Australian Government

Australian Pesticides and Veterinary Medicines Authority

PUBLIC RELEASE SUMMARY

on the evaluation of the new active clitoria ternatea in the product Sero-X Insecticide

NOVEMBER 2016

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Director Public Affairs and Communication
Australian Pesticides and Veterinary Medicines Authority
PO Box 6182
KINGSTON ACT 2604 Australia

Telephone: +61 2 6210 4701

Email: communications@apvma.gov.au

This publication is available from the APVMA website: www.apvma.gov.au.

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PREFACE

The Australian Pesticides and Veterinary Medicines Authority (APVMA) is the Australian Government regulator responsible for assessing and approving agricultural and veterinary chemical products prior to their sale and use in Australia.

In undertaking this task, the APVMA works in close cooperation with advisory agencies, including the Department of Environment (DoE) and State Departments of Primary Industries.

The APVMA has a policy of encouraging transparency in its activities and seeking community involvement in decision making. Part of that process is the publication of Public Release Summaries for products containing new active constituents.

The information and technical data required by the APVMA to assess the safety of new chemical products, and the methods of assessment, must be consistent with accepted scientific principles and processes. Details are outlined on the APVMA website.

This Public Release Summary is intended as a brief overview of the assessment that has been conducted by the APVMA and of the specialist advice received from its advisory agencies. It has been deliberately presented in a manner that is likely to be informative to the widest possible audience to encourage public comment.

About this document

This is a Public Release Summary.

It indicates that the APVMA is considering an application for registration of an agricultural or veterinary chemical. It provides a summary of the APVMA's assessment, which may include details of:

- the toxicology of both the active constituent and product
- the residues and trade assessment
- occupational exposure aspects
- environmental fate, toxicity, potential exposure and hazard
- efficacy and target crop or animal safety.

Comment is sought from interested stakeholders on the information contained within this document.

Making a submission

In accordance with section 13 of the Agvet Code, the APVMA invites any person to submit a relevant written submission as to whether the application for registration of Sero-X Insecticide should be granted. Submissions should relate only to matters that the APVMA is required, by legislation, to take into account in deciding whether to grant the application. These matters include aspects of public health, occupational health and safety, chemistry and manufacture, residues in food, environmental safety, trade, and efficacy and target crop or animal safety. Submissions should state the grounds on which they are based. Comments received that address issues outside the relevant matters cannot be considered by the APVMA.

Submissions must be received by the APVMA by close of business on 29 December 2016 and be directed to the contact listed below. All submissions to the APVMA will be acknowledged in writing via email or by post.

Relevant comments will be taken into account by the APVMA in deciding whether the product should be registered and in determining appropriate conditions of registration and product labelling.

When making a submission please include:

- contact name
- company or group name (if relevant)
- email or postal address (if available)
- the date you made the submission.

All personal information, and confidential information judged by the APVMA to be confidential commercial information (CCI)[1] contained in submissions will be treated confidentially.

Written submissions on the APVMA's proposal to grant the application for registration that relate to the grounds for registration should be addressed in writing to:

Case Management and Administration Unit
Australian Pesticides and Veterinary Medicines Authority
PO Box 6182
Kingston ACT 2604

Phone: +61 2 6210 4701
Fax: +61 2 6210 4721
Email: enquiries@apvma.gov.au

[1] A full definition of 'confidential commercial information' is contained in the Agvet Code.

Further information

Further information can be obtained via the contact details provided above.

Further information on Public Release Summaries can be found on the APVMA website at www.apvma.gov.au.

1 INTRODUCTION

1.1 Applicant

Innovate Ag Pty Limited.

1.2 Details of the product

It is proposed to register Sero-X Insecticide, containing 400 g/L clitoria ternatea extract, as an emulsifiable concentrate for the control or suppression of Green mirid, Silverleaf whitefly (biotype B) and heliothis in cotton (Queensland and New South Wales only).

Repeat applications of Sero-X Insecticide are to be made of 2 L/ha (800 g clitoria ternatea extract/ha) at a 7 day interval for Heliothis and at a 14–20 day interval for green mirid and silverleaf whitefly. The product is to be applied by ground rig in an application volume of 50–200 L/ha.

Sero-X Insecticide is the first product containing clitoria ternatea extract to be introduced to the Australian market. The active constituent is a biological extract manufactured from clitoria ternatea plant material. Clitoria ternatea extract has not been allocated with a mode of action for resistance management.

Both clitoria ternatea extract and Sero-X Insecticide will be manufactured and formulated in Australia. Sero-X Insecticide will be available in 1–1000 L High density polyethylene (HDPE) containers.

1.3 Overseas registrations

There are currently no overseas registrations for clitoria ternatea extract.

This publication provides a summary of the data reviewed and an outline of the regulatory considerations for the proposed registration of Sero-X Insecticide and approval of the new active constituent, clitoria ternatea extract.

2 CHEMISTRY AND MANUFACTURE

2.1 Active constituent

Table 1 and 2 summarise key properties of clitoria ternatea extract.

