Australian Government

Australian Pesticides and Veterinary Medicines Authority

PUBLIC RELEASE SUMMARY

on the evaluation of the new active Bacillus thuringiensis subsp. kurstaki Cry1Ac (synpro) gene and Bacillus thuringiensis subsp. aizawai Cry1F (synpro) gene and their controlling sequences in the product Widestrike Insect Protection Cotton Event 281-24-236/3006-210-23

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PREFACE

The Australian Pesticides and Veterinary Medicines Authority (APVMA) is the Australian Government regulator with responsibility 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 Health and Ageing, Office of Chemical Safety (OCS), Department of Sustainability, Environment, Water, Population and Communities (DSEWPaC previously Department of Environment, Water, Heritage and Arts DEWHA), and State Departments of Primary Industries.

The APVMA has a policy of encouraging openness and transparency in its activities and of 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 undertaken according to accepted scientific principles. Details are outlined in the APVMA's publications *Ag MORAG: Manual of Requirements and Guidelines* and *Vet MORAG: Manual of Requirements and Guidelines*.

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 thereby encouraging public comment.

About this document

This is a Public Release Summary.

It indicates that the Australian Pesticides and Veterinary Medicines Authority (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 persons on the information contained within this document.

Making a submission

In accordance with sections 12 and 13 of the Agvet Code, the APVMA invites any person to submit a relevant written submission as to whether the application for registration of **WIDESTRIKE INSECT PROTECTION** 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 grounds are **public health aspects, occupational health and safety, chemistry and manufacture, residues in food, environmental safety, trade** and **efficacy**. Submissions should state the grounds on which they are based. Comments received outside these grounds cannot be considered by the APVMA.

Submissions must be received by the APVMA by close of business on **Tuesday 22/11/2011** 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)
- Postal Address
- Email Address (if available)
- The date you made the submission.

All personal and ***confidential commercial information (CCI)***[1] material 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:

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

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Further information

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

Copies of full technical evaluation reports covering toxicology, occupational health and safety aspects, residues in food and environmental aspects are available from the APVMA on request.

Further information on public release summaries can be found on the APVMA website: http://www.apvma.gov.au

1 INTRODUCTION

The Australian Pesticides and Veterinary Medicines Authority (APVMA) has before it an application from Dow AgroSciences Australia Ltd for registration of a new product, Widestrike Insect Protection containing the new active constituent *Bacillus thuringiensis subsp. kurstaki Cry1Ac* (synpro) gene and *Bacillus thuringiensis subsp. aizawai Cry1F* (synpro) gene and their controlling sequences.

Cotton is an important fibre crop and lepidopteran insects are the main insect problem for that crop. Dow AgroSciences Australia Ltd is seeking registration of this product to provide the Australian cotton industry with an alternative mode of action in transgenic cotton and as an alternative supplier of transgenic cotton seed.

Widestrike Insect Protection is for the control of lepidopteran insect pests in cotton.

Widestrike Insect Protection is a genetically modified cotton plant that confers resistance of the cotton to lepidopteran insect pests.

Bacillus thuringiensis subsp. kurstaki Cry1Ac (synpro) gene and *Bacillus thuringiensis subsp. aizawai Cry1F* (synpro) gene and their controlling sequences are currently licensed for release by the OGTR, DIR 091/2008. Oil and linters derived from this GM cotton have been approved by Food Standards Australia New Zealand for use in human food. Widestrike cotton has been registered for use in the U.S.A. since 2004. Widestrike Insect Protection has food import approval for Mexico, Canada, Japan and Korea.

This publication provides a summary of the data reviewed and an outline of the regulatory considerations for the proposed registration of Widestrike Insect Protection, and approval of the new active constituent, *Bacillus thuringiensis subsp. kurstaki Cry1Ac* (synpro) gene and *Bacillus thuringiensis subsp aizawai Cry1F* (synpro) gene and their controlling sequences.

2 CHEMISTRY AND MANUFACTURE

2.1 Active Constituent

The chemical active constituent of Widestrike Insect Protection is *Bacillus thuringiensis* subsp*. kurstaki Cry1Ac* (synpro) gene and *Bacillus thuringiensis subsp. aizawai Cry1F* (synpro) gene and their controlling sequences.

The APVMA has evaluated the chemistry aspects of *Bacillus thuringiensis* subsp. *kurstaki* delta endotoxin as produced by the *Cry1Ac* (synpro) gene and its controlling sequence and *Bacillus thuringiensis* subsp. *aizawai* delta endotoxin as produced by the*Cry1F* (synpro) gene and its controlling sequence, including the manufacturing/transformation process, quality control procedures and analytical method, and found them to be acceptable.

For additional information relating to the genes and methods used to transfer the genes to cotton, please refer to the Office of the Gene Technology Regulator (OGTR) Risk Assessment and Risk Management Plan (RARMP) for DIR 0912/2009 involving Widestrike Insect Protection (see http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir091). A brief summary of the APVMA assessment is provided below.

Manufacture

Widestrike Insect Protection contains genes derived from the common soil bacterium *Bacillus thuringiensis* (Bt), a non-pathogenic bacterium *Streptomyces viridochromogenes*, corn (*Zea mays*) and *Agrobacterium tumefacien.* The genes from *Bacillus thuringiensis* are synthetic genes; *cry1Ac (synpro)* and *cry1F (synpro)* that encode the Bt toxins Cry1Ac and Cry1F.

The *cry1F* (synpro) gene is composed of parts of the *cry1Fa*, *cry1Ca3* and *cry1Ab1* genes and encodes the insecticidal protein, Cry1F, and is derived from the common soil bacterium *Bacillus thuringiensis* subsp. *aizawai*. Cry1F protein exhibits insecticidal activity to specific lepidopteran caterpillar insects.

The *cry1Ac* (synpro) gene is composed of part of the *cry1Ac*, *cry1Ca3* and *cry1Ab1* genes and encodes the insecticidal protein, Cry1Ac, and is derived from the common soil bacterium *Bacillus thuringiensis* subsp. *kurstaki*. Cry1Ac protein exhibits insecticidal activity specific to specific lepidopteran caterpillar insects.

The *pat* gene encodes phosphinothricin acetyltransferase (PAT) derived from a non-pathogenic bacterium *Streptomyces viridochromogenes*. PAT is a plant selectable marker conferring tolerance to herbicide glufosinate-ammonium.

The controlling sequences are derived from corn (*Zea mays)* for *ubiZm1* and *Agrobacterium tumefaciens* for *4OCSmas2* and *ORF25.* These genes are responsible for the optimal expression of Cry1F and Cry1Ac proteins in transgenic plants. Although *A.*

tumefaciens is a plant pathogen, the controlling sequences comprise only a small part of its total genome, and are not in themselves capable of causing disease.

The *cry 1F* (synpro) gene in combination with the *pat* gene was introduced into cotton genome by *Agrobacterium*-mediated transformation to create transgenic cotton event 281-24-236. The *cry1A* (synpro) gene in combination with the *pat* gene was introduced into cotton genome by *Agrobacterium*-mediated transformation to create transgenic cotton event 3006-210-23, Cotton events 281 and 3006 were then crossed to generate Widestrike cotton event Cotton Event 281-24-236/3006-210-23 (Cotton event 281/3006).

Molecular Characterization of Transgenic Cotton Event 281/3006

Genomic DNA extracted from the cotton was assayed by Southern blot analysis to determine the integration, copy number and integrity of the inserted genes. Studies show that the cotton contained a single integration of transgenic DNA from *cry1F (synpro)* and *cry1A (synpro)* transgenes. The insert from Cry1F event 281 contained one intact copy of *cry1F (synpro)* and *pat* genes. The insert from Cry1Ac event 3006 contained one intact copy of *cry1A (synpro)* and *pat* genes. Expression of the Cry1F and Cry1Ac proteins in transgenic plants was confirmed by ELISA.

Stability of Gene Insertion in Transgenic Cotton Plants

Stable inheritance of the inserted *cry1F* (synpro) and *cry1Ac* (synpro) genes in the transgenic cotton was analysed to compare the frequency of observed to expected number of progeny that expressed Cry1F and Cry1Ac proteins determined by ELISA. The studies showed that the transgenic cotton were tolerant to the herbicide glufosinate ammonium and yielded the expected segregation ratios for the different crossings in relation to the Cry1F and Cry1Ac proteins. Genetic stability was also confirmed by Southern blot analysis of the inserted genes across two generations.

Executive Summary for Chemistry and Manufacture

Adequate information has been provided to demonstrate the method by which the active constituent (the endotoxin) and the product (the genes and their controlling sequences) have been produced. Assessment of the analysis of the genetic modification and the protein produced in cotton found that no additional genetic materials or proteins of concern were produced via the introduction of the *cry1F* (synpro) and *cry1Ac* (synpro) genes and their controlling sequences. The stability of the inserted genes was found to be acceptable as the presence and position of the genes were unchanged after two generations.

Recommendation

Based on a review of the data provided by the applicant, the APVMA is satisfied that the chemistry and manufacturing details of *Bacillus thuringiensis* subsp*. kurstaki Cry1Ac* (synpro) gene and *Bacillus thuringiensis* subsp*. aizawai Cry1F* (synpro) gene and their controlling sequences are acceptable.

3 TOXICOLOGICAL ASSESSMENT

3.1 Summary

Public Health Aspects

Bacillus thuringiensis (*B. thuringiensis*) is a gram-positive sporulating bacterium used extensively as a biological insecticide. The bacterium exerts its insecticidal activity through the production of proteinaceous crystal inclusion bodies (Cry delta-endotoxin proteins) during sporulation, which disrupt the osmotic regulation of cellular membranes in the midgut cells of insect pests, resulting in gut paralysis, starvation, septicaemia and eventual death.

The product Widestrike Insect Protection consists of transgenes, which are inserted into cotton to control *in plant* protein expression of two Cry delta-endotoxins, Cry1F (derived from *B. thuringiensis* var. *aizawai*) and Cry1Ac (derived from *B. thuringiensis* var. *kurstaki*). These Cry proteins are used as an insecticide to protect against infestation of cotton crops by cotton bollworm (*Heliothis zea*), tobacco budworm (*Heliothis virescens*) and pink bollworm (*Pectinophora gossypiella*).

B. thuringiensis bacteria were not pathogenic or infective in rodent species, and are of low acute oral, dermal and inhalational toxicity. Bacteria were non-sensitising. Skin and eye irritancy potential of the bacteria are unknown; however, formulated products containing *B. thuringiensis* bacterial strains were generally considered slight to moderate eye irritants and slight skin irritants.

Repeat-dose oral toxicity studies did not reveal clinical signs of toxicity, and physical, clinical and histopathological parameters were similar to control animals. Short-term inhalation studies reported findings of bronchial/interstitial pneumonia and histopathological effects in lung tissue; however, these findings were considered equivocal due to the nature of the formulated material used in the study. Overall, the repeat-dose toxicity potential of *B. thuringiensis* strains is considered to be low.

