton

Copper effects zooplankton such as Daphnia by decreasing fecundity, survival, body length, weight, carbon uptake, mobility, and delays maturation (see page 79-80) (Prosnier et al., 2015).

Prawns

The metabolism and kinetics of copper sulphate in prawns is detailed above

Residues

The effects of copper sulphate residue on human health has been review in detail in the overseas report the 2009 US EPA Re-registration Eligibility Decision (RED) report (page 25) (USEPA, 2009). The applicant considers that data generated under Australian conditions should not be required as the effect the product will be the same as overseas, and there are no residue differences between the active product in Australia and the overseas countries that would be relevant to the residue effect of the product on human health.

Holding of treated copper sulphate treated water for 2 days (estimated time required for over 99% of copper to bind to sediment in Liu 2006's study in catfish ponds (page 159) (R. Liu et al., 2006)) to allow for active copper to sediment and bind to organic matter in the influent water and in ponds prior to use/ discharge. An estimated less than 0.004mg/L of copper would likely be present in water after a two day holding period. The

toxicity of copper to prawns is detailed in Module 3 where the 60 day EC50 of copper to *P.monodon* was reported to be 1.89 mg L^{-1} which is 472.5 times greater than 0.004 mg L^{-1}.

Copper has been shown to accumulate in the hepatopancreas of *Penaeus monodon* (page 228- Vogt et al., 1994) and is likely excreted in faeces (page 230- Vogt et al., 1994). In decapods, as copper concentration increases, the rate of copper excretion increases until a threshold is reached where copper is accumulated in the body (see 3.1.3.1. of (Rainbow, 2002)). One residue study in *Penaeus stylirostris* found that there was no accumulation of copper when the prawns were exposed to 6 treatments of copper sulphate over a 6 week period (page 197) (Williams et al., 1982). Considering the proposed use pattern of this minor use permit does not involve direct exposure of cultured stock to copper sulphate it is not considered that the risk of residue accumulation is negligible.

Pharmacokinetic and residue kinetic studies

Copper can accumulate in the hepatopancreas of *P.monodon* (page 228) and is likely excreted in faeces (page 230) (Vogt et al., 1994). In decapods, as copper concentration increases, the rate of copper excretion increases until a threshold is reached where copper is accumulated in the body (see 3.1.3.1. of (Rainbow, 2002)). The level of exposure the prawns would likely be exposed to may not result in any significant accumulation of copper in their hepatopancreas with the proposed use pattern. One residue study in *Penaeus stylirostris* found that there was no accumulation of copper when the prawns were exposed to 6 treatments of copper sulphate over a 6 week period (page 197) (Williams et al., 1982).

Food-safety (marker residue depletion) trials

Copper sulphate is currently permitted to treat of protozoan and trematode ectoparasitic infections in freshwater finfish in Australia (See PER14748). An acceptable daily intake of 0.2 mg kg^{-1} bw^{-1} d^{-1} has been established by the APVMA (page 26) (APVMA, 2017). One residue study in *Penaeus stylirostris* found that there was no accumulation of copper when the prawns were exposed to 6 treatments of copper sulphate over a 6 week period (page 197) (Williams et al., 1982). The level of exposure the prawns would likely be exposed to may not result in any significant accumulation of copper in their hepatopancreas with the proposed use pattern. It is considered that data to demonstrate marker residue depletion is not required.

MRL

The proposed MRL is 20 mg L^{-1} which is the proposed 90th percentile generally expected levels of copper expected in crustacea reported by Food Standards Australia New Zealand's 2001 report titled "Generally Expected Levels (GELs) for Metal Contaminants" (page 5) (FSANZ, 2001).

Copper testing methods

If testing of prawn tissue is required, residue of copper can be assessed using atomic absorption spectrometry of tissues (page 1) (Barbera et al., 2003).