Table 1: Nomenclature, chemistry and key identifiers of the active constituent clitoria ternatea extract

COMMON NAME (AS 1719-1994):	Clitoria ternatea extract
BOTANICAL NAME:	Clitoria ternatea
FAMILY NAME:	Fabaceace (formally Leguminosae)
ACTIVE COMPONENTS:	Cyclotides Flavonyl glycosides Protein
CAS REGISTRY NUMBER:	Not available
STRUCTURAL FORMULA:	Not available
CHEMICAL FAMILY:	Plant extract

Physico-chemical properties of active constituent

Table 2: Summary of key physio-chemical properties of the active constituent clitoria ternatea extract

PHYSICAL FORM:	Viscous syrupy liquid verging to paste
APPEARANCE:	Dark green, resinous
ODOUR:	Herbaceous characteristics
RELATIVE DENSITY, SPECIFIC GRAVITY:	not less than 0.9
PH (IN AQUEOUS SOLUTION):	4-6
OXIDISING OR REDUCING POTENTIAL:	Not available
FLAMMABILITY LIMITS:	3.5% – 19.0% (ethanol)
FLASH POINT:	70°C (sample with ethanol not more than 19%)

2.2 Formulated product

The product Sero-X Insecticide will be manufactured and formulated in Australia in HDPE containers.

Table 3: Key aspects of the identity of the product Sero-X Insecticide

DISTINGUISHING NAME:	Sero-X Insecticide
FORMULATION TYPE:	Emulsifiable Concentrate (EC)
ACTIVE CONSTITUENT CONCENTRATION:	400 g/L clitoria ternatea extract

Physical and chemical properties of product

Table 4: Summary of key physico-chemical parameters of the Sero-X Insecticide

PHYSICAL FORM:	Dark green viscous liquid
ODOUR:	Herbaceous odour
PH VALUE (1% IN WATER):	4.97
RELATIVE DENSITY:	1.08 at 20°C
KINEMATIC VISCOSITY:	157.2 cSt at 40°C
FLASH POINT:	No flash point
OXIDISING PROPERTIES:	Negligible oxidising potential
EXPLOSIVE PROPERTIES:	Not explosive
FLAMMABILITY:	Not flammable
CORROSIVE HAZARD:	Negligible corrosive potential to HDPE container

2.3 Recommendations

The APVMA has evaluated the chemistry aspects of clitoria ternatea extract active constituent and associated product Sero-X Insecticide (manufacturing process, quality control procedures, batch analysis results, stability and analytical methods) and found them to be acceptable. Stability data was supported by a bioassay study comparing efficacy of the product stored at ambient temperature to that of a sample batch subjected to accelerated stability treatment. Mortality for the target insect, helicoverpa armigera, when treated with the accelerated storage sample was 100% at 96 hours after treatment and this efficacy was comparable to results demonstrated for product sample stored at ambient temperature. Results for both treatments were significantly greater than the negative control sample. This data confirmed that storage under normal conditions is not likely to impact on product stability and resulting efficacy.

3 TOXICOLOGICAL ASSESSMENT

3.1 Evaluation of toxicology

The toxicology of clitoria ternatea extract has been considered by the APVMA when the active constituent was approved (80403) in Australia in 2016.

It should be noted that toxicity tests generally use doses that are high compared with likely human exposures. The use of high doses increases the likelihood that potentially significant toxic effects will be identified. Findings of adverse effects in any one species do not necessarily indicate such effects might be generated in humans. From a conservative risk assessment perspective however, adverse findings in animal species are assumed to represent potential effects in humans, unless convincing evidence of species specificity is available.

Where possible, considerations of the species specific mechanisms of adverse reactions weigh heavily in the extrapolation of animal data to likely human hazard. Equally, consideration of the risks to human health must take into account the likely human exposure levels compared with those, usually many times higher, which produce effects in animal studies. Toxicity tests should also indicate dose levels at which the specific toxic effects are unlikely to occur. Such dose levels as the NOEL are used to develop acceptable limits for dietary or other intakes (Acceptable Daily Intake (ADI) and Acute Reference Dose (ARfD)) at which no adverse effects in humans would be expected.

Chemical class

Clitoria ternatea extract is a plant based ethanolic extract.

Toxicokinetics, metabolism and dermal absorption

No toxicokinetic, metabolism or dermal absorption studies with the clitoria ternatea extract were available for assessment. Clitoria ternatea extract consists of a range of plant based compounds including flavonoid glycosides, essential amino acids, pigments, cyclic peptides, lipids, mineral salts and carbohydrates common to legumes; all of which would be expected to have different absorption, distribution, metabolism and excretion properties.

Acute toxicity

Clitoria ternatea extract has low acute oral (LD50 > 2000 mg/kg bw) and dermal (LD50 > 2000 mg/kg bw) toxicity in rats. Clitoria ternatea extract is not a skin irritant in rabbits and the results of a skin sensitisation study (local lymph node assay [LLNA]) did not provide evidence of a sensitisation potential. The acute inhalational toxicity of clitoria ternatea extract is likely to be low. The chemical composition of clitoria ternatea extract indicates it will be a moderate eye irritant due to the physical and chemical properties of the extract.

Repeat-dose toxicity

In a GLP-compliant, non-guideline 12–day pilot oral toxicity study, rats (n=3/sex/dose) were administered 0, 100, 250, 500, 750 and 1000 mg/kg bw/d clitoria ternatea extract with no test-item related toxicity or abnormal clinical signs observed. However, an oral NOEL was not established due to limitations in the study design.

In a 21–day dermal toxicity study with 7 day recovery period rats (n=15/sex; 5/sex recovery group) were topically exposed to 1000 mg/kg bw clitoria ternatea extract for 6 hours 5 days/week. No toxic effects were noted. There were no test-item related effects on body weight gain or food consumption. No test-item related effects were noted on functional behavioural, haematology, clinical chemistry or urinalysis parameters. There were no test-item related abnormalities seen during gross or microscopic examinations.
A dermal NOEL of 1000 mg/kg was determined.