B. thuringiensis bacteria were not mutagenic and did not express any exotoxins of potential health concern. Human epidemiological studies and worker exposure data reported no ill effects attributable to exposure over extended timeframes; however, minor dermal and ocular irritation was recorded in one study, consistent with the toxicological profile of *B. thuringiensis* formulations.

The toxicological profile of the product Widestrike Insect Protection, containing the Cry1F (synpro) and Cry1Ac (synpro) transgenes inserted into the cotton plant, is expected to be identical to non-transgenic cotton material, with minimal toxicological hazards attributable to the presence of the Cry delta-endotoxins in transgenic cotton seeds and other plant material.

Occupational Health and Safety

Workers may be exposed to transgenic seed during planting, and to plant material containing the transgenes during normal cotton crop maintenance and harvesting.

Based on the risk assessment conducted, exposure to Cry delta-endotoxin proteins is expected to be minimal, as cotton seeds used in planting do not contain these proteins, and human contact with transgenic cotton plants is primarily limited to plant surfaces, seed coats or cotton fibres which are essentially free of the delta-endotoxin proteins. Given the low hazards associated with use of the product, minimal occupational health and safety concerns are expected.

Conclusion

Based on an assessment of the toxicology and occupational health and safety, it was considered that there should be no adverse effects on human health from the use of Widestrike Insect Protection when used in accordance with the label directions.

3.2 Evaluation of toxicology

The toxicological database for *Bacillus thuringiensis* bacteria and associated bacterial products, which consists primarily of toxicity tests conducted using animals, is quite extensive. In interpreting the data, 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 No-Observable-Effect-Level (NOEL) are used to develop acceptable limits for dietary or other intakes (ADI and ARfD) at which no adverse health effects in humans would be expected.

Toxicokinetics and Metabolism

White mice injected with *B. thuringiensis* var. *kurstaki* by the intra-peritoneal route showed no bacterial presence in blood at 72 hours post-injection.

After administration of *B. thuringiensis* var. *aizawai* by the intra-venous route in rats, a moderate number (up to 10^4 colony forming units (cfu)/g) of viable *B. thuringiensis* bacteria

were found in the spleen after 66 days. Administration of an acute dose of *B. thuringiensis* (10^8 cfu) by the intra-tracheal route resulted in a detectable number of bacteria in the lungs up to 35 days, and in kidney and spleen for up to 14 days. Single dose oral administration of *B. thuringiensis* (~ 10^{11} cfu) to rats indicated that complete clearance of bacteria was achieved from 10 to 24 days post-dosing. In each case, cfu counts decreased with time, and no signs of infectivity or pathogenicity were observed.

In *in vitro* simulated gastric fluid, *B. thuringiensis* delta-endotoxin protein was rapidly digested and degraded into non-active amino acid fragments within 1 minute of incubation. In *in vitro* simulated intestinal fluid, *B. thuringiensis* delta-endotoxin degrades to the stable enzyme-resistant toxin core, with insecticidal activity decreased by 95 % after 24 hours of exposure.

Acute toxicity studies

Studies on a number of TGAC *B. thuringiensis* var. *kurstaki* strains indicated that the viable spores were of low acute oral (LD_{50} > 5000 mg/kg bw), low acute dermal (LD_{50} > 2000 mg/kg bw) and low acute inhalational toxicity (LC_{50} > 3190 mg/m^3). It was a slight eye irritant in rabbit eye, a non-irritant on rabbit skin and a non-sensitiser in guinea pig. Acute sub-cutaneous (~10^7 cfu), intra-peritoneal (~10^8 cfu), intra-venous (~10^{11} cfu) and intra-tracheal (~10^9 cfu) injections in rats did not produce clinically significant signs of toxicity.

Cry1Ac and Cry2Ab2 delta-endotoxins from *B. thuringiensis* var. *kurstaki* expressed in a transgenic cotton plant (Cotton event 15985) were shown to be chemically and biologically equivalent to bacterially produced delta-endotoxins, which were of low acute oral toxicity in mice (LD_{50} > 4000 mg/kg bw). Cottonseed meal and refined oil products produced from this transgenic cotton plant did not contain detectable levels of either delta-endotoxin, while whole plant material contained up to 17 mg/kg dry weight of the delta-endotoxins.

Acute toxicological studies conducted with TGAC *B. thuringiensis* var. *aizawai* bacteria indicated that this strain was of low toxicity and pathogenicity in rodent species when delivered by intra-venous, intra-tracheal, intra-peritoneal and subcutaneous routes. Products formulated with *B. thuringiensis* var. *aizawai* (at 540 g/kg) as the active constituent were of low acute oral (LD_{50} > 5000 mg/kg bw) and inhalational toxicity (LC_{50} > 3050 mg/m^3) in rats, low dermal toxicity (LD_{50} > 2000 mg/kg bw) in rabbits, and slight skin and moderate eye irritants in rabbits.

Acute oral toxicity studies conducted using microbial protein mixtures containing purified Cry1Ac and Cry1F were of low toxicity (LD_{50} > 700 mg/kg bw for Cry1Ac and > 375 mg/kg bw Cry1F, with no clinical signs or mortalities).

Repeat dose studies

A study carried out by the Canadian Wildlife Service in 1961 revealed that technical grade *B. thuringiensis* var. *kurstaki* was of low toxicity in rats, guinea pigs and mice after 5 days

oral administration of a 24 % suspension, or as an active broth culture (in which case there was no evidence of infection). A further study in rats showed that there was little difference in sub-acute toxicity when the substance was given by gavage or in the diet, but the former gave more reliable control of dose given.

Albino guinea pigs were exposed to dust containing ~5 × 10^9 spores/g of *Bacillus thuringiensis* subsp. *kurstaki* for 6 hours/day, 5 days/week for 4 consecutive weeks at mean exposure concentrations of 0, 1 and 10 g/m^3. One high dose male died during the experimental period, 2 high dose females lost weight during weeks 3 and 4 and one of these females died immediately prior to sacrifice. Four males and four females in the high dose group had white spots on one or both of their eyes immediately prior to sacrifice. The relative and absolute adrenal weights in high dose animals were 40 to 50 % lower than controls. An increased incidence of dark spots and areas of consolidation on the lungs of low and high dose animals was observed at necropsy; otherwise gross pathology observation did not reveal any clearly treatment related abnormalities. Microscopic examination revealed an increased incidence of bronchial and/or interstitial pneumonia in treated animals. The severity of the pneumonia in the low dose groups was comparable to the control but was more pronounced in the high dose groups. As controls received only filtered air, non-specific effects from the chronic inhalation of dust may have contributed to the observations in treated groups.

B. thuringiensis var. *kurstaki* was given to rats by oral gavage at daily doses of 0, 1–3 × 10^7; 1–3 × 10^8 or 1–3 × 10^9 spores/kg bw/day for 13 weeks. There was little evidence of any toxicity caused by the treatment. At the highest dose there was a 35–40 % increase in white blood cells and an increase in serum glucose. A relative and significant increase in heart weight was noted in females at terminal sacrifice, for all doses of the bacillus. Infectivity analyses showed only very few numbers of the bacteria in organs outside the gastrointestinal tract and only in the case of the highest dose group were any substantial numbers found in the small intestine. Because of the effect on the female heart at all doses, it was difficult to set a NOEL. The NOEL in males was 1–3 × 10^8 spores/kg bw/day.

Rats were administered *B. thuringiensis (Berliner)* var. *kurstaki* at 0, 0.84/840, and 8.4/8400 mg/kg bw/day in their diet (the lower dosage in each treatment group was administered in the first week only). No animal died during the study and no treatment related abnormalities were observed in clinical signs, weight gain, food consumption, haematology, urinalysis, blood biochemistry, gross pathology, or microscopic pathology. An increase in the relative heart weight was observed in high dose females. *B. thuringiensis* was not pathogenic in this study.

A *B. thuringiensis* formulation was administered to groups of albino rats at doses of 0 and 8.4 g/kg bw/day for 104 weeks, incorporated into the basal diet. Body weights in treated males and females were approximately 10 % below controls by the end of the study. Treated females had twice the incidence of chromophobe adenoma of controls although this was reportedly within historical control values. All other tumour incidences were comparable between treated and control groups. Despite the age and experimental design limitations of this study, the conclusions that *B. thuringiensis* in the diet was not pathogenic

in rats at this dose, and that it did not affect survival over a two year period are reasonable. Because of those limitations however, the data is insufficient to provide other than weak support to the conclusion that *B. thuringiensis* is unlikely to be carcinogenic at doses or probable exposure levels encountered by users or by consumers of treated produce.

Genotoxicity Studies

Two mutagenicity studies (Ames tests) were conducted with a product containing *B. thuringiensis* (unknown strains). Neither test elicited a mutagenic response from exposure to the bacteria.

Special Studies

A trial was reported in which culture supernatants prepared from *B. thuringiensis* var. *kurstaki* Strain HD-1, serotype 3a and 3b were administered orally, intra-peritoneally or intravenously to mice. No deaths occurred, and there appeared to be no gross pathological effects, indicating that this strain of *B. thuringiensis* does not produce a toxic exotoxin.

A number of studies are also reported on the effect of *B. thuringiensis* on production workers at the manufacturing plant of Pacific Yeast Products Inc., in California. Medical records of personnel involved in production for 1–20 years showed no illness attributable to the working environment. In addition, 6 volunteers exposed for a total of 289 hours to the bacillus during production of a microbial insecticide showed no ill-effects. In a further study, 18 volunteers ingested 1 g of microbial insecticide daily for 5 days and five of the subjects also inhaled 100 mg of powder daily. No ill-effects were observed immediately after exposure or during a 4-5 week follow-up period.

An epidemiological study was conducted during an aerial spray program in Oregon, USA, over 2 years (1985–86). A total of 120,000 people lived in the spray areas and were potentially exposed to the bacteria. Cultures taken for routine purposes were examined for the presence of *B. thuringiensis* var. *kurstaki*. A total of 55 specimens grew *B. thuringiensis* var. *kurstaki*. In 52 of these, the bacterium was judged to be a contaminant or a commensal rather than a pathogen. In the other three cases, *B. thuringiensis* var. *kurstaki*. could not be ruled out as the causative agent. However, no illness was directly attributed to the bacteria.

An extensive epidemiological study was conducted in Vancouver during a 1992 aerial and ground spray program with *B. thuringiensis* var. *kurstaki*. The population studied comprised 1,400,000 people. Results indicated that there were no illnesses in the general population that could be linked to the spray program. Almost two-thirds of exposed workers (compared with one-third of 'non-exposed controls') developed symptoms of headache, nose, throat and eye irritation, dry skin and chapped lips. Some remained culture-positive for 'prolonged periods of time', but most individuals remained culture-positive for 'little more than a few days'. However, no days of work loss were attributable to *B. thuringiensis* var. *kurstaki*. It was also demonstrated that the public was readily exposed

to the bacterium through consumption of commercially available fresh vegetables, “either organically or conventionally grown”.