If testing of water is required, a range of colorimetric photometers can be used to determine free copper levels (e.g. Palintest Copper (coppercol) PHOT.10.AUTO) at concentrations between 0-5mg/L (https://www.palintest.com/wp-content/uploads/2019/04/Phot.10.AUTO-Copper-Coppercol-v3.pdf). Further greater accuracy at lower copper levels flame atomic absorption spectrometry after cloud point extraction. The limit of detection with this method is 0.27 ng L^{-1} (page 219) (J Chen et al., 2001).

Occupational health and safety

This proposed minor use permit involves a use pattern similar to approved permits (See PER14748), and using products whereby APVMA has previous assessed Occupational health and safety risks. It is considered that APVMA hold safety data sheets (SDs) for these registered chemicals and any other pertinent data required to ensure adequate work health and safety for use of this chemical.

Exposure and Risk characterization

1. Occupational exposure to copper sulphate can occur through application and post-application of the chemical. Acute adverse effects resulting from dermal, oral or inhalation exposures are due to the irritating properties of copper, rather than a result of systemic toxicity. No dermal, oral or inhalation endpoints were established to determine any potential systemic toxicity resulting from occupational uses of copper products (USEPA, 2009).
2. The USEPA's 2009 Re-registration Eligibility Decision (RED) assessment of copper sulphate and other copper compounds concluded that there was no evidence of copper or its salts being carcinogenic or posing any other systemic toxicity in animals having normal copper homeostasis (USEPA, 2009). No endpoint was established to quantify any potential risks from exposure to copper (USEPA, 2009).
3. The USEPA's 2009 Re-registration Eligibility Decision (RED) report concluded that there was no evidence that warranted determining any dietary, oral, dermal or inhalation endpoints to quantify sub-chronic and chronic toxicity. Available short-term feeding studies with rats and mice indicate decreased food and water intake with increasing oral concentrations of copper, with irritation of the stomach at higher copper concentrations (USEPA, 2009).
4. Ingestion or consumption of 10-20g of copper sulphate is toxic to humans (Saravu et al., 2007).
5. Acute copper sulphate toxicity by consumption or ingestion can cause gastrointestinal, cardiovascular, haematological, hepatic, renal, central nervous system, muscular disturbances in humans (Saravu et al., 2007).
6. The average half-life of copper in a healthy individual is approximately 26 days in humans (Saravu et al., 2007).
7. Exposure threshold:
    a. Nausea is reported when 4 mg L^{-1} of copper was consumed orally, and vomiting was noted at 6mg/L (Olivares et al., 2001).

Risk management and workplace information

1. Appropriate personal protective equipment can be used to limit the irritating effects of copper (USEPA, 2009).
2. This application for minor-use permit involves a registered product(s). It is considered that APVMA hold safety data sheets (SDs) for these registered chemicals and any other pertinent data required to ensure adequate work health and safety for use of this chemical.

Environmental

Environmental Fate

Background copper levels of Australian marine waters is considered 0.003 – 0.38 µg/L and 0.06-1.3 µg/L in estuarine water (ANZECC, 2000). No data could be found on the time required for active copper to fall below the ANZECC guidelines for copper in marine waters.

In soil, copper sulphate is partly washed down to lower levels, partly bound by soil components, and partly oxidatively transformed

Copper sulphate can be neutralize with agricultural lime (CaO), crushed limestone (CaCO3), or sodium bicarbonate (NaHCO3) (Toxicology Data Network., 2019). The pH should be adjusted to neutral (pH= 7) (Toxicology Data Network., 2019). The use mechanical dredges or lifts to remove immobilized masses of pollutants and precipitates can reduce environmental contamination (Toxicology Data Network., 2019).