In a 28–day oral toxicity study, rats (n=4/sex/dose) were administered 0, 250, 500, 750 and 1000 mg/kg bw/d clitoria ternatea extract with no test-item related systemic toxicity or terminal examination abnormalities observed at any of the test doses. There were no test-item related effects on body weight gain or food consumption between treatment and control groups. There were no test-item related abnormalities noted in haematology, clinical chemistry or urinalysis parameters. No gross abnormalities were seen at necropsy and no test-item related effects on organ weights were noted. An oral NOEL of 1000 mg/kg bw/d was determined.

In a GLP and guideline compliant 90-day oral toxicity study with 14 day recovery period rats were administered 0, 500 and 1000 mg/kg bw/5/7d clitoria ternatea extract. No toxic effects were observed up to the highest dose. There were no effects on body weight gain or food and water consumption between treatment and control groups. There were no test-item related abnormalities noted in functional behavioural, ophthalmological, haematology, clinical chemistry or urinalysis parameters. No test-item related abnormalities were seen on organ weights, or during gross or microscopic examinations. The study used a 5 out of 7 day dosing regimen due to inappetence seen in previous studies. An oral NOEL of 1000 mg/kg bw/d (5 days/week for 90 days) was established.

Genotoxicity

The genotoxic potential of the compound was tested in an Ames test using five strains of S. typhimurium gave negative results both with and without metabolic activation. An in vivo mouse micronucleus test also produced negative results.

Reproductive and developmental toxicity

There were no reproductive toxicity studies available. However, in the 90 day repeat-dose oral toxicity study, there were no gross or histopathological lesions in the male and female reproductive organs, noting that oestrous cycling and sperm parameters were not assessed. Available information does not indicate a concern for reproductive toxicity of clitoria ternatea extract.

Neurotoxicity

There were no neurotoxicity studies available. However, clinical examinations in the 90–day oral repeat dose study with 14 day recovery showed no clinical signs of a central nervous system effect, based on posture, gait, convulsions and reactivity to handling. Functional observations in the same study also showed no abnormalities. Available information does not indicate a neurotoxic concern for clitoria ternatea extract.

Toxicity of Sero-X Insecticide

Based on the evaluated acute toxicity studies, Sero-X Insecticide has low acute oral and acute dermal toxicity. It is not a skin irritant or a skin sensitiser. The acute inhalational toxicity and eye irritation were estimated based on the data on clitoria ternatea extract and the excipients in Sero-X Insecticide. It is expected the product has low inhalational toxicity and is a moderate eye irritant.

3.2 Public health standards

Poisons scheduling

Clitoria ternatea extract was considered at the August 2015 Advisory Committee on Chemicals Scheduling (ACCS) meeting. The Scheduling Delegate's final decision in November 2015 was to include clitoria ternatea in Appendix B (Substances considered not to require control by scheduling) of the Standard for the Uniform Scheduling of Medicines and Poisons (SUSMP) due to its low toxicity for the proposed use pattern (use pattern restricts hazard and area of use).

Sero-X Insecticide contains clitoria ternatea extract at 400 g/L and is, therefore, classified as a product considered not requiring control by scheduling.

No Observable Effect Level (NOEL)/Acceptable Daily Intake (ADI)

The ADI is that quantity of a compound which can safely be consumed on a daily basis for a lifetime and is based on the lowest NOEL obtained in the most sensitive species. This NOEL is then divided by a safety (uncertainty) factor which reflects the quality of the toxicological database and takes into account the variability in responses between species and individuals.

An ADI for clitoria ternatea extract was not considered necessary as no effects were observed in a 90–day oral toxicity study in rats at the NOEL of 1000 mg/kg bw/d.

Acute Reference Dose (ARfD)

An ARfD has not been established for clitoria ternatea extract as it was not deemed necessary due to low acute toxicity in animal studies.

4 RESIDUES ASSESSMENT

4.1 Introduction

Sero-X Insecticide contains the active constituent clitoria ternatea extract (see section 2.1) for use in cotton to control or supress green mirid, silver leaf whitefly (biotype B) and heliothis. The proposed use is described in section 1.2.

The following withholding periods and restraints are proposed:

WHP
Harvest: Not required when used as directed

Restraints

DO NOT apply with aircraft.

SPRAY DRIFT RESTRAINTS

DO NOT apply with spray droplets smaller than a FINE spray droplet size category according to nozzle manufacturer specifications that refer to the ASAE S572 Standard or the British Crop Production Council guideline.
DO NOT apply when wind speed is less than 3 or more than 20 kilometres, as measured at the application site.
DO NOT apply during surface temperature inversion conditions at the application site.

4.2 Residues consideration

Clitoria ternatea extract is a biological extract manufactured from clitoria ternatea plant material. The relevant physiologically active compounds have been identified as belonging to the flavonoids and cyclotides group of compounds and are considered appropriate markers for the extract.

The Applicant has supplied information concerning the levels of flavonoid aglycones (non-sugar component of a glycoside molecule) in cotton foliage from application of Sero-X Insecticide in comparison to the loadings naturally present in the environment. It was shown that the overall contribution of Sero-X Insecticide on the environment loading of flavonoid aglycones in cotton production areas was several orders of magnitude lower than the contribution of the cotton crop itself. It was concluded that this was not unexpected considering the same aglycones occur in both cotton and clitoria ternatea. It was noted that these two aglycones are shown to be present in a large range for crops from different plant families including those of fruits, vegetables and cereals including wheat, sorghum, rice, tea, broccoli, cabbage, kale, beans, endive, leek, tomato, strawberries and grapes.