Exposure of eight employees manufacturing the active constituent and end-use products during a 7 month period of observation did not result in any ill effects.

Fleece from sheep treated with a concentrate, containing 2.9×10^6 spores of *Bacillus thuringiensis* var*. thuringiensis* (serotype 1) per gram of fleece, did not produce cumulative irritation of the skin of human subjects after 14 days of contact under an occlusive dressing.

In a published review of data submitted to the US FDA during 1958 for the registration of a preparation of *B. thuringiensis* var. *kurstaki* and diatomaceous earth, the TGAC was found not to be pathogenic in serial passages through mice, after oral administration to rats, or inhalation by humans or mice, and not to persist in the blood of mice beyond 48 hours following intra-peritoneal administration. *B. thuringiensis* was not more persistent than non-pathogenic *B. cereus* in mice. Death in guinea pigs was caused only by massive parenteral doses (quantitative figures not stated) of *B. thuringiensis*.

In planta studies

Comparison of Cry1Ac and Cry2Ab2 delta-endotoxins expressed in transgenic cotton plants with native delta-endotoxins from *B. thuringiensis* indicated that the delta-endotoxin from the different sources are essentially identical. delta-endotoxins expressed in transgenic plants were considered biologically and chemically equivalent to those found in bacterially-derived formulations.

3.3 Public Health Standards

Poisons Scheduling

Bacillus thuringiensis is listed in Appendix B of the Standard for the Uniform Scheduling of Medicines and Poisons (SUSMP).

NOEL/ADI/ArfD

The Acceptable Daily Intake is that quantity of an agricultural 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 factor which reflects the quality of the toxicological database and takes into account the variability in responses between species and individuals.

The acute reference dose is the maximum quantity of an agricultural or veterinary chemical that can safely be consumed as a single, isolated, event. The ARfD is derived from the lowest single or short term dose which causes no effect in the most sensitive species of experimental animal tested, together with a safety factor which reflects the

quality of the toxicological database and takes into account the variability in responses between species and individuals.

The establishment of an ADI or ARfD is not considered necessary, as the Cry1 delta-endotoxins will not be present in processed cotton products used for human consumption. Furthermore, delta-endotoxins from *B. thuringiensis* var. *kurstaki*, and viable *B. thuringiensis* var. *aizawai* and *kurstaki* strains, whose mode of action is through the production of Cry delta-endotoxins, are currently listed in Table 5 of the MRL Standard (i.e. maximum residue limits are not necessary), when for use as an insecticide on crops.

4 RESIDUES ASSESSMENT

4.1 Introduction

The Cry1Ac gene is included in another variety of genetically modified cotton which is currently registered in Australia (Bollgard II Cotton Event 15985, product number 55786). After reviewing the available toxicological data the TGA determined that no ADI or ARfD were necessary as the *Bacillus thuringiensis kustaki* delta endotoxin Cry1Ac would not be found in commodities available for human consumption (processing to cotton seed oil and linters removes or deactivates all proteins). There were no residue concerns for animal commodities resulting from feeding of recombinant cotton to livestock as Cry proteins are rapidly degraded in gastric fluid. The following Table 5 entry was therefore established to account for the presence of *Bacillus thuringiensis kustaki* delta endotoxin protein in recombinant cotton.

Table 5

COMPOUND	USE
Bacillus thuringiensis kurstaki delta endotoxin protein	Insecticide expressed in recombinant cotton

The Cry1F gene encodes for an insecticidal delta-endotoxin produced by *Bacillus thuringiensis aizawai.* As for the Cry1Ac protein, it can be argued that the Cry1F endotoxin is unlikely to occur in commodities for human consumption (cotton seed oil and linters). In addition, the bacterial strain that produces the Cry1F protein is currently registered as an insecticide in Australia (eg. Product number 53435). With respect to the genetically modified cotton, the US EPA has established an exemption from the requirement of a tolerance for residues of Bacillus thuringiensis var. aizawai strain PS811 (Cry1F insecticidal protein) and the genetic material necessary for its production in cotton when applied/used as a plant-incorporated protectant. The EPA noted a lack of mammalian toxicity at high levels of exposure to the pure Cry1F protein and that the Cry1F protein is rapidly degraded by gastric fluid. FSANZ have also conducted an assessment of the genetically modified cotton line (MXB-13) which included consideration of (i) the genetic modification to the plant; (ii) the safety of any transferred antibiotic resistance genes; (iii) the potential toxicity and allergenicity of any new proteins; and (iv) the composition and nutritional adequacy of the food. No potential public health or safety concerns were identified by FSANZ who concluded that food (oil and linters) derived from the cotton line was as safe and wholesome as food from other cotton varieties. As the food was highly refined it would contain no novel DNA or proteins. FSANZ therefore amended Standard 1.5.2 to include oil and linters derived from insect-protected, glufosinate ammonimum-tolerant cotton line MXB-13.

As the Cry1F protein will not occur in food for human consumption and transfer of its residues to animal commodities through feeding of recombinant cotton to livestock should

not be an issue due to its rapid digestion in gastric fluid, no further residues assessment of Cry1F is required. However, it is recommended that a Table 5 entry be established to cover the use of Cry1F as an insecticide in recombinant cotton.

The PAT gene included in Widestrike Insect Protection cotton enables the plant to rapidly metabolise the herbicidally-active glufosinate ammonium into a relatively non-herbicidally active metabolite, N-acetyl glufosinate. The applicant has stated that the PAT gene was only included to allow selection during breeding. It is not present to allow glufosinate to be used as a herbicide during the growth of the cotton plant. In any case the residue definition for glufosinate-ammonium was previously amended to include N-acetyl glufosinate due to its possible formation by genetically modified canola containing the PAT gene.

As the Cry1F protein should not occur in commodities for human consumption and transfer of residues to animal commodities through feeding of recombinant cotton to livestock should not be an issue, a detailed residues assessment of Widestrike Insect Protection is not required. An entry to Table 5 of the MRL Standard is recommended to cover the use of the Cry1F protein as an insecticide in recombinant cotton. The following amendment is recommended to the MRL Standard:

Table 5

COMPOUND	USE
ADD:	
Bacillus thuringiensis aizawai delta endotoxin protein	Insecticide expressed in recombinant cotton

5 ASSESSMENT OF OVERSEAS TRADE ASPECTS OF RESIDUES IN FOOD

Cottonseed and meal are significant export commodities. In 2008-9 Australia exported 37 kt of cottonseed, 9.98 kt cottonseed oil and 10.72 kt of cottonseed and sunflowerseed oildseed meal (Australian Commodity Statistics 2009, ABARE).

Cattle, pigs, sheep and poultry are all significant export commodities. All of these species may consume straw, forage and/or grain from cotton crops containing Widestrike Insect Protection.

5.1 Overseas registration status

The United States, Canada, Mexico, Korea and Japan have assessed Widestrike Insect Protection cotton with respect to the risks from consuming the resultant food commodities. The United States determined that the *Cry1Ac (synpro)* and Cry1F (synpro) proteins and the genetic material necessary for its production in cotton are exempt from the requirement of a tolerance when used as plant-pesticides in the food and feed commodities of cotton seed, cotton oil, cotton meal, cotton hay, cotton hulls, cotton forage, and cotton gin by-products'. Japan, Canada, Mexico and Korea have accepted WideStrike Insect Protection cotton as safe for human consumption.

Potential risk to trade

As the U.S.A, Canada, Japan, Mexico and Korea have established exemptions for commodities originating from WideStrike Insect Protection cotton, no undue prejudice to trade exists from the export of commodities incorporating WideStrike Insect Protection cotton.

In addition, as Australia has been exporting food commodities resultant from the use of other transgenic Bt cotton without prejudice due to its genetically engineered origins, no undue prejudice to Australia's trade with other countries is expected from the use of Widestrike Insect Protection in cotton.

6 OCCUPATIONAL HEALTH AND SAFETY ASSESSMENT

6.1 Health hazards

Bacillus thuringiensis Cry1Ac and Cry1F delta-endotoxins are not listed on the Safe Work Australia (SWA) Hazardous Substances Information System (HSIS) Database (SWA, 2009).

With the available toxicology information, OCSEH classifies *Bacillus thuringiensis* Cry1Ac and Cry1F delta-endotoxins as non-hazardous substances according to NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

Based on the product toxicology information and concentrations of active and other ingredients, transgenic cotton plants containing WideStrike Insect Protection are 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

Cotton seeds containing WideStrike Insect Protection will be packaged in 20 kg paper bags. Transport workers and store persons will handle the packaged products, though contact with the Cry1 delta-endotoxin proteins are not expected, as the proteins will not be expressed in seeds for planting.

6.3 Use pattern and Exposure during use

As Widestrike Insect Protection consists of genetic elements directly incorporated into the transgenic cotton genome, there is no direct application method associated with the use of this product apart from planting. Direct exposure to the active constituents (Cry1Ac and Cry1F delta-endotoxins from *Bacillus thuringiensis*) is unlikely to occur except when damaged unprocessed cotton plant material grown from cotton seeds containing Widestrike Insect Protection is handled or ingested. As the level of Cry1 delta-endotoxin active constituents expressed in transgenic cotton is very low (maximum of ~ 25 mg/kg plant weight), the overall exposure to Cry1 delta-endotoxins is considered to be minimal.

6.4 Exposure during re-entry

Post-application exposure to transgenic cotton containing Widestrike Insect Protection will be limited to post-harvest handling of transgenic plant material. As the level of Cry1 delta-endotoxin active constituents expressed in transgenic cotton is very low (maximum of ~ 25 mg/kg plant weight), and direct exposure is limited to the handling of damaged plant material, the overall exposure to Cry1 delta-endotoxins is considered to be minimal.

6.5 Recommendations for safe use

Users should follow the First Aid Instructions and Safety Directions on the product label.

6.6 Conclusion

The approval of synthetic Cry1Ac delta-endotoxin protein (derived from *Bacillus thuringiensis* var. *kurstaki*) and synthetic Cry1F delta-endotoxin protein (derived from *Bacillus thuringiensis* var. *aizawai*), which are expressed in the stacked transgenic cotton plant line 281-24-236/3006-210-23, is supported.

The registration of the product Widestrike Insect Protection, consisting of the transgenes enabling the expression of synthetic Cry1Ac and Cry1F delta-endotoxins in the stacked transgenic cotton plant line 281-24-236/3006-210-23, is supported.

Widestrike Insect Protection 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 MSDS.

7 ENVIRONMENTAL ASSESSMENT

Limited studies on the environmental fate and persistence of the synthetic Cry1Ac (synpro) and Cry1F (synpro) insecticidal proteins in the Australian environment have been provided in the application. To inform this risk assessment, DEWHA has considered data available for other Cry proteins.