Copper sulphate is partly washed down to lower soil levels by water percolating through the ground, called groundwater; partly bound to soil components; and partly changed into different metabolites, or breakdown products. Copper sulphate is bound, or adsorbed, to organic materials, and to clay and mineral surfaces (Extoxnet, 1994). The degree of copper adsorption to soils depends on the level of acidity or alkalinity of the soil (Extoxnet, 1994). The distance that it can travel in soil is limited by its strong adsorption to many types of

surfaces (Extoxnet, 1994). All applied copper will become a part of the soil copper content. Although copper sulphate is highly water soluble-that is, it dissolves very easily in water-the copper ions are strongly adsorbed or precipitated to soil particles when it is applied to soil (Extoxnet, 1994). The leaching potential of this material is low in all but sandy soils. When applied to irrigation water, copper sulphate does not accumulate in the surrounding soils. 60% of applied copper was deposited in the sediments at the bottom of the irrigation ditch, where it became adsorbed to clay, mineral and organic particles (Extoxnet, 1994). Copper compounds, or precipitates, also settle out of solution, in a process called precipitation (Extoxnet, 1994). This occurs less often than adsorption (Extoxnet, 1994).. Usually, precipitates are biologically inactive, meaning that they do not undergo further biological changes (Extoxnet, 1994).

An Australian assessment of copper in freshwater and marine water is detailed in Section 8.3.7 'Detailed descriptions of chemicals' of the ANZECC & ARMCANZ (2000) guidelines. Markich and Camilleri (1997) ((ANZECC, 2000) concluded that the toxicity of copper to freshwater crustacea and fish, normalized for major differences in water chemistry, was not significantly ($P \leq 0.05$) different between tropical Australia and temperate North America. Further toxicological and environmental effects data on copper sulphate can be found in the 2009 US EPA Re-registration Eligibility Decision (RED) report (USEPA, 2009).

The environmental effects of copper sulphate has been review in detail in the overseas report in the 2009 US EPA Re-registration Eligibility Decision (RED) report (USEPA, 2009). Additionally, an ecological risk assessment performed for copper in European saltwater details the hazard quotients of copper in 101 sites with only 3 out of 101 sites with a hazard quotient greater than 1 (Hall et al., 1999).. The assessment from the study concluded that the probability of ecological risk from acute dissolved water column copper exposure to saltwater environments to be generally low (Hall et al., 1999).

The applicant considers that data generated under Australian conditions should not be required as the environmental effect the product will be the same as overseas, and there are no environmental differences between Australia and the overseas countries that would be relevant to the toxicological effect of the product. Additionally, an Australian assessment of copper in freshwater and marine water is detailed in Section 8.3.7 'Detailed descriptions of chemicals' of the ANZECC & ARMCANZ (2000) guidelines. A summary of the environmental impacts of the product will also be provided using the range of published literature available across a range of taxa.

Table 17. Copper sulphate environmental exposure

Expected exposure	Low
Behaviour and fate	The bioavailability and toxicity of copper is dependent on the speciation of copper. In seawater, copper speciation is dominated by naturally occurring organic ligands (Hall et al., 1999). The bioavailable copper concentrations are reduced by inorganic speciation (Hall et al., 1999). In total, cupric ion (Cu(II)) accounts for less than 0.2% of total dissolved copper (Hall et al., 1999). Copper ions are highly reactive and likely to be bound to other inactive forms.
Potential harmful effects on aquatic organisms	Any direct application of copper to any water body is likely to have an effect on invertebrates and reduce primary production (USEPA, 2009). The hazard quotient (observable environmental concentration / predicted no effect concentration (5.6 $\mu g\ L^{-1}$)) was greater than the value of 1 in only 3 out of 101 sites examined (Hall et al., 1999).
Potential harmful effects on terrestrial organisms	Low – the amount of copper sulphate required to cause toxicity in mammals and birds is significantly higher than aquatic animals. It is not expected that toxicity would occur with the proposed use pattern.