Neither an ADI nor an ARfD for clitoria ternatea have been established. Dietary exposure associated with the proposed use on cotton is likely to be minimal. Residues of clitoria ternatea in treated cotton seed are therefore of no toxicological significance.

The proposed use is considered acceptable from a residues perspective because the residues of the individual extract components relating to the proposed use of clitoria ternatea extract in Sero-X Insecticide will not occur in foods and animal feeds above background levels of these or similar compounds; the extract components are the same or closely similar to those found in food; cattle are already consuming clitoria ternatea plant material and, when the product is used as directed, clitoria ternatea extract is unlikely to pose a toxicological concern. A withholding period of 'Not required when used as directed' is appropriate.

It is recommended that a Table 5 entry be established for the use of clitoria ternatea extract on food and non-food producing crops, as clitoria ternatea extract is indistinguishable from natural food components and is of no toxicological significance.

4.3 Recommendations

The following amendment is proposed to Table 5 of the APVMA MRL Standard.

Table 5: Proposed amendments to MRL Standard Table 5—uses of substances where MRLs are not necessary

SUBSTANCE	USE
ADD:	
Clitoria ternatea extract	For use as an insecticide on food and non-food producing crops

5 ASSESSMENT OF OVERSEAS TRADE ASPECTS OF RESIDUES IN FOOD

5.1 Recommendations

The APVMA is satisfied that the proposed use of Sero-X Insecticide containing the active clitorea ternatea extract will meet the relevant trade criteria. The trade risk associated with the proposed use of clitoria ternatea extract on cotton is considered to be low and not undue.

6 OCCUPATIONAL HEALTH AND SAFETY ASSESSMENT

6.1 Health hazards

Clitoria ternatea extract is currently not listed on the Safe Work Australia Hazardous Substances Information System (HSIS) Database (SWA, 2016). However, based on the available toxicology information, clitoria ternatea extract is not classified as a hazardous substance in accordance with NOHSC Approved Criteria for Classifying Hazardous Substances (NOHSC, 2004).

Based on the product toxicology information and/or concentrations of active and other constituents in the product Sero-X Insecticide is not classified as a hazardous substance in accordance with NOHSC Approved Criteria for Classifying Hazardous Substances (NOHSC, 2004).

6.2 Formulation, packaging, transport, storage and retailing

Both clitoria ternatea extract and Sero-X Insecticide will be manufactured and formulated in Australia. Sero-X Insecticide will be available in 1–1000 L high density polyethylene (HDPE) containers.

6.3 Use pattern

Sero-X Insecticide is proposed for use on cotton crops from early growth stages until late growth stages to assist control of heliothis, silverleaf white fly and green mirids.

Sero-X Insecticide will be applied at intervals of 7–20 days with a maximum of five applications per growing season at a rate of 2 L/ha by ground boom application equipment. It is proposed that a maximum of 300 ha/d will be treated. The product is not to be applied by air.

6.4 Exposure during use

Farmers and their employees will be the main users of the product. For professional users, the duration of potential exposure will be on a medium-term (sub-chronic) basis. Workers may be exposed to the product when opening containers; mixing/loading, application, cleaning up spills, maintaining equipment and re-entering treated areas. The main routes of exposure to the product and spray will be dermal and inhalational, with possible ocular exposure.

This WHS information provided by the applicant was considered when estimating the exposure of the proposed product during use. In the absence of exposure data for the proposed mode of application, the US Environmental Protection Agency (EPA) Pesticide Handler Exposure Database (PHED) Surrogate Exposure

Guide (1998) was used to estimate exposure. The toxic endpoint of concern and identified NOEL was derived has been derived from animal toxicity testing, and in this instance a margin of exposure (MOE) of 100 or above will be considered acceptable. The MOE takes into account both interspecies extrapolation and intraspecies variability and the seriousness of the critical health effect of concern.

The MOE values associated with repeated use of Sero-X Insecticide when preparing and applying to cotton crops are considered acceptable (i.e. MOE ≥ 100), when users wearing appropriate PPE comprising of cotton overalls buttoned to the neck and wrist and elbow length chemical resistant gloves.

Bystander risk is possible during spraying activities, but is expected to be limited. Adherence to good agricultural practice and spray drift minimisation strategies will minimise potential risks.

6.5 Exposure during re-entry

Workers entering treated areas may be exposed to product residues, degradation products during post-application activities. The most likely route of exposure upon re-entry is considered to be dermal. Post-application activities may include crop inspection, checking efficacy of control agent and harvesting.

The MOE estimate for workers re-entering treated areas to conduct all exposure activities is considered acceptable on day zero after treatment. (i.e. MOE >100). Therefore, it is expected that the risk associated with re-entry into areas where the product has been used according to label instructions will be low.

As the re-entry risks associated with Sero-X Insecticide are considered to be low, a re-entry statement was not considered necessary.

6.6 Recommendations for safe use

Taking into consideration the potential toxicological hazard, use pattern and likelihood of handler exposure, the following First Aid and Safety Directions are considered appropriate:

First Aid Instructions:

If poisoning occurs, contact a doctor or Poisons Information Centre. Phone Australia 131126; New Zealand 0800 764

Safety Directions:

Will irritate the eyes. Avoid contact with eyes. When opening the container, preparing spray and using the prepared spray, wear cotton overalls buttoned to the neck and wrist (or equivalent clothing), elbow length chemical resistant gloves and goggles. If product in eyes, wash it out immediately with water. Wash hands after use. After each day's use wash gloves, goggles and contaminated clothing.