The Cry1Ac (synpro) and Cry1F (synpro) proteins in Widestrike Insect Protection cotton will most likely be consumed by target and non-target organisms, either directly through feeding on plant tissue or indirectly through feeding on insects that have fed on the GM cotton. The Cry1 (synpro) proteins will also enter the soil environment through pollen dispersal, root exudation and decomposition of plant materials. Their persistence in the soil environment will depend on a number of factors, as discussed below.

DEWHA has also considered the fate of the Widestrike Insect Protection cotton and the synthetic genes as a means of spreading the insecticidal proteins in the environment and exposing a broader spectrum of non-target organisms than would occur in the cropping situation. The possibility that the insecticidal proteins could confer a selective advantage to the cotton in certain habitats, leading to weediness and persistence in the environment, was also considered

7.1 Environmental fate summary

Amount of Cry toxins entering soil

Cry toxins derived from GM crops enter the soil through decomposition of crop residues, pollen release, sloughing of root cells and root exudates. Some *Bt* plants[2] (corn, rice and potato), but not others (canola, cotton and tobacco) release Cry proteins in root exudates. This disparity is probably due to the different root anatomy and physiology of these plant species.

The concentration of Cry1Ac and Cry1F entering soil under Widestrike Insect Protection cotton cultivation has been estimated at 0.0196 mg a.c./kg soil and 0.317 mg a.c./kg soil respectively under US conditions. These values are likely to be higher in Australian cotton fields as Australian cotton density is higher than in the USA, and the level of expression of the Cry proteins under Australian conditions has been shown to be higher than that in the US.

[2] In this document, "*Bt* plants" refers to plants genetically modified to contain *cry* genes from *Bacillus thuringiensis,* e.g. Bollgard II® and Ingard ® cotton. Other examples include "*Bt* corn" and "*Bt* cotton". Similarly, "*Bt* toxins" refers to Cry toxins from *B. thuringiensis*.

Data supplied by the applicant has shown that the Cry1Ac (synpro) and Cry1F (synpro) proteins are expressed at varying levels in Widestrike Insect Protection cotton tissues (leaves, squares and roots) throughout the season under Australian field conditions, with Cry1F (synpro) being expressed at higher levels than Cry1Ac (synpro), particularly towards the end of the cotton growing season. Thus the Cry1Ac (synpro) and Cry1F (synpro) proteins will be available to the soil environment via degradation of plant material and, to a smaller extent, root exudation. Although these Australian studies did not measure the expression of these proteins in pollen, it is expected that the proteins will be present in this tissue since they are known to be expressed in pollen under USA field conditions.

Soil binding

Once in the soil, Cry toxins are rapidly absorbed or bound on clay particles and humic substances in less than 30 minutes, and therefore only remain in a free state susceptible to biodegradation for a brief period of time. Rapid binding of *Bt*-purified toxin to montmorillonite-humic acid-aluminium hydroxypolymers appears to protect the toxin from biodegradation. Both the extent of adsorption and the resulting conformational changes of Cry proteins are often strongly dependent on pH. Adsorption of the Cry proteins on clays decreases with increasing pH to above their isoelectric point.

Clay-Cry toxins interaction may be partly reversible as water and alkaline buffers can desorb a significant amount of toxin. This indicates that Cry toxins could be desorbed under environmental conditions and lead to the contamination of surface and underground waters. One study has reported that Cry1Ab protein was below the detection limit in most of the aquatic environments examined, and was only present in trace concentrations when it was detected.

Soil environment

Degradation and persistence of Cry proteins in soil depend largely on microbial activity, which is affected by soil type, pH, temperature and other physicochemical and biological characteristics of soil. The type and amount of clay minerals and humic acids affect the availability and degradation of Cry toxins in soil. For example, the Cry3Bb1 protein from *Bt* maize was detected for approximately 40 days in soil amended with kaolinite and for only 21 days in soil amended with montmorillonite. The more rapid degradation of the protein in the soil containing montmorillonite is likely to be the result of a greater microbial metabolic activity.

Cry proteins have been reported to persist longer in acidic soil because of a decrease in microbial activity at lower pH. Temperature has a major influence on the decomposition of Cry proteins. An increase of 10˚C in soil temperature can result in a two- to three-fold increase in microbial activity and higher rates of decomposition. Other factors affecting degradation and persistence of Cry proteins include the type and amount of protein released, the crop species, crop management practices and climatic conditions. In field trials of *Bt* maize expressing Cry3Bb1 and Cry1Ab, while the concentration of Cry3Bb1 in senescent leaves was higher than that of Cry1Ab, degradation of Cry3Bb1 was faster,

indicating that this protein has a shorter persistence in plant residues. Faster degradation of Cry3Bb1 compared to Cry1Ab has also been reported. Crop management practices have been shown to affect *Bt* toxin degradation in soil. A faster initial degradation of the Cry1Ab toxin in a no-tillage system than in a tillage system during autumn and winter has been reported. When leaf residues were left on the soil surface, approximately 62% of the initial Cry1Ab toxin concentration was degraded after 40 days, probably because of sunlight inactivation of the toxin.

Accumulation of Cry proteins in soil

Cry proteins generally do not accumulate in soil to detectable levels after repeated cultivation of *Bt* plants and do not affect soil non-target organisms. However, conflicting results have been reported about the persistence of *Bt* proteins in soil. Several soil model studies have suggested that Cry proteins degrade rapidly in soil with a half-life of 20 days or less, however, when purified Cry1Ab toxin was added to non-sterile soil, it remained active for at least 234 days. The same toxin was detected in soil for 180 days after growth of *Bt* corn. Cry1Ab protein from *Bt* maize tissues could still be detected after 240 and 200 days under tillage and no-tillage conditions, respectively. During both field experiments, the average soil temperatures were 7.6 and 8.2˚C, respectively, resulting in slower toxin degradation rates. In contrast to the Cry3Bb1 protein, the Cry1Ab protein was still detectable in rhizosphere soil cultivated with *Bt* maize over four consecutive years.

The variability in the reported data on the persistence of the Cry proteins under field conditions may be due to the studies being conducted on different soil types under different environmental conditions with the use of different sources of Cry proteins.

Cry1Ac in soil

Laboratory and field studies on the persistence of Cry1Ac in soil indicate that the toxin does not persist in soil for an extended period of time. Immunological and insecticidal activity of purified Cry1Ac protein has been observed for up to 56 days in soil. Between 10-40% of the Cry1Ac protein remained at the end of a 28 day period. Levels of Cry1Ac in USA field sites that had been used for Bollgard II® cotton crops for 3–6 years were examined and soil cores collected three months post-tillage contained no detectable toxin using enzyme-linked immunosorbent assay (ELISA) and bioassays with a detection limit of 3.68 ng and 8 ng of toxin per gram of soil, respectively.

Soil degradation of Cry1Ac in buried Ingard® and Ingard®/RoundupReady® cotton tissue at an Australian field site was examined using 10 litter bags containing leaves, crop stubble or dried stems and leaves, and 20 cores containing leaf residues and dried leaf samples. Levels of endotoxin in Ingard® and Ingard®/RoundupReady® cotton leaves in litter bags declined from 1.14 and 0.98 µg/g dry weight respectively at week 0, to 0.12 and 0.25 µg/g dry weight after two weeks, and to levels that were not quantifiable thereafter due to soil inhibition of the assay method used. For cotton stubble in litter bags, Cry1Ac levels were 3.47 µg/g dry weight at week 0, 0.013 µg/g dry weight after four weeks and below quantifiable levels thereafter. The soil used for these experiments contained 31% clay.

This suggests that Cry1Ac does not degrade at a significantly slower rate in high clay soils than in soils with low clay minerals, and that clay minerals may not cause increased persistence of Cry toxins. However, this remains to be shown in other soil types.

Cry1F in soil

A half-life in soils for Cry1F of less than 1 day has been estimated using a bioassay for tobacco budworm (*Heliothis virescens*). However, this study used purified proteins rather than plant material, resulting in an unrealistic exposure scenario. A recent study of multiple sites in the USA indicates that negligible levels of Cry1F protein accumulated in soil after three consecutive years of planting GM corn expressing Cry1F. The GM corn plants were incorporated into the soil through post-season tillage or no tillage, and samples were taken from each plot at two time points during the season each year. Bulk soil samples were taken at least 10 cm from the plant, and rhizosphere soil samples consisted of soil clinging to the plant roots. The level of Cry1F protein was determined using an improved extraction system using synthetic gut fluid and ELISA. The ELISA was validated in soil matrices over the concentration range of 18–180 ng/g dry weight, with a limit of detection of 4.5 ng/g dry weight. The assay was shown to have good accuracy and precision. No detectable Cry1F protein was found in any of the soil samples collected from the GM corn fields and no biological activity was observed against *H. virescens* neonates.

No persistence, accumulation or degradation data are available for Cry1F or Cry1F (synpro) in Australian soils.

UV degradation

Cry proteins degrade rapidly in the presence of sunlight, losing their biological activity. The UV-A/B range (280–380 nm) of sunlight is considered responsible for their photodegradation and consequent loss of toxicity. Photodegradation of Cry proteins from GM plants is more likely to occur when plant residues are left on the soil surface. A faster initial degradation of the Cry1Ab toxin has been reported in a no-tillage system than in a tillage system during autumn and winter, probably because of sunlight inactivation of the toxin.

Dispersal of reproductive material and introduced genes

Some dispersal of cotton seed may occur during transport, stockfeeding, adverse weather conditions and through animals. While cotton seed meal is consumed by stock, if whole seed is fed, a small percentage of whole cotton seed can pass through the digestive system intact and is able to germinate. Flooding or other extreme environmental conditions such as cyclones can disperse cotton seeds far from the area where they are grown.

Should Widestrike Insect Protection cotton seed be dispersed to favourable environments outside of cultivation, it is possible that feral populations could establish in the absence of any management practices. Favourable environments are far more likely to occur in northern Australia than in southern Australia. Any establishment of feral populations in

these environments could increase the distribution of the Cry1Ac (synpro) and Cry1F (synpro) insecticidal toxins in the environment, both in terms of exposure routes (insect feeding, plant degradation) and further dispersal of reproductive material (seeds, pollen and gene transfer to sexually compatible cotton plants).

The insecticidal trait in Widestrike Insect Protection cotton is expressed in cotton pollen, hence there is the potential for both exposure of non-target organisms relating to direct contact with the pollen or for cotton pollen to fertilise sexually compatible cotton plants and so establish the trait outside of cultivation.