Environmental exposure assessment

Environmental effects

A study of copper movement in catfish aquaculture ponds in the USA found that 99% of the free copper was transferred to the sediment sample within 2 days after its addition (R. Liu et al., 2006). 90% of the copper applied to the ponds became associated with the suspended solids within 2 hours (R. Liu et al., 2006). Nearly all of the copper accumulated in the top 16cm of sediment in the pond (R. Liu et al., 2006). Similar results were seen in another study in 2016. The study found that >90% of copper applied transferred to sediment in 2 days (Willis et al., 2016). In another study in catfish aquaculture ponds, it was found that the toxicity to amphipods (*Hyallela azteca*) and common cattail (*Typha latifolia L*.) were no different in ponds that were treated with copper sulphate and were measured with a high copper sediment concentration compared to ponds that did not receive copper sulphate treatment and had a low copper sediment concentration (Han et al., 2001). The effluent of aquaculture ponds treated with copper did not appear to adversely impact receiving water ways (Huggett, 2001).

Fairmont Lakes in southern Minnesota has received copper sulphate treatment for 58 years to reduce excessive algal growth. 1,500T of copper sulphate was added to the lakes during the period. Short term and long term effects were seen (Hanson et al., 1985).

Environmental Fate and behaviour

5. General
    a. Copper naturally occurs in the environment, and continuously cycles through natural geothermodynamic processes that binds or releases copper ions. Copper ions cannot break down any further via hydrolysis, metabolism, or any other degradation process as it is an element (USEPA, 2009).
    b. Free cupric ion has a high sorption affinity for soil, sediments and organic matter, and copper applied to the surface is not expected to move into groundwater (USEPA, 2009).
    c. Copper toxicity decreases with increasing hardness and alkalinity (ANZECC, 2000).
    d. The environmental trigger for copper in marine water in Australia is 1.3 µg/L (ANZECC, 2000).
6. Aqueous
    a. A study of copper movement in catfish aquaculture ponds in the USA found that 99% of the free copper was transferred to the sediment sample within 2 days after its addition (R. Liu et al., 2006). 90% of the copper applied to the ponds became associated with the suspended solids within 2 hours (R. Liu et al., 2006). Nearly all of the copper accumulated in the top 16cm of sediment in the pond (R. Liu et al., 2006).
    b. Similar results were seen in another study in 2016. The study found that >90% of copper applied transferred to sediment in 2 days (Willis et al., 2016). Copper rapidly partitions to suspended phytoplankton and particulates (Willis et al., 2016). Copper shifted to less bioavailable forms after being transferred to sediment and its toxicity to non-target species decreased significantly (Willis et al., 2016). The elevated copper concentration in the sediment did not appear to have adverse effects to non-target species (Willis et al., 2016).
    c. In another study in catfish aquaculture ponds, it was found that the toxicity to amphipods (*Hyallela azteca*) and common cattail (*Typha latifolia L*.) were no different in ponds that were treated with copper sulphate and were measured with a high copper sediment concentration compared to ponds that did not receive copper sulphate treatment and had a low copper sediment concentration (Han et al., 2001).
    d. The effluent of aquaculture ponds treated with copper did not appear to adversely impact receiving water ways (Huggett, 2001).
    e. The biodiversity of sulphate reducing prokaryotes in sediment samples exposed to long term copper-mining residue were lower than non-copper-contaminated sediment samples, however the total abundance was not affected (Besaury et al., 2012).
    f. The USEPA's model predicts that 21 days is required for an application of 1 ppm of copper to reach 522 ppb (USEPA, 2009).

g. Any direct application of copper to any water body is likely to have an effect on invertebrates and reduce primary production (USEPA, 2009).
h. The bioavailability and toxicity of copper is dependent on the speciation of copper. In seawater, copper speciation is dominated by naturally occurring organic ligands (Hall et al., 1999). The bioavailable copper concentrations are reduced by inorganic speciation (Hall et al., 1999). In total, cupric ion (Cu(II)) accounts for less than 0.2% of total dissolved copper (Hall et al., 1999).