6.7 Conclusion

The registration of Sero-X Insecticide, containing 400 g/L clitoria ternatea extract, for the control or suppression of various insect pests in cotton is supported. Sero-X Insecticide can be used safely if handled in accordance with the instructions on the product label and any other control measures described above. Additional information is available on the product Safety Data Sheet (SDS).

7 ENVIRONMENTAL ASSESSMENT

7.1 Introduction

It is proposed to register Sero-X Insecticide containing the new active constituent clitoria ternatea extract for the control of various insect pests in cotton. Section 1.2 describes the proposed use in greater detail.

7.2 Environmental fate and behaviour

Natural occurrence in Australia

Clitoria ternatea extract is an ethanolic extract of the butterfly pea (clitoria ternatea). It comprises of a complex mixture of many different compounds. The most prominent and biologically active constituents within the extract belong to the cyclotides and flavonoid groups.

Cyclotides are only found in four families of plant rubiaceae, violaceae, rabacae and cucurbitaceae. However these families are widespread in Australia. Contrastingly there are thousands of flavonoids present in plants, and of the six identified in clitoria ternatea extract (kaempferol, quercetin, myricetin, astragalin, kaempferol 3-neohesperiodoside and clitorin) only three (quercetin and kaemperol and myricetin) were positively identified in plants in the cotton growing regions of Australia.

Fate and behaviour

The cyclotide group consists of small proteins (chain of approximately 30 amino acids—molecular weight around 3000). These proteins are extremely water soluble (around 1–10 g/L), not readily degraded except in sunlight over weeks when the amino acid tryptophan is present. These chemicals are readily sorbed onto clays and humic acids but less readily onto inorganic minerals such as goethite. This means these chemicals are likely to remain in soils to which they are applied and breakdown over time due to the action of sunlight.

The flavonoid group is characterised by chemicals with extremely low volatility, relatively high water solubility and ready biodegradability. This means that they are likely to be mobile and may move through soil into groundwater or surface waters but they are unlikely to persist or bioaccumulate.

7.3 Environmental effects

The environmental effects of clitoria ternatea extract and the product Sero-X insecticide, were tested on a number of species and in-line with international guidance on naturally occurring substances consideration was also given to background levels and other uses such as human or animal feed.

Table 6: Toxicity of active constituent *Clitoria ternatea* extract and the product Sero-X insecticide for various organisms

ORGANISM		MEASURE OF TOXICITY OR EFFECT	PARAMETER (TEST PERIOD)	TOXICITY (UNIT)
TERRESTRIAL SPECIES				
Mammal	Sprague dawley rat	Acute toxicity (oral)	LD_{50}	> 2000 mg/kg bw
		Acute Contact	LD_{50}	> 2000 mg/kg bw
		Repeat dose	NOAEL (21 d)	1000 mg/kg bw
		Repeat dose	NOAEL (21 d)	1000 mg/kg bw
		Repeat dose	LD_{50} (90 d)	> 1000 mg/kg bw
Bird	Bobwhite quail	Not Tested		
	Bobwhite quail, mallard duck			
	Bobwhite quail			
Bees	Honeybee (apis mellifera)	Oral and contact toxicity	LD_{50}	> 150 µg/bee
Non-target arthropods	predatory mite (hypoaspis aculeifer)	Tier 1 dose/response	LR_{50}	733 mg/kg soil
	Earthworm	Acute toxicity	LC_{50}	≥ 1000 mg/kg
		Reproduction	Not tested	
Aquatic species				
Fish	Zebrafish danio rerio	Acute toxicity	LD_{50} (96 h)	>100 mg extract/L
	Rainbowfish melanotaenia splendida	Acute toxicity	LD_{50} (96 h)	>100 mg extract/L
Aquatic invertebrate	Daphnia magna	Acute toxicity	EC_{50} (48 h)	>100 mg extract/L

ORGANISM		MEASURE OF TOXICITY OR EFFECT	PARAMETER (TEST PERIOD)	TOXICITY (UNIT)
	Americamysis bahia	Acute toxicity	EC_{50} (48 h)	>100 mg extract/L
	Daphnia magna	Reproduction	Not tested	
	Chironomus riparius	Acute exposure	EC_{50} 48 h	9.5 mg/L
Aquatic plants	Lemna gibba	Growth inhibition	EC_{50} (unspecified)	30 mg ac/L*
Algae	Pseudokirchneriella subcapitata	Growth inhibition	E_rC_{50} (72 h)	mg/L

*** Value based on cyclotides only assuming average molecular weight—3000 g/mol**

Terrestrial organisms

The toxicity of clitoria ternatea extract to a number of terrestrial organisms based on the results (Table 9) from submitted studies, can be summarised as:

Mammals

Clitoria ternatea extract is practically non-toxic to mammals on an acute basis, with no signs of toxicity when mammals were exposed to the highest level tested, through the oral and dermal routes. Similar findings were made when mammals were exposed on a short term basis (≤ 90 days) through repeat doses, with no toxicity exhibited.

Birds

No eco-toxicity studies were conducted on birds. However, it is likely that birds are already exposed to the cyclotide group present in plants widespread in Australia, with no known toxic effect. Birds are likely to have only been exposed to some of the flavonoids present in clitoria ternatea extract, from background levels; but flavonoids all have the same mode of toxic action, which does not seem detrimental to vertebrate species. Additionally there is anecdotal evidence that there are no toxic effect to birds when butterfly pea (clitoria ternatea), is used as a feed supplement for chickens.