Cotton is primarily self-pollinating with large and sticky pollen not easily dispersed by wind and it is released before flower opening. Pollen viability decreases rapidly after eight hours. Out-crossing is usually the result of insect pollination. Out-crossing rates depend on insect pollinator species presence and density that varies geographically and seasonally. Experiments conducted in Narrabri (Australia) showed that cross-pollination in this area relied mainly on hibiscus beetles (Family *Nitidulidae*). Several species of native bees, including the black stingless bee and *Trigona* spp., are present in eastern Australia and are likely to be involved in cross-pollination. The level of out-crossing observed in Australia is in the order of 1 to 2% between plants in adjacent rows. Out-crossing distances of more than 20 metres have been rarely recorded with out-crossing rates averaging 0.0035% of seed tested.

Given the low production and brief nature of pollen production and viability in cotton, long distance transport of Widestrike Insect Protection cotton pollen via target and non-target organisms or by weather events (e.g. wind) is not likely. However, fertilisation of sexually compatible cotton plants outside of cultivation remains a possible means of establishing the trait in the environment.

Gene transfer to sexually compatible cotton populations

Under commercial cropping it is likely that gene transfer from GM crops or volunteer plants to any nearby sexually compatible feral cotton populations would successfully occur at some time and to some degree. The frequency of gene transfer to feral cotton would depend on a range of factors, including the occurrence of feral cotton, survival and reproduction rate of GM plants, and abundance and behaviour of insect pollen vectors. Whatever the frequency, the key requirement to evaluate the significance of gene transfer is information on whether or not the trait confers a selective advantage to feral cotton. Feral cotton containing the insecticidal proteins may be protected against lepidopteran herbivory and become more invasive or weedy in habitats where lepidopteran herbivory may currently control feral cotton populations. In these habitats, an increase in the occurrence of cotton containing the insecticidal proteins would result in their persistence in the environment and an increased likelihood of a broader spectrum of non-target organisms being exposed to the insecticidal proteins than would occur in the commercial cotton crop situation.

Gene transfer to *G. barbadense*

In Australia *G. hirsutum* represents 99% of the commercial cotton crop and its growing areas partially overlap that of *G. barbadense*. The two cotton species share the AD genome and can hybridise to produce fertile F1 progeny. Several agronomic traits have been incorporated by crossing these cotton species. Spontaneous gene introgression occurs mainly from *G. barbadense* to *G. hirsutum* because of genetic and physical isolation mechanisms. However, commercial cultivars have been obtained by using *G. hirsutum* as the male parent, resulting in *G. barbadense* cultivars with an average 8-12% introgressed *G. hirsutum* chromatin.

Given the frequent breeding exchange of alleles between *G. hirsutum* and *G. barbadense*, transfer of the Widestrike Insect Protection cotton traits to either species represents an equivalent environmental risk. Therefore in the following discussion any comments made relating to *G. hirsutum* also apply to *G. barbadense*.

Gene transfer to feral cotton

Some Widestrike Insect Protection cotton seeds are likely to be dispersed following cultivation and therefore volunteers may establish in suitable ecological niches. *G. hirsutum* volunteers are found in all regions of Australia where seed has been widely dispersed from current large-scale commercial operations, by harvesting and transport of cotton bolls and the use of cotton seed meal as animal feed. These volunteers appear to be transient in nature and are found in disturbed areas where adequate water and nutrients occur (e.g. ditches, roadside drains). Such volunteers are generally removed by roadside management practices and are unlikely to establish and persist. Widestrike Insect Protection cotton volunteers could establish in areas where livestock is fed cotton seed meal or graze after being fed meal. Cotton volunteers generally do not reach maturity in dairy farms where stock were fed cotton seed meal due to trampling, grazing and competition.

Feral cotton populations occur rarely in the current cotton growing regions in southern Australia and their growth, survival and persistence is limited by a number of factors other than lepidopteran herbivory. However, a study of the distribution of feral cotton in northern Australia showed that there are a considerable number of self-sustaining populations of feral cotton distributed throughout the Northern Territory, in isolated areas as well as areas in or near proposed cotton growing regions. These habitats favour growth and persistence of cotton away from agricultural sites due to adequate water, nutrient-rich soils and low fire frequency. The existing feral cotton populations are believed to be primarily derived from varieties and cultivars introduced early in the NT by European settlers. These findings suggest that habitats favourable for feral cotton growth are not uncommon in this region and that populations are more common than previously understood.

Any dispersal of Widestrike Insect Protection cotton beyond commercial cultivation could result in Widestrike Insect Protection cotton plants, or feral cotton containing the insecticidal proteins (following successful gene transfer from Widestrike Insect Protection

cotton), establishing in favourable habitats. Lepidopteran herbivory in northern Australia may limit survival and persistence of feral cotton to a greater extent than elsewhere. Consequently, Widestrike Insect Protection cotton or feral cotton containing the insecticidal proteins would be protected against lepidopteran herbivory and become more invasive or weedy in these habitats. Should this occur, the synthetic genes and insecticidal proteins would persist in the environment and would result in an increased likelihood of a broader spectrum of non-target organisms being exposed to the insecticidal proteins than would occur in the commercial crop situation.

Gene transfer to native cottons

There are 17 native species of *Gossypium*, all unique to Australia. These species comprise the subgenus Sturtia and are grouped according to the composition of their genomes; C, G or K. The C-genome species of the section Sturtia are *G*. *sturtianum* (Sturt's Desert Rose) and *G*. *robinsonii*. The former species is widely, though sparsely, distributed across most of inland Australia except for the south-west and south-east of Australia and far Northern QLD.

It is thought that some native species of *Gossypium* arose via hybridisation of different native *Gossypium* species and that hybrids occur naturally between some closely related native species (for example, *G*. *pilosum* and *G. exiguum*).

The feasibility of producing inter-specific hybrids between various cotton species, including cultivated and native Australian cottons has been investigated under glasshouse conditions. The G-genome native species did not form viable hybrids when the G-genome was the male parent. The C-genome native species in contrast readily formed hybrids with *G. hirsutum* when the C-genome was the male parent. The 12 K-genome species (male parent) showed variable hybrid formation intermediate between that of the G- and C-genome species when crossed with *G. hirsutum*. Pollen from the C- and K- hybrids was sterile, functional pollen was obtained from two of the K- hybrid species. However, female fertility was reported for at least six of the K-species hybrids when back-crossed to *G. hirsutum*. This unexpected hybrid fertility was proposed to be due to unreduced gamete formation. This compatibility between native K-genome species and *G. hirsutum* represents a potential mechanism for gene flow between native and cultivated GM cotton grown in northern Australia, although the recombination rate between chromosomes of different genome origin in a hybrid is likely to be extremely low.

The extent and range of out-crossing of the 17 native Australian *Gossypium* species is not known and cannot be assumed to be similar to that of *G. hirsutum*.

Hybrids formed in cultivated cotton fields as a result of pollen flow from wild K-genome species to *G. hirsutum* would be harvested and therefore represent an evolutionary dead-end. However, large-scale cultivation, harvesting and transportation of GM cotton in northern Australia would give rise to ephemeral populations of the GM cotton, a known occurrence in the comparatively less favourable conditions south of latitude 22°S. These ephemeral populations could become geographically widespread along waterways and

transportation routes and represent a route for hybrid formation with native cottons, especially those of the C- and K-genome compositions, when these native species are the pollen donors. These perennial GM-native cotton hybrids could potentially persist in the environment for several years. It is not known whether such hybrids would possess a fitness advantage over the parental species. In the case of the AD-K hybrids, these would be female fertile and thus represent a potential source of gene flow to K-genome native species or naturalised cottons. The consequences of this gene flow could be the acquisition of traits by native or naturalised cottons, such as insect resistance, that confer a fitness advantage and result in increased weediness of these species. In the case of native cotton species, especially the K-genome species that are rare, the formation of hybrids represents an extinction risk by 'gene swamping', even in the absence of any fitness advantage of the hybrids.

7.2 Environmental Effects Summary

Widestrike Insect Protection cotton has been genetically modified to express the synthetic *Bt* proteins Cry1Ac (synpro) and Cry1F (synpro) for targeting cotton bollworm (*H. armigera*) and native budworm (*H. punctigera*) in Australian cotton fields.

The Cry1Ac (synpro) and Cry1F (synpro) proteins are expressed in different tissues of Widestrike Insect Protection cotton at varying levels, thus non-target organisms will be exposed to these proteins throughout the cotton growing season and also in areas where Widestrike Insect Protection cotton and/or its introduced genes occur outside of cultivation (e.g. feral populations). Non-target organisms present in both the cotton crop and outside of cultivation will be exposed to Cry1Ac (synpro) and Cry1F (synpro) via consumption of the GM cotton or of insects that have fed on the cotton, and via pollen dispersal, plant degradation and root exudation. The effects of exposure to Cry1Ac (synpro) and Cry1F (synpro) on terrestrial invertebrates, soil microorganisms, aquatic invertebrates, fish, birds and mammals are considered below.

The dispersal of Widestrike Insect Protection cotton reproductive material beyond areas of cultivation is likely to occur under commercial cropping via transport, stockfeeding, extreme weather events and via target and non-target organisms and the environmental effects resulting from this are also considered.

Pest Terrestrial Invertebrates

Aside from the targeted *Helicoverpa* spp. (*H. armigera* and *H. punctigera*), there are a large number of other invertebrate species present in Australian cotton fields. In using Widestrike Insect Protection cotton according to the draft label, there is potential for some non-target species to be exposed to Cry1Ac (synpro) and Cry1F (synpro) proteins derived from the cotton plant and its residues.

Field trials conducted in the US between 2001 and 2006 showed that Widestrike Insect Protection cotton was effective in controlling tobacco budworm (*Heliothis virescens*) and

cotton bollworm (*Helicoverpa zea*). In the USA, GM corn expressing Cry1F protected the corn from certain lepidopteran insect larvae including European corn borer (*Ostrinia nubilalis*), southwestern corn borer (*Diatraea grandiosella*), fall armyworm (*Spodoptera frugiperda*) and black cutworm (*Agrotis ipsilon*).

Widestrike Insect Protection cotton tested in the USA was claimed to be effective in controlling the following non-Heliothine insects: *Pectinophora gossypiella*, *Spodoptera frugiperda*, *Pseudoplusia includens*, *Estigmene acrea* and *Ostrania nubilalis*, and some control of *Agrotis ipsilon*.

In Australian field trials, Widestrike Insect Protection cotton has been shown to have activity against cotton looper (*Anomis planalis*), the cluster caterpillar (*Spodoptera litura*), and rough bollworm (*Earias huegeliana*).

Some published literature suggests that Cry1Ac, Cry1Fa and Cry1Ja have a common binding site in *H. armigera*, *H. zea* and *Spodoptera exigua*, suggesting that such a pattern may be widespread among lepidopteran species. Thus there is potential for target and non-target insects to eventually develop cross-resistance to Widestrike Insect Protection cotton and survive exposure to the Cry1Ac (synpro) and Cry1F (synpro) proteins, providing another route of exposure to the insecticidal proteins for predatory non-target organisms.