7. Terrestrial
    a. Copper ions from copper sulphate can be released to the environmental soil. This process is affected by pH, redox potential, dissolved organic carbons, and ligands in the soil (USEPA, 2009).
    b. Copper tends to accumulate in the surface soil. Copper can persist in soil as it binds to organic matter, minerals, and some metal oxides. In acidic or sandy soil, copper may leech from the soil (Flores-Velez et al., 1996).
    c. Less copper is bound at low pH. 30% of copper was bound at pH 3.9 c.f. 99% of copper was bound at pH 6.6 (Temminghoff et al., 1997).

Environmental hazard (effects on non-target species)

An assessment in European saltwater environments found that the probability of ecological risk from acute dissolved water column copper exposure to saltwater environments to be generally low (Hall et al., 1999). In this study, the hazard quotient (observable environmental concentration / predicted no effect concentration (5.6 µg L^{-1})) was greater than the value of 1 in only 3 out of 101 sites examined (Hall et al., 1999). The concentration of copper was generally higher in marinas/harbours (1.53 µg L^{-1}) which was slightly higher than the estuary concentration of 1.49 µg L^{-1} (Hall et al., 1999).

Manufacturing plant (active constituent) and formulating plant (product)

Not considered relevant to this application

Predicted Environmental Concentration (PEC)

The environmental trigger for copper in marine water in Australia is 1.3 µg L^{-1} (ANZECC, 2000).

The bioavailability and toxicity of copper is dependent on the speciation of copper. In seawater, copper speciation is dominated by naturally occurring organic ligands (Hall et al., 1999). The bioavailable copper concentrations are reduced by inorganic speciation (Hall et al., 1999). In total, cupric ion (Cu(II)) accounts for less than 0.2% of total dissolved copper (Hall et al., 1999).

The predicted no effect concentration (5.6 µg/L)) (Hall et al., 1999).

Environmental effects assessment

1.1.1 Quantitative structure activity relationships

treatment of protozoan and trematode ectoparasitic infections.

This application is not a new product, but is a new application, relative to previous permits approved by APVMA for use of this product in other agriculture industries. It is considered that a large component of the environmental data of this product is already held by APVMA.

This section is considered not relevant for the proposed use pattern.

Environmental risk characterisation

Risk quotient (RQ) method

The environmental trigger for copper in marine water in Australia is 1.3 µg L^{-1} (ANZECC, 2000).

The bioavailability and toxicity of copper is dependent on the speciation of copper. In seawater, copper speciation is dominated by naturally occurring organic ligands (Hall et al., 1999). The bioavailable copper concentrations are reduced by inorganic speciation (Hall et al., 1999). In total, cupric ion (Cu(II)) accounts for less than 0.2% of total dissolved copper (Hall et al., 1999).

The predicted no effect concentration (5.6 μg L^{-1}) (Hall et al., 1999). An ecological risk assessment performed for copper in European saltwater details the hazard quotients of copper in 101 sites with only 3 out of 101 sites with a hazard quotient greater than 1 (Hall et al., 1999). The assessment from the study concluded that the probability of ecological risk from acute dissolved water column copper exposure to saltwater environments to be generally low (Hall et al., 1999).

Efficacy and target animal safety

Efficacy and safety are demonstrated using peer-reviewed literature and scientific studies on copper sulphate to support assessment based on the two proposed purposes of use of this chemical.

The proposed use pattern involves killing of the target animals (zooplankton and phytoplankton). This section will detail the efficacy of copper sulphate on the target animals. The effects of copper sulphate on prawns will be detailed in

Pharmacological data/studies

Summary

The active component of copper sulphate pentahydrate is copper (II) ions. Its affects phytoplankton by inhibiting photosynthesis, cell division, respiration, ATP production, electron transport and cellular ultrastructure (Stauber et al., 1987 pages 517 to 518 – see page 518 for conclusion). Additionally, copper can decrease concentrations of glucides, proteins, amino-acids, and chlorophyll in algal cells, and decreased alkaline phosphatase activity (Prosnier et al., 2015 pages 79-80).