Bees

Bees demonstrated some sensitivity to clitoria ternatea extract when tested at the highest concentrations. However an LD50 could not be established and it may be concluded that clitoria ternatea extract is virtually non-toxic to bees, on an acute contact basis.

Other non-target arthropod species

Other non-target arthropod species also showed some sensitivity to clitoria ternatea extract. An EC50 of 733 mg/kg dry soil, based on the production of juveniles was calculated for soil dwelling predatory mites. No adverse effects on adult mites were observed.

Earthworms

Earthworms also showed some sensitivity to clitoria ternatea extract for acute exposure at the highest concentrations in soils tested. However, an LC50 could not be established and clitoria ternatea extract is considered very slightly toxic to earthworms.

Terrestrial plants

No studies were presented on clitoria ternatea extract for the phytotoxicity to plants. However, no phytotoxicity is expected as the most prominent and biologically active constituents are naturally occurring in a wide range of plants. Additionally anecdotal evidence from efficacy field trials, found no phytotoxicity to cotton plants.

Soil Microorganisms

No studies were presented on clitoria ternatea extract for the effects on soil micro-organisms. However, given that these chemicals are naturally produced in plants, soil micro-organisms are expected to be exposed continuously from background levels. Additionally the flavonoids are readily biodegradable.

Aquatic organisms

Effects on fish

Clitoria ternatea extract on an acute basis was practically non-toxic to fish.

Effects on aquatic invertebrates

Similarly clitoria ternatea extract on an acute basis was practically non-toxic to crustaceans, but was moderately toxic to sediment dwelling organisms.

Effects on algae and aquatic plants

Clitoria ternatea extract was practically non-toxic to algae, but based on the cyclotide component only was slightly toxic to aquatic plants (lemna spp.).

Aquatic No Observed Effect Concentration (NOEC) and regulatory acceptable level

The NOEC was established on the basis of the most sensitive organism to clitoria ternatea extract, (sediment dwelling organisms). As this was an acute effect a safety factor of 10 was applied and a NOEC of 0.95 mg/L is expected. This value was established as the regulatory acceptable level and was used for the determination in spray-drift and run-off modelling.

7.4 Risk assessment

An environmental risk assessment of the application of Sero-X insecticide to the foliage of mature cotton for the control or suppression of certain insects using high ground boom equipment in a FINE spray quality at up to 5 × 2 L product/ha (5 × 800 g ac/ha) with a minimum of 14 days between applications was conducted. As this was a natural product a modified risk assessment was conducted taking into account background levels of the active constituents of clitoria ternatea extract and other uses such as human or animal feed. The assessment found that the environmental risks posed by the use of this product as specified are acceptable to terrestrial vertebrates and invertebrates, soil organisms and terrestrial plants, as no adverse effects are expected. In a worst-case scenario of direct overspray, risk was shown to the aquatic environment. However, with a downwind no-spray zone of 5 m to aquatic and wetland areas, this risk was considered acceptable.

7.5 Conclusions

The APVMA is satisfied that the proposed use of this product is unlikely to have an unintended effect that is harmful to animals, plants or things or the environment.

8 EFFICACY AND CROP SAFETY ASSESSMENT

8.1 Proposed product use pattern

The proposed use of Sero-X insecticide is for the control or suppression of green mirid, silverleaf whitefly (biotype B) and heliothis in cotton (Queensland and New South Wales only).

More details on the proposed use are provided in Section 1.

8.2 Summary of evaluation of efficacy and crop safety

Efficacy

Data from 7 laboratory and 18 screen-house and field trials conducted in New South Wales undertaken between 2001 and 2007 were provided to demonstrate efficacy and activity of developmental formulations containing clitoria ternatea extract for control of cotton pests. Trials on cotton plants indicated that toxins were located in all parts of the plant and extracts showed significant reduction in oviposition by helicoverpa spp. on treated leaves in laboratory and screen-house trials and also discouraged feeding by 1st and 2nd instar larvae causing lower larval weight gain and less leaf consumed. Extracted fractions were shown to be toxic to heliothis larvae within 48 hours. One hundred percent mortality of green mirid was achieved in 9 days after treatment.

In addition, to demonstrate efficacy of Sero-X Insecticide in the proposed use situations, data from 10 field trials conducted in major cotton growing regions of Queensland and NSW during 2011 and 2013 were submitted.

The trials used scientific methodology and appropriate assessment parameters, insect counts and weights and leaf consumed in feeding studies. The rates applied in the trials encompassed the proposed label rate (2 L/ha), used appropriate trial design, with multiple replicates, industry standards and untreated controls. Results were analysed using standard statistical procedures (ANOVA, LSD, Tukey-Kramer Multiple Comparison tests).

Laboratory and small scale trial data, demonstrate that the Sero-X Insecticide is toxic to insects and is a deterrent to oviposition and feeding of heliothis larvae on cotton leaves.

Field trials indicate variable efficacy when Sero X Insecticide is used as a knockdown insecticide ranging from suppression to significant levels of control that are equivalent to industry standards. Trials confirm that a higher level of control is achieved for heliothis, green mirids and silverleaf whitefly where a repeated application is carried out. The trial results are consistent with the proposed label which identifies that

suppression rather than control may be observed where sufficient exposure is not possible and that best results are obtained with repeat applications and when product is applied before pest populations build up to damaging levels.

Beneficial insects including lacewings and spiders were not significantly affected by the various extracts tested.