Non-target Terrestrial Invertebrates

Apart from pest terrestrial invertebrates, there are also various non-target terrestrial invertebrates found in cotton fields and feral cotton populations, such as predatory beetles, wasps, spiders and others (e.g. honey bees). Insect predators are important in integrated pest management, preying on both target species and secondary pests, in addition to being important components of the overall ecosystem. Non-target terrestrial invertebrates may be exposed to the Cry1Ac (synpro) and Cry1F (synpro) proteins either directly via consumption of Widestrike Insect Protection cotton plant material or indirectly via consumption of other insects that have fed on Widestrike cotton.

In a laboratory bioassay Cry1F was found not to have acute effects on the larvae of Monarch butterfly (*Danaus plexippus*), but Cry1Ac did. Other laboratory bioassays have shown that Widestrike Insect Protection cotton did not have significant effects on ladybird beetles (*Hippodamia convergens*) or parasitic *Hymenoptera* (*Nasonia vitripennis*).

Given the potential for altered specificity due to the synthetic nature of the Cry1Ac (synpro) and Cry1F (synpro) genes, there is a risk that some non-target terrestrial invertebrates will be susceptible to Widestrike Insect Protection cotton. Depending on the extent to which they are affected by Widestrike Insect Protection cotton, commercial cultivation could impact not only on integrated pest management strategies, but also on general ecosystems (e.g. food webs, population dynamics) in which these non-target terrestrial invertebrates play a role.

Soil-dwelling invertebrates

Soil-dwelling invertebrates are an essential link in food webs as decomposers, and the health and quality of soil are directly related to the number and diversity of invertebrates present. Therefore, evaluation of the impacts of *Bt* crops on soil organisms, including invertebrates (e.g. earthworms, isopods, collembolans, mites and nematodes) is essential in assessing the environmental risks of *Bt* plants. Most studies on lethal or sub-lethal effects of Cry proteins on soil invertebrates, including earthworms (*Lumbricus terrestris*, *Eisenia fetida* and *Aporrectodea caliginosa*), woodlice, collembolans and mites, have shown only minimal to no effects on these species. This is consistent with the reported specificity of Cry toxins which is largely restricted to insects within the orders *Lepidoptera* (moths and butterflies), *Diptera* (flies and mosquitoes), *Coleoptera* (beetles and weevils), *Hymenoptera* (wasps and bees) and *Homoptera* (aphids, hoppers) as well as activity against nematodes.

Earthworms (*E. fetida*) exposed to the toxins in Widestrike Insect Protection cotton were found to be normal in appearance and behaviour throughout the test period and no mortality was reported.

A diet containing 709 mg Cry1F/kg and a combination of 702 mg Cry1F plus 22.6 mg Cry1Ac/kg (724.6 mg/kg) did not adversely affect reproduction of collembola when compared to organisms exposed to the assay control. This was also confirmed by an assay conducted by substituting the original diet formulation with lyophilized Cry1Ac cotton leaves at a diet concentration of 5% and 50% in the same study.

Soil Microorganisms

Microorganisms are the dominant organisms in soil, both in terms of biomass and activity, and they are involved in numerous important processes, including decomposition of organic matter, nutrient mineralization, regulation of plant pathogens, decomposition of agricultural chemicals and improvement of soil structure. Any change in the quality and quantity of root exudates could potentially modify the composition (biodiversity) and activity of the soil microbiota and may cause changes in both deleterious and beneficial microorganisms.

While *Bt* occurs naturally in soil, growth of *Bt* corn causes a large increase in the amount of Cry endotoxin present in agricultural ecosystems (eg. roughly 0.25 g/ha produced naturally versus 650 g/ha in a *Bt* corn crop, excluding grain). Stubble could be a major reservoir of the persistent Cry protein in soil, as found in *Bt* maize fields. Therefore there is some concern that introducing *Bt* plants may increase levels of insecticidal Cry proteins in soil and may have an effect on non-target soil organisms and a change in microbe-mediated processes and functions in soil.

There are some reports that Cry proteins may affect soil microorganisms such as fungi, actinomycetes, bacteria, and protozoa. However, the Cry proteins released in root exudates and from plant residues of *Bt* crops generally appear to have no consistent,

significant or long-term effects on the microbiota and their activities in soil. Some studies have shown that the bacterial community structure was more affected by other environmental factors, such as the age of the plants, field heterogeneities and soil moisture, than by the Cry proteins accumulated in the soil.

Aquatic Invertebrates

Cry proteins present in GM crop debris or accumulated in top soil may reach a natural water body through flooding, run-off, or soil erosion. Aquatic invertebrates may therefore be exposed to the Cry1Ac (synpro) and Cry1F (synpro) toxins expressed in Widestrike Insect Protection cotton, with potential negative effects.

A single study on the acute toxicity of Cry1F (synpro) and Cry1Ac (synpro), tested on the daphnid (*Daphnia magna*), found no adverse effects such as immobility and sub-lethal effects. The level of the Cry1F (synpro) and Cry1Ac (synpro) proteins in aquatic systems is likely to be considerably less than the predicted NOEC (510 µg Cry1F (synpro) and 2500 µg Cry1Ac (synpro) per L).

Fish

Aquatic vertebrates, such as fish, may be exposed to the Cry1Ac (synpro) and Cry1F (synpro) proteins via direct consumption of plant material reaching the water body or indirectly via consumption of insects that have fed on Widestrike Insect Protection cotton plants.

The acute dietary toxicity of cotton seed expressing Cry1F and Cry1Ac, and Cry1F (synpro) and Cry1Ac (synpro) proteins was tested in rainbow trout *(Oncorhynchus mykiss* Walbaum). No fish mortality or sub-lethal effects were observed. The level of the Cry1F (synpro) and Cry1Ac (synpro) proteins in aquatic systems is likely to be considerably less than the predicted NOEC (100 mg a.c./kg diet containing a mixture of the two proteins).

Avian Species

Birds may be exposed to the Cry1Ac (synpro) and Cry1F (synpro) proteins directly, via consumption of Widestrike Insect Protection cotton plant material or inhalation of pollen, or indirectly via consumption of insects that have fed on Widestrike Insect Protection cotton. The acute toxicity of these proteins was tested on both juvenile and adult northern bobwhites (*Colinus virginianus*) and found to have no adverse effects on these birds. The level of the Cry1Ac (synpro) and Cry1F (synpro) proteins both produced in Widestrike Insect Protection cotton plant parts and predicted to enter soil (Section 6) is considerably less than the predicted NOEC of a combined Cry1Ac (synpro) and Cry1F (synpro) protein diet of 128 mg a.c./kg body weight.

Mammals

Mammals may also be exposed to the Cry1Ac (synpro) and Cry1F (synpro) proteins directly, via consumption of Widestrike Insect Protection cotton plant material or inhalation of pollen, or indirectly via consumption of insects that have fed on Widestrike Insect Protection cotton.

Pure Cry1F and Cry1Ac microbial proteins were evaluated separately or mixed (50:50) for acute oral toxicity to mice and no adverse effects were observed. The level of the Cry1Ac (synpro) and Cry1F (synpro) proteins produced in Widestrike Insect Protection cotton plant parts is considerably less than the predicted LD50s of a combined Cry1Ac (synpro) (350 mg/kg body weight) and Cry1F (synpro) (375 mg/kg body weight) protein diet.

Dispersal of Widestrike Insect Protection reproductive material and weediness

Cotton cultivars do not possess many of the attributes typical of problematic weeds such as seed dormancy, rapid vegetative growth, a short life cycle, and very high seed output and dispersal, however there are problematic weeds which do not display such characters including many woody weeds. In Australia, no *Gossypium* species are recognised as problematic weeds, although *G. sturtianum* can be locally weedy and isolated populations of *G. hirsutum* and *G. barbadense* are present in conservation areas where they are considered weeds. In Kakadu National Park, *G. hirsutum* is listed under the category “moderate to minor weed”.

Favourable environments for cotton are far more likely to occur in northern Australia (north of latitude 22°S) than in southern Australia. A considerable number of persistent populations of feral cotton are known to be distributed throughout the NT, suggesting that habitats favourable for feral cotton growth are not uncommon in this region and that populations are more common than previously understood. Native cotton species also occur throughout the region.

Some dispersal of cotton seed to areas outside of cultivation may occur during transport, stock feeding, adverse weather conditions and through animals. If Widestrike Insect Protection cotton seed is dispersed to favourable environments outside of cultivation, feral populations of Widestrike Insect Protection cotton could establish in the absence of any management practices. Dispersal of pollen containing the Widestrike Insect Protection trait (from cultivated or feral cotton plants) may also fertilise any nearby sexually compatible cotton plants outside of cultivation, providing another means of establishing the trait in the broader environment. The resulting hybrids could then establish in favourable habitats, particularly those where Lepidopteran insects may currently limit survival and persistence of feral cotton to a greater extent than elsewhere. Consequently, if the *cry1Ac (synpro)* and *cry1F (synpro)* genes confer a selective advantage to cotton in these habitats, feral populations may increase in size, become more invasive or weedy, and provide a persistent source of the insecticidal proteins to a different spectrum of potentially susceptible non-target terrestrial invertebrates than those encountered in-field.

7.3 Risk Assessment

The *cry1Ac (synpro)* and *cry1F (synpro)* genes and their encoded proteins are unlikely to pose a significant risk to the environment or to most non-target organisms. This is because the proteins are not expected to persist or accumulate in soil or water, and they will only be present during cultivation of the cotton crop itself, thereby limiting the likelihood of exposure. In the event that non-target organisms are exposed to the proteins, studies have shown that they are unlikely to have any significant toxicity effects on non-target organisms including soil invertebrates, soil microorganisms, aquatic invertebrates, fish and other aquatic vertebrates, birds or mammals.

However, risks to the environment from an unmanaged commercial release of Widestrike Insect Protection cotton were identified for the following:

- toxicity to non-target terrestrial invertebrates, both in-field and outside of cultivation; and
- feral cotton populations containing the Widestrike Insect Protection insect-resistance trait establishing in favourable habitats in northern Australia, resulting in increased weediness, persistence of the insecticidal proteins in the environment and exposure of a different spectrum of non-target terrestrial invertebrates than would occur in the crop situation.

Toxicity to non-target terrestrial invertebrates

The Cry1Ac (synpro) and Cry1F (synpro) proteins are synthetic proteins and are only available to the environment through the cultivation of Widestrike Insect Protection cotton. Protein expression patterns measured under Australian field conditions show that higher levels of Cry1Ac (synpro) are expressed at the beginning of the cotton growing season compared to the end, while the level of Cry1F (synpro) expression increases throughout the season. This means that non-target terrestrial invertebrates will likely be exposed to the insecticidal proteins throughout the life of Widestrike Insect Protection cotton plants. These non-target terrestrial invertebrates would likely encounter Widestrike Insect Protection cotton both in-field and in any populations growing outside of cultivation.