The efficacy of copper sulphate on phytoplankton can be dependent on the initial phytoplankton concentrations, and it is expected that a higher algal density would require a larger amount of copper to have an effect (Moreno-Garrido et al., 2000 page 116).

Copper effects zooplankton such as Daphnia by decreasing fecundity, survival, body length, weight, carbon uptake, mobility, and delays maturation (Prosnier et al., 2015 pages 79-80,). Additionally, copper may impact zooplankton numbers by altering the availability of phytoplankton food sources (Luna-Andrade et al., 2002).

Pharmacokinetics

This section is considered not relevant for the proposed use pattern.

Pharmacodynamics

The active component of copper sulphate pentahydrate is copper (II) ions. Its affects phytoplankton by inhibiting photosynthesis, cell division, respiration, ATP production, electron transport and cellular ultrastructure (Stauber et al., 1987 pages 517 to 518 – see page 518 for conclusion). Additionally, copper can decrease concentrations of glucides, proteins, amino-acids, and chlorophyll in algal cells, and decreased alkaline phosphatase activity (Prosnier et al., 2015 pages 79-80).

Copper effects zooplankton such as Daphnia by decreasing fecundity, survival, body length, weight, carbon uptake, mobility, and delays maturation (see page 79-80) (Prosnier et al., 2015). Additionally, copper may impact zooplankton numbers by altering the availability of phytoplankton food sources (Luna-Andrade et al., 2002).

Efficacy studies

Summary

There is a wide range of dose rates reported in the literature that explore the effects of copper on phytoplankton and zooplankton. The efficacy detailed in this section reflect the efficacy required to kill phytoplankton and zooplankton.

Laboratory model efficacy studies

There is a wide range of dose rates reported in the literature that explore the effects of copper on phytoplankton and zooplankton. The efficacy detailed in this section reflect the efficacy required to kill phytoplankton and zooplankton. Additional details regarding the effects of copper sulphate on prawns will be detailed in "**Error! Reference source not found. Error! Reference source not found.**".

Target organism efficacy studies

EC50, IC50 and LC50 for phytoplankton exposed to copper are detailed in Table 18.