Crop safety

Eight of the submitted trials specifically investigated crop phytotoxicity at rates of up to 5 L/ha (2.5 times the proposed rate). Trials involved a number of different cotton varieties, including Sicot 189, Sicot 74 BRF, Bollgard II and Roundup Ready Flex. Early, mid and late season applications were made. No detectable phytotoxic symptoms were observed following any treatment.

Resistance management

Clitoria ternatea extract and Sero-X Insecticide have not been allocated with a mode of action for resistance management. As part of a resistance management program, treatments with Sero-X should be used in rotation with alternative treatment/control methods.

8.3 Conclusions

Trial data support that Sero-X Insecticide will provide acceptable control or suppression of green mirid, silverleaf whitefly (biotype B) and heliothis in cotton when used as directed.

Acceptable crop safety is expected when the product is used as directed. The directions for use are appropriate and consistent with insecticide use in commercial agriculture in Australia.

The application for registration of Sero-X Insecticide is supported on efficacy and crop safety grounds when used in accordance with label directions.

9 LABELLING REQUIREMENTS

Label Name: Sero-X Insecticide

Signal Headings: **KEEP OUT OF REACH OF CHILDREN**

READ SAFETY DIRECTIONS BEFORE OPENING OR USING

Constituent Statements: **ACTIVE CONSTITUENT:** 400 g/L *CLITORIA TERNATEA* EXTRACT

Statement of Claims: For the control or suppression of Green mirids, Silver leaf white fly (Biotype b) and Heliothis in cotton as specified in the Directions for Use table.

Net Contents: 1–1000 L

Restraints: DO NOT apply with aircraft

SPRAY DRIFT RESTRAINTS
DO NOT apply with spray droplets smaller than a FINE spray droplet size category according to nozzle manufacturer specifications that refer to the ASAE S572 Standard or the British Crop Production Council guideline.
DO NOT apply when wind speed is less than 3 or more than 20 kilometres per hour, as measured at the application site.
DO NOT apply during surface temperature inversion conditions at the application site.
Users of this product MUST make an accurate written record of the details of each spray application within 24 hours following application, and must KEEP this record for at least 2 years. The spray application details that must be recorded are: 1 date with start and finish times of application, 2 location address and paddock(s) sprayed, 3 full name of this product, 4 amount of product used per hectare and number of hectares applied to, 5 crop or situation and weed or pest, 6 wind speed and direction during application, 7 air temperature and relative humidity during application, 8 nozzle brand, type, spray angle, nozzle capacity and spray system pressure measured during application, 9 name and address of person applying this product. (Additional record details may be required by the state or territory where this product is used.)
MANDATORY NO-SPRAY ZONES
DO NOT apply if there are aquatic and wetland areas including aquacultural ponds, surface streams and rivers within 5 metres downwind from the application area.

DIRECTIONS FOR USE

QLD and NSW only

CROP	**PEST**	**RATE**	**CRITICAL COMMENTS**
Cotton	Cotton bollworm (helicoverpa armigera) Native budworm (helicoverpa punctigera) Silverleaf whitefly (biotype b) (bemisia tabaci) Green mirid (creontiades dilutus)	2 L / ha	Apply as indicated by field checks and pest presence thresholds to a maximum of 5 applications per growing season. Ensure good coverage. Note treatment effects may not be seen for 3 or more days. Suppression of pest numbers rather than control may occur if sufficient exposure is not possible **Budworm and Bollworm:** Applications should be timed to coincide with egg hatch and when small larvae up to 5 mm are present. A second application at 7 days may

be required if conditions favour pest development.
Silver leaf white fly, Green mirid: Apply at recommended threshold levels as indicated by field checks. A repeat application may be required at 14–20 days if conditions favour pest development.

Withholding Periods: HARVEST: Not required when used as directed.

General Instructions:

Sero-X Insecticide contains the active constituent *Clitoria ternatea* extract. *Clitoria ternatea* extract includes many different biologically active compounds which in combination reduce the economic damage caused by target pests through insecticidal and behaviour modification activity such as egg laying disruption and anti feeding. Sero-X Insecticide does not rely solely on direct mortality to provide crop protection. It can be used as a protective treatment when applied at regular intervals or as a knockdown treatment to control existing pests. Best results are obtained when Sero-X Insecticide is applied before pest populations build up to damaging levels. This product is suitable for use in Integrated Pest Management (IPM) programs. Effects on insect predators or parasites have been demonstrated as low.

The biological extract in Sero-X is comprised of many bioactive compounds and as a result will have different modes of action. The likelihood of resistance developing to all of the bioactive compounds is considered low. However, as the product requires repeated treatment for good results, it is suggested that this product is incorporated into an Integrated Pest Management plan where insect management products are rotated during the growing season to minimise resistance development to any insecticide product being used.

MONITORING

Detailed checks of pest numbers as per best practice pest management requirements are recommended to ensure application can be made at the earliest suitable time to achieve the best result.

MIXING

Shake or agitate the container prior to mixing with water. Add the required quantity of Sero-X Insecticide to clean water in half filled spray tank with agitator or by-pass in operation. Maintain agitation while filling tank with remainder of water. Agitation must also be maintained throughout the spray operation.

COMPATIBILITY

Sero-X Insecticide is an emulsifiable concentrate and is likely compatible with commonly used organic liquid fertilizers. Always check the physical compatibility with other products using a jar test in the correct proportions.

SURFACTANTS

Sero-X Insecticide contains a surfactant. Additional surfactant such as esterified vegetable oils may only be necessary on hard to wet plants. Use as per surfactant label instructions.