There is potential for target and non-target terrestrial invertebrates to develop cross-resistance to Widestrike Insect Protection cotton, thus they would survive exposure to the crop or to feral cotton populations containing the insect-resistance trait, and could represent a further means of exposing potentially susceptible non-target terrestrial invertebrates to the insecticidal toxins if they are used as a food source by these organisms.

Depending on their susceptibility to Cry1Ac (synpro) and Cry1F (synpro), a proportion of the non-target terrestrial invertebrates that visit Widestrike Insect Protection cotton crops during cultivation would likely be adversely affected. Non-target terrestrial invertebrates that are not susceptible to Cry1Ac (synpro) and Cry1F (synpro) but prey on susceptible

insects may be adversely affected by a reduced prey source if cotton crops harbour a significant source of their prey. However, in-field exposure of non-target insects to Cry1Ac (synpro) and Cry1F (synpro) during Widestrike Insect Protection cotton cultivation would be restricted by the length of the cotton growing season (approximately 6 months), the amount of Widestrike Insect Protection cotton grown and resistance management plans followed. As cultivated cotton is harvested at the end of the season and the insecticidal proteins are expected to rapidly degrade in plant litter, no continual source of the synthetic insecticidal proteins would be available for direct or indirect consumption by non-target terrestrial invertebrates.

Any establishment of feral cotton populations containing the Widestrike insect-resistance trait in favourable environments outside of cultivation could provide a continual source of the *cry1Ac* (synpro) and *cry1F* (synpro) genes and their encoded insecticidal proteins in the environment and expose a different spectrum of non-target terrestrial invertebrates to the Cry1Ac (synpro) and Cry1F (synpro) insecticidal proteins than those occurring in-field. Persistent populations of feral cotton have only been found in northern Australia. Should non-target terrestrial invertebrates in these environments be susceptible to the insecticidal proteins, this may have further effects such as interfering with ecosystem processes, e.g. food webs and population dynamics. The extent of this unintended environmental risk would depend on several factors including the size of any established feral cotton populations expressing the insect-resistance trait, herbivory of the feral cotton plants by the non-target terrestrial invertebrates (and predation of insects that have fed on the feral cotton plants by other insects), and the susceptibility of the non-target terrestrial invertebrates to the insecticidal proteins.

DEWHA considers that, until more information regarding the effects of Cry1Ac (synpro) and Cry1F (synpro) on non-target terrestrial invertebrates under Australian conditions are known, conditions that minimise the length and extent of exposure of these organisms in-field and the likelihood of exposure in environments suitable for feral cotton establishment in northern Australia are required to manage the risks to non-target terrestrial invertebrates posed by the commercial use of Widestrike Insect Protection cotton. The applicant has proposed to restrict the commercial release of Widestrike Insect Protection cotton to the southern cotton growing regions of Australia, south of latitude 22°S, and to a maximum 10% on-farm area as recommended by the Australian Cotton Industry's Transgenic and Insect Management Strategy committee. DEWHA considers that these limitations are appropriate to manage the risks to non-target terrestrial invertebrates.

Weediness

Some Widestrike Insect Protection cotton seeds are likely to be dispersed along transport routes, from fields by flooding and from the use of cotton seed as stock feed. It is highly likely that, as a minimum, ephemeral populations of volunteer Widestrike Insect Protection cotton plants will establish in suitable ecological niches such as ditches and roadside drains. These volunteers would generally be removed by roadside management practices (i.e. annual slashing/spraying) and would be unlikely to persist, but they may act as a source of seed to establish further populations.

Gene transfer of *cry1Ac* (synpro) and *cry1F* (synpro) to sexually compatible feral cotton populations is likely to occur under commercial cropping, and the resulting hybrids could establish in favourable environments. Environments that support persistent feral cotton populations are known only to occur in northern Australia (north of latitude 22°S) near previous and potential cotton growing regions. If lepidopteran insects play a significant role in limiting survival and persistence of cotton in these environments, the introduced *cry1Ac* (synpro) and *cry1F* (synpro) genes may confer a selective advantage to the feral cotton in these habitats by protecting it from lepidopteran herbivory. Consequently, cotton plants containing the insecticidal proteins could persist and become more invasive or weedy, and provide a continual source of the *cry1Ac* (synpro) and *cry1F* (synpro) genes and encoded insecticidal proteins in the environment.

As any risk of Widestrike Insect Protection cotton or its insect-resistance traits establishing in the wild is more likely to occur in northern Australia (where more favourable habitats exist) than in southern Australia, DEWHA considers that conditions that reduce the likelihood of the dispersal of Widestrike Insect Protection cotton reproductive material into environments suitable for feral cotton establishment in northern Australia would minimise the weediness risk posed by the commercial use of Widestrike Insect Protection cotton, until more information on the impact and importance of Lepidoptera herbivory on limiting existing feral cotton populations in this area is known. The restrictions proposed by the applicant, as above, are considered by DEWHA to be appropriate management conditions to mitigate the risk of cotton containing the insecticidal proteins spreading and persisting in the environment in favourable habitats.

7.4 Conclusions and Recommendations

The applicant has proposed to restrict the commercial release of Widestrike Insect Protection cotton to the southern cotton growing regions of Australia, south of latitude 22°S, and to a maximum 10% on-farm area as recommended by the Australian Cotton industry's Transgenic and Insect Management Strategy Committee. DEWHA considers that these limitations are appropriate to manage the environmental risks through minimising exposure of non-target terrestrial invertebrates and the spread of Widestrike Insect Protection cotton and its synthetic genes into favourable habitats in northern Australia.

These management conditions are consistent with those imposed on the approved commercial release of Widestrike Insect Protection cotton by the Gene Technology Regulator, and likely to be imposed by key management strategies developed and enforced by the Australian cotton industry.

DEWHA recommends that the product label be amended as follows:

- adding the condition that Widestrike Insect Protection cotton should be grown south of latitude 22°S

- replacing the current designation “Cotton Events 281/3006” with the full designation “Cotton Event 281-24-236/3006-210-23”

- replacing “the *Cry1Ac* gene” and “the *Cry1F* gene” with “the *Cry1Ac (synpro)* gene” and “the *Cry1F (synpro)* gene”, respectively, to reflect the synthetic nature of the introduced genes in Widestrike Insect Protection cotton.

In conclusion, DEWHA recommends that the APVMA be satisfied that the proposed registration of Widestrike Insect Protection cotton for controlling *H. armigera* and *H. punctigera* insect pests in cotton would not be likely to have an unintended effect that is harmful to animals, plants or things or to the environment under Section 14 subsection 1 of the Agvet Codes provided the product is applied according to the proposed label use pattern and when it is applied in accordance with its revised label directions.

8 EFFICACY AND SAFETY ASSESSMENT

8.1 Proposed use pattern

The intended use of Widestrike Insect Protection is to control Helicoverpa spp. in cotton.

8.2 Summary of Evaluation of Efficacy and Crop safety

A total of 33 references were presented, including 26 trial reports. One trial presented extensive data on toxin expression in numerous plant parts across the season (USA). A number of other trials also included less extensive expression data from trials conducted in Australia. Nineteen trials were presented comparing Widestrike Insect Protection efficacy against *Helicoverpa armigera* and/or *H. punctigera* compared with sprayed conventional cotton. Of these, 5 trials also compared efficacy of single gene transgenic cotton and 11 trials investigated if spraying Widestrike Insect Protection, cotton improved yields. In these 11 trials, different pest thresholds were used to initiate spraying, including spraying on conventional cotton thresholds, spraying on Bollgard II thresholds and spraying on a proposed Widestrike Insect Protection threshold which differed from the Bollgard II threshold in that spraying was conducted when above threshold observations were made once, compared with two consecutive checks above thresholds for Bollgard II. One trial also included efficacy against *Anomis planalis* (cotton looper), two trials against *Erias huegeliana* (rough bollworm) and two trials against *Spodptera litura* (cluster caterpillar).

Three trials were presented investigating non-target effects of Widestrike Insect Protection, against both beneficial insects and other Lepidopteran pests, comprised of two trials conducted in the USA, and one in Australia. An additional trial conducted in the USA was presented showing efficacy against two Heliothine pest species (in USA), *Helicoverpa zea* and *Heliothis virescens*.

Widestrike Insect Protection cotton, containing Cry 1Ac (synpro) and Cry1F (synpro) genes and their controlling sequences provides effective control of *Helicoverpa armigera* and *H. punctigera*, comparable to sprayed conventional cotton, under both light and heavy insect pressure.

Both toxins were expressed in aerial plant parts throughout the season. While expression varied, there was evidence that Cry1Ac (synpro) expression steadily increased or remained generally constant, and Cry 1F (synpro) expression increased during the growing season.

8.3 Efficacy Conclusion

In terms of the evidence for the efficacy of the product and its safety to target and non-target species, the application by Dow AgroSciences Australia Ltd for the registration of

Widestrike Insect Protection is supported when used in accordance with the proposed label instructions and Good Agricultural Practice (GAP).

8.4 Resistance Management

The applicant provided a resistance management plan and support from the TIMs committee with planting up to a maximum 10% of the on-farm area on the proportion of WideStrike grown (relative to total cotton area), to allow for further evaluation of the product in regard to expression, efficacy and cross resistance between the two Cry (synpro) toxins in Widestrike Insect Protection. The RMP would have to be modified and updated through consultation with TIMS to reflect changes in other transgenic Bt cotton RMPs, as well as in response to changes in knowledge of Cry resistance frequencies and mechanisms, and other data relating to WideStrike presented by Dow since the 2007 RMP.

9 CONCLUSION

From the risk assessment summary provided above, it can be demonstrated that the use of the active constituents and the product in accordance with the recommendations for their use:

- would not be an undue hazard to the safety of people via occupational or dietary exposure due to the inherent low toxicity of the endotoxins and low exposure to them; and
- would not be likely to have an effect that is harmful to people due to the low toxicity; and
- would not be likely to have an unintended effect to the environment due to the high specificity, low toxicity and exposure to the endotoxins, or the cotton plant it is part of; and
- would not unduly prejudice Australia's trade with other countries due to the similarity with to other transgenic Bt cotton; and
- would be efficacious according to the label claims and APVMA requirements (*i.e*. when used according to the RMP).

Unless further information is provided to the APVMA to discount these conclusions, the APVMA will grant the application to approve the active Bacillus thuringiensis subsp. kurstaki Cry1Ac (synpro) gene and Bacillus thuringiensis subsp. aizawai Cry1F (synpro) gene and their controlling sequences (the endotoxins), register the product Widestrike Insect Protection Cotton Events 281/3006 and approve the associated label.

10 LABELLING REQUIREMENTS

A label is proposed to be attached to the containers of seeds that carry the product (Widestrike Insect Protection). The label carries the required information for product identification and use instructions. In addition to the label instructions, the applicant is proposing to require users sign a Technology User Agreement (TUA), which includes the Resistance Management Plan (RMP). It is proposed the TUA and RMP be required as Conditions of Registration, thereby contributing to the APVMA's satisfaction that the product can be used safely and effectively.