Table 18. EC50, IC50, LC 50 of phytoplankton exposed to copper

Algae	EC50/IC50/LC50	Lowest observable effect	No observable effect	Additional comments	Reference
Chaetoceros gracilis	63.75 $\mu gCuL^{-1}$ (IC50)	17 $\mu gCuL^{-1}$	10 $\mu gCuL^{-1}$	96h growth	(Suratno et al., 2015)
Chaetoceros sp	88 $\mu gCuL^{-1}$ (IC50)	-	-	72h growth	(Debelius et al., 2009)
Chlamydomonas bullosa	0.78 $\mu mol\ L^{-1}$ (EC50)	-	-	96hr growth	(Visviki et al., 1994)
Chlorella sp	150 $\mu mol\ L^{-1}$ (EC50)			96h growth	(Wan et al., 2018)
Chlorococcum litorale	10,200 $\mu gCuL^{-1}$ (IC50)	-	-	72h growth	(Satoh et al., 2005)
Chlorococcum sp	11,700 $\mu gCuL^{-1}$ (IC50)	-	-	72h growth	(Satoh et al., 2005)
Cylindrotheca	7700 $\mu gCuL^{-1}$ (IC50)	-	-	72h growth	(Satoh et al., 2005)
Dinaliela tertiolecta	530 $\mu gCuL^{-1}$ (IC50)	42 $\mu gCuL^{-1}$	8 $\mu gCuL^{-1}$	72h growth	(Levy et al., 2007)
Dunaliella salina	5.94 $\mu molL^{-1}$ (EC50)	-	-	96hr growth	(Visviki et al., 1994)
Emiliania huxleyi (cocoliths)	17 $\mu gCuL^{-1}$ (IC50)	1 $\mu gCuL^{-1}$	<1 $\mu gCuL^{-1}$	72h growth	(Levy et al., 2007)
Emiliania huxleyi (non cocoliths)	20 $\mu gCuL^{-1}$ (IC50)	-	9 $\mu gCuL^{-1}$	72h growth	(Levy et al., 2007)
Gephyrocapsa oceanic (non cocoliths)	>25 $\mu gCuL^{-1}$ (IC50)	2.6 $\mu gCuL^{-1}$	1.3 $\mu gCuL^{-1}$	72h growth	(Levy et al., 2007)
Heterocapsa niei	4.8 $\mu gCuL^{-1}$ (IC50)	-	-	72h growth	(Levy et al., 2007)
Heterocapsa sp	11,600 $\mu gCuL^{-1}$ (IC50)	-	-	72h growth	(Satoh et al., 2005)
Isochrysis galbana	31.4 $\mu molL^{-1}$ (EC50)	-	-	Percentage of motile	(G. Liu et al., 2011)
Isochrysis galbana	910 $\mu gCuL^{-1}$ (EC50)	-	-	5d growth	(Yap et al., 2004)
Isochrysis galbana	4,200 $\mu gCuL^{-1}$ (IC50)	-	-	72h growth	(Satoh et al., 2005)
Isochrysis galbana (T-iso)	58 $\mu gCuL^{-1}$ (IC50)	-	-	72h growth	(Debelius et al., 2009)
Isochrysis sp	4.0 $\mu gCuL^{-1}$ (IC50)	1.1 $\mu gCuL^{-1}$	<1.1 $\mu gCuL^{-1}$	72h growth	(Levy et al., 2007)
Isochrysis sp	31.80 $\mu gCuL^{-1}$ (IC50)	10 $\mu gCuL^{-1}$	5 $\mu gCuL^{-1}$	96h growth	(Suratno et al., 2015)
Karenia breve	0.05-0.10 $mgCuL^{-1}$	-	-	-	(Rounsefell, 1959)
LPP-group	5,400 $\mu gCuL^{-1}$ (IC50)	-	-	72h growth	(Satoh et al., 2005)
Micromonas pusilla	1.2 $\mu gCuL^{-1}$ (IC50)	0.6 $\mu gCuL^{-1}$	0.3 $\mu gCuL^{-1}$	72h growth	(Levy et al., 2007)

Minutocellus polymorphus	0.6 µgCuL^{-1} (IC50)	0.2 µgCuL^{-1}	<0.2 µgCuL^{-1}	72h growth	(Levy et al., 2007)
Nannochloropsis gaditana	137 µgCuL^{-1} (IC50)	-	-	72h growth	(Debelius et al., 2009)
Nitzia closterium	18 µgCuL^{-1} (IC50)	5.8 µgCuL^{-1}	4.4 µgCuL^{-1}	72h growth	(Levy et al., 2007)
Nitzschia thermalis	0.6 µmol/L^{-1}	-	-	No growth	(Metaxas et al., 1991)
Pavlova sp	51.46 µgCuL^{-1} (IC50)	29 µgCuL^{-1}	-	96h growth	(Purbonegoro et al., 2018)
Phaeodactylum sulcata	4.2 µgCuL^{-1} (IC50)	-	<5 µgCuL^{-1}	72h growth	(Levy et al., 2007)
Phaeodactylum tricornutum	8 µgCuL^{-1} (IC50)	1.5 µgCuL^{-1}	<1.5 µgCuL^{-1}	72h growth	(Levy et al., 2007)
Prasinococcus sp	5,400 µgCu/L^{-1} (IC50)	-	-	72h growth	(Satoh et al., 2005)
Rhodomonas salina	48 µgCuL^{-1} (IC50)	-	-	72h growth	(Debelius et al., 2009)
Scrippsiella trochoidea	N/A				
Skeletonema costatum	-	-	<0.0032 µmolL^{-1}	-	(Morel et al., 1978)
Skeletonema costatum	0.045 mgCuL^{-1} (IC50)	-	-	96h growth	(Nassiri et al., 1995)
Skeletonema costatum	0.5 µmolL^{-1}	-	-	No growth	(Metaxas et al., 1991)
Synechoccus sp	5,300 µgCuL^{-1} (IC50)	-	-	72h growth	(Satoh et al., 2005)
Tetraselmis chui	1.3 µmolL^{-1} (EC50)	-		Percentage of motile	(G. Liu et al., 2011)
Tetraselmis chuii	330 µgCuL^{-1} (IC50)	-	-	72h growth	(Debelius et al., 2009)
Tetraselmis sp	47 µgCuL^{-1} (IC50)	22 µgCuL^{-1}	7 µgCuL^{-1}	72h growth	(Levy et al., 2007)
Tetraselmis tetathele	7,400 µgCuL^{-1} (IC50)	-	-	72h growth	(Satoh et al., 2005)