APPLICATION

Sero-X Insecticide may be applied by ground rig. Thorough coverage is essential to ensure adequate control. Applications should be made using nozzles, pressures and other spray conditions to produce a fine / medium spray. An application volume of 50-200 L/ha is recommended.

Protections: PROTECTION OF WILDLIFE, FISH, CRUSTACEANS AND THE ENVIRONMENT

DO NOT contaminate streams, rivers or waterways with the product or the used containers. Tail drains which flow from treated areas should be prevented from entering river systems.

Storage and Disposal: Store in the closed, original container in a cool, well-ventilated area. Do not store for prolonged periods in direct sunlight. Triple-rinse containers before disposal. Add rinsings to spray tank. Do not dispose of undiluted chemicals on site. If not recycling, break, crush, or puncture and deliver empty packaging to an approved waste management facility. If an approved waste management facility is not available, bury the empty packaging 500 mm below the surface in a disposal pit specifically marked and set up for this purpose, clear of waterways, desirable vegetation and tree roots, in compliance with relevant local, state or territory government regulations. Do not burn empty containers or product.

Safety Directions: Will irritate eyes. Avoid contact with eyes. When opening the container and preparing spray and using prepared spray, wear cotton overalls buttoned to the neck and wrist (or equivalent clothing), elbow length chemical resistant gloves and goggles. If product in eyes, wash it out immediately with water. Wash hands after use. After each day's use, wash gloves, goggles and contaminated clothing.

First Aid Instructions: If poisoning occurs, contact a doctor or Poisons Information Centre. Phone Australia 13 11 26.

ABBREVIATIONS

ac	active constituent
ADI	Acceptable Daily Intake (for humans)
AHMAC	Australian Health Ministers Advisory Council
ai	active ingredient
ANOVA	Analysis of Variance
ARfD	Acute Reference Dose
bw	bodyweight
^{o}C	Degrees Celsius
CAS	Chemistry Abstracts Service
cST	Centistokes (physical unit for kinematic viscosity)
d	day
DAT	Days After Treatment
DNA	Deoxyribonucleic acid
EC	Emulsifiable Concentrate
EC_{50}	concentration at which 50% of the test population are immobilised
E_rC_{50}	concentration at which the rate of growth of 50% of the test population is impacted
EI	Export Interval
Eq	equivalent
EU	European Union
g	gram
GLP	Good Laboratory Practice
h	hour
ha	hectare
HDPE	High Density Polyethylene
HR	Highest Residue

HSIS	Hazardous Substances Information System (Safe Work Australia)
im	intramuscular
IPM	Integrated Pest Management
iv	intravenous
in vitro	outside the living body and in an artificial environment
in vivo	inside the living body of a plant or animal
kg	kilogram
L	Litre
LC_{50}	concentration that kills 50% of the test population of organisms
LD_{50}	dosage of chemical that kills 50% of the test population of organisms
LOAEL	Lowest Observable Adverse Effect Level
LOEL	Lowest Observable Effect Level
LR_{50}	tissue concentration that causes mortality of 50% of the test population of organisms
LSD	Least Significant Difference
mg	milligram
mL	millilitre
MoA	Mode of Action
MOE	Margin of Exposure
MRL	Maximum Residue Limit
MSDS	Material Safety Data Sheet (see also SDS)
ND	Not Detectable
NOEC/NOEL	No Observable Effect Concentration Level
NOHSC	National Occupational Health and Safety Commission
OC	Organic Carbon
OCS	Office of Chemical Safety
OD	Oil Dispersion (oil-based suspension concentrate)

OECD	Organisation of Economic Cooperation and Development
OM	Organic Matter
Pa	Pascals
PEC	Predicted Environmental Concentration
pH	Measure of acidity/alkalinity of an aqueous solution
PHED	Pesticide Handler Exposure Database
po	oral
PPE	Personal Protective Equipment
ppm	parts per million
RNA	Ribonucleic acid
s	second
sc	subcutaneous
SDS	Safety Data Sheet (see also MSDS)
SUSDP	Standard for the Uniform Scheduling of Drugs and Poisons
SUSMP	Standard for the Uniform Scheduling of Medicines and Poisons
SWA	Safe Work Australia
µg	microgram
UV	Ultra Violet light
WHP	Withholding Period
w/v	Weight/volume

GLOSSARY

Active constituent	The substance that is primarily responsible for the effect produced by a chemical product
Acute	Having rapid onset and of short duration.
Carcinogenicity	The ability to cause cancer
Chronic	Of long duration
Codex MRL	Internationally published standard maximum residue limit
Desorption	Removal of a material from or through a surface
Efficacy	Production of the desired effect
Formulation	A combination of both active and inactive constituents to form the end use product
Genotoxicity	The ability to damage genetic material
Metabolism	The chemical processes that maintain living organisms
Subcutaneous	Under the skin
Toxicokinetics	The study of the movement of toxins through the body
Toxicology	The study of the nature and effects of poisons

REFERENCES

National Occupational Health and Safety Commission (2004). *NOHSC Approved Criteria for Classifying Hazardous Substances*.

SWA (2016). Safe Work Australia (SWA), *Hazardous Substances Information System (HSIS) Database*, SWA, Canberra, 2015. Available at: hsis:safeworkaustralia.gov.au/

US EPA (1998). United States Environmental Protection Agency (US EPA). *The Pesticide Handlers Exposure Database (PHED), version 1.1-PHED Surrogate Exposure Guide, Estimates of Worker Exposure.* US EPA, Washington DC, United States, 1998.