The TUA is a legal contract binding the applicant and the user to undertake certain actions. These actions include such things as undertaking the necessary resistance management measures outlined in the RMP, informing any purchaser of Widestrike Insect Protection cotton produce that it is from a GMO, allowing the applicant and their representatives to undertake audits of a growers property to confirm that they are compliant with the RMP and other requirements necessary to ensure safe and effective use.

It is proposed that the registration of Widestrike Insect Protection be subject to conditions relating to the following, in order for the APVMA to be satisfied the relevant requirements of the Agvet Codes have been met:

1. Dow AgroSciences ensure seed containing Widestrike Insect Protection is only supplied to or used by, persons holding a current TUA with Dow AgroSciences. The TUA must legally allow Dow AgroSciences to:

    a. audit growers at any time to ensure they are compliant in accordance with the RMP; and

    b. take or enforce remedial action in instances of non-compliance.

2. Dow AgroSciences provide to users of Widestrike Insect Protection a RMP that has been authorised by the APVMA. The RMP must be endorsed by a Stakeholder Committee. For the purposes of this condition, the Stakeholder Committee is the TIMS Committee. The RMP must include adequate information to ensure users can effectively manage Widestrike Insect Protection cotton to minimise the development of resistance to the active constituent by *Helicoverpa* spp. Such information must include, but is not limited to:

    a. the types and sizes of refuges required;

    b. fixed planting windows;

    c. post harvest crop destruction and control of volunteer and ratoon cotton; and

    d. pupae destruction and or trap cropping.

3. Should the text or format of the RMP be amended in any manner, Dow AgroSciences must have the RMP approved by the APVMA before providing the amended RMP to users;

4. Dow AgroSciences must take all reasonable steps to ensure cotton containing Widestrike Insect Protection is not grown north of latitude 22° South;

5. Dow AgroSciences must ensure that all suppliers of cotton seed containing Widestrike Insect Protection and agents acting on their behalf are fully aware of all conditions of registration for Widestrike Insect Protection;

6. Dow AgroSciences must ensure that any containers holding seed containing Widestrike Insect Protection carry a label approved by the APVMA for Widestrike Insect Protection;

7. Dow AgroSciences must keep a record of or have access to:

    a. each TUA made, including details of the size and location of cotton containing Widestrike Insect Protection, the resistance management strategy used as per the RMP, if the user was compliant with the TUA and if not, what steps were taken to ensure compliance; and

    b. each seed supplier's records of seed supplied containing Widestrike Insect Protection, including the details of the purchaser, the relevant TUA, the date of supply and quantity supplied;

8. Dow AgroSciences must keep for a period of three years, the records made pursuant to conditions listed herein and make these available to the APVMA on request;

9. Dow AgroSciences must provide reports to the APVMA at defined times throughout the season, which detail:

    a. the hectares of total cotton and cotton containing Widestrike Insect Protection planted per farm;

    b. grower compliance with the TUA and RMP;

    c. the adequacy of the RMP to manage resistance via, but not limited to, resistance bioassays with target pests.

10. Dow AgroSciences ensure through the TUA that Plantings of commercial irrigated and dryland (rain-fed) cotton containing the Widestrike Insect Protection genes are limited to a maximum of 10% of the total cotton being grown by one grower (as defined by the growers trading name) on a farm. Total cotton is defined as all cotton being grown on a farm unit and includes all insect-tolerant and herbicide tolerant and conventional varieties including sprayed and unsprayed cotton refuges.

Condition 10 is required to manage risk for resistance management as well as risk to non-target organisms.

10.1 Proposed label

*WideStrike**
Insect Protection

Cotton Events 281-24-236/3006-210-23

Active Constituent: *Bacillus thuringiensis* subsp. *kurstaki* delta endotoxin as produced by the *Cry1Ac* gene and its controlling sequence and the *Bacillus thuringiensis* subsp. *aizawi* delta endotoxin as produced by the *Cry1F* gene and its controlling sequence

GROUP	11	INSECTICIDE

For in-built protection of cotton against the Cotton Bollworm and Native Budworm

READ LABEL BEFORE OPENING THIS BAG

Cotton seed in this bag contains WideStrike technology by Dow AgroSciences

Dow AgroSciences Australia Limited
ABN 24 003 771 659
20 Rodborough Road
FRENCHS FOREST NSW 2086
www.dowagrosciences.com.au
CUSTOMER SERVICE TOLL FREE: 1-800 700 096

* Trademark of Dow AgroSciences

DIRECTIONS FOR USE

DO NOT plant or move seed north of latitude 22° S

Cotton containing WideStrike technology must be grown in accordance with these directions and the conditions set out in the current WideStrike Technology User Agreement. Read before planting.

Control of Cotton bollworm (*Helicoverpa armigera)* and Native budworm (*H. punctigera)*

Cotton containing the WideStrike technology will provide significant protection of cotton against the cotton bollworm and native budworm. Supplemental insecticide control may still be required under conditions of high pest pressure or high plant stress to maintain *Helicoverpa'* populations below economically damaging levels.

Monitor crops regularly and apply insecticide treatments if necessary. Recommendations developed by the Australian Cotton Research Institute may be used as a guide for treatment thresholds. Insecticide selection should comply with the Insecticide Resistance Management Strategy for Conventional Cotton for the current year. For optimum efficacy, manage crops and inputs to avoid plant stress. Note that eggs and very small larvae (neonates/first instar) will be observed in the crop, as larvae must feed before being controlled by the WideStrike technology.

The WideStrike technology is not registered to control other pests. Other pests should be monitored and treated using the recommended thresholds.

Resistance management
Preventative resistance management is designed to keep Helicoverpa resistance to non-detectable levels and so extend the benefits of GM cotton into the future. Growers must practice preventative resistance management as detailed in the 'Resistance Management Plan for WideStrike Cotton' (the Plan) for the current year, as recommended by the TIMS Committee and included in the WideStrike Technology User Agreement. Note that separate Plans apply in different geographic regions. Additional copies of the current year Plan may be obtained from any WideStrike Agent or Dow AgroSciences.

Crop management
Always grow a WideStrike cotton variety that is appropriate for the local area. Use the best agronomic and crop management practices for the area. For optimum efficacy, manage agronomic inputs to avoid plant stress and to achieve early plant maturity. Follow integrated pest management principles and utilise crop management practices that minimise pest incidence.

APVMA Approval Number No 62268/42746

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ABBREVIATIONS

ac	active constituent
ADI	Acceptable Daily Intake (for humans)
ai	active ingredient
ArfD	Acute Reference Dose
Bt	*Bacillus thuringiensis*
bw	bodyweight
C	Celsius
cfu	colony forming unit
cry	Crystalline toxin from *Bacillus thuringiensis*
d	day
DEWHA	Department of Environment Water Heritage and Arts now Department of Sustainability, Environment, Water, Population and Communities (DSEWPaC)
DNA	Deoxyribonucleic acid
DSEWPaC	Department of Sustainability, Environment, Water, Population and Communities. Formally called Department of Environment Water Heritage and Arts (DEWHA)
ELISA	Enzyme linked immunosorbent assay
F1	The first filial generation i.e. the offspring resulting from a cross mating of distinctly different parental types
g	gram
GAP	Good Agricultural Practice
GMO	Genetically modified organism
h	hour
ha	hectare
HSIS	Hazardous Substances Information System
kg	kilogram
L	Litre
m	metre

mg	milligram
mL	millilitre
MRL	Maximum Residue Limit
MSDS	Material Safety Data Sheet
ng	nanogram
NOEC/NOEL	No Observable Effect Concentration / Level
NOHSC	National occupational Health and Safety Commission
OGTR	Office of the Gene Technology Regulator
pat	phosphinothricin acetyl transferase
RMP	Resistance Management Plan
SUSMP	Standard for the Uniform Scheduling of Medicines and Poisons
subsp	sub species
SWA	Safe Work Australia
synpro	Synthetically produced
TGAC	technical grade active constituent
TIMS	Transgenic and Insect Management Strategy
TUA	Technology Users Agreement
µg	microgram
USA	United States of America
US FDA	United States of America Food and Drug Administration
UV	Ultra violet
var.	variety

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.
adsorb	To gather on a surface in a condensed layer
allele	Any of the alternative forms of a gene
Carcinogenicity	The ability to cause cancer
Causative agent	The organisms causing the effect
chromatin	The part of the nucleus that consists of DNA and proteins, forms the chromosomes, and stains with basic dyes
Chronic	Of long duration
Codex MRL	Internationally published standard maximum residue limit
commensal	Symbiotic relationship in which one species is benefited while the other is unaffected.
Cotton event	An insertion of a genetic material into cotton
Desorption	Removal of an absorbed material from a surface
Efficacy	Production of the desired effect
endotoxin	A toxin contained within the protoplasm of an organism, esp a bacterium, and liberated only at death
ephemeral	Short lived
exotoxin	A toxin produced by a microorganism and secreted into the surrounding medium
Feral cotton	Cotton plants not grown under cultivation / not farmed
Formulation	A combination of both active and inactive constituents to form the end use product
gavage	Forced feeding, as by a flexible tube and a force pump
genome	A full set of chromosomes; all the inheritable traits of an organism.
Genotoxicity	The ability to damage genetic material
herbivory	Feeding on plants
humic	Pertaining to the dark organic matter in soils
Hydrophobic	Water repelling

intraperitoneal	Administered by entering the peritoneum
intravenous	Administered by entering the vein
introgression	The introduction of genes from the gene pool of one species into that of another during hybridization
In vitro	Made to occur in a laboratory vessel or other controlled experimental environment
In vivo	Occurring or made to occur within a living organism or natural setting
isoelectric	Having the same electric potential
Leaching	Removal of a compound by use of a solvent
Log Pow	Log to base 10 of octonol water partitioning co-efficient
lyophilized	To dry by freezing in a high vacuum
Metabolism	The conversion of food into energy
Occlusive dressing	A dressing that seals a wound from air or bacteria.
parenteral	Taken into the body in a manner other than through the digestive canal.
pathogen	Any agent that can cause disease
Photodegradation	Breakdown of chemicals due to the action of light
Photolysis	Breakdown of chemicals due to the action of light
rhizosphere	The region of the soil in contact with the roots of a plant. It contains many microorganisms and its composition is affected by root activities
serotype	A group of organisms, microorganisms, or cells distinguished by their shared specific antigens as determined by serologic testing
Southern blot analysis	An electrophoretic procedure used to separate and identify DNA sequences
Subcutaneous	Under the skin
supernatant	Liquid lying above a sediment or settled precipitate
Toxicokinetics	The study of the movement of toxins through the body
Toxicology	The study of the nature and effects of poisons
transgenic	Pertaining to, or containing a gene or genes transferred from another species

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