Dose determination studies

A list of EC50, IC50 and LC50 concentrations are detailed in **Error! Reference source not found.** and **Error! Reference source not found.** for zooplanktons and phytoplanktons.

The maximum application rate of copper detailed in the USEPA RED report is 0.4 mg L^{-1} Cu^{2+}.

A recommended dose of 0.15-0.20 mg L^{-1} Cu^{2+} is suggested by Yanong's report "Use of Copper in Marine Aquaculture and Aquarium Systems" (Yanong, 2013).

The efficacy of copper sulphate on phytoplankton can be dependent on the initial phytoplankton concentrations, and it is expected that a higher algal density would require a larger amount of copper to have an effect (page 116) (Moreno-Garrido et al., 2000).

Still to be determined. A dose rate of 0.4 mg L^{-1} Cu^{2+} is being considered for the proposed use patterns. Additional data required.

Confirmatory clinical/field studies

Additional data required.

Palatability studies

This section is considered not relevant for this application.

Target organism safety studies

The proposed use pattern involves killing of the target organism (zooplankton and phytoplankton). This section will detail the efficacy of copper sulphate on the target organism. The effects of copper sulphate on prawns are detailed in "**Error! Reference source not found. Error! Reference source not found.**".

Margin of safety studies

The proposed use pattern involves killing of the target organisms (zooplankton and phytoplankton). This section will detail the efficacy of copper sulphate on the target organisms. The effects of copper sulphate on prawns are detailed in "**Error! Reference source not found. Error! Reference source not found.**".

Dose rate

Still to be determined. A dose rate of 0.4 mg L^{-1} Cu^{2+} is being considered for the proposed use patterns. Additional data required.

Duration of treatment

The proposed use pattern involves killing of the target organisms (phytoplankton). This section will detail the efficacy of copper sulphate on the target animals. See toxicology section above.

Related studies

Compatibility studies

This section is considered not relevant for the proposed use pattern. The proposed use pattern will not be used in conjunction with any other treatment(s).

Effects on hides and fleeces

This is considered not relevant for this application.

Accidental administration or exposure to non-target organisms

See toxicology section above.

Effects on taste or produce (organoleptic effects)

This is considered not relevant for this application.

Other studies or data

In vivo or *in vitro* bioequivalence studies

No in vivo or in vitro studies were used to demonstrate bioequivalence in this application. This section is considered irrelevant for the proposed use pattern application.

Clinical case studies

No clinical case studies were used in this application. This is considered not relevant for this application.

Scientific references or extrapolated scientific argument

See above and below.

Topical studies, inhalation studies, tissue irritation studies

This is considered not relevant for this application.

Reproductive function studies

This is considered not relevant for this application.

Minimum inhibitory concentration studies

This is considered not relevant for this application.

Non-food Trade

This is considered not relevant for this application.

Special Data

This module is considered not relevant for this application. The product does not involve killing or suppressing growth of bacteria, GMOs or nanotechnology.

Freshwater cyanobacteria *Microcystis aeruginosa* have been shown to develop resistant to copper sulphate (García-Villada et al., 2004). This finding has not been demonstrated in saltwater phytoplankton.

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