

**Public Release Summary  
on the**

**Evaluation of the new active**

***Bacillus thuringiensis* var. *kurstaki* delta-endotoxins  
as produced by the *Cry1Ac* and *Cry2Ab* genes and  
their controlling sequences**

**in the new product**

**BOLLGARD II COTTON EVENT 15985**

**Australian Pesticides and Veterinary Medicines Authority**

**June 2003**

**Canberra  
Australia**

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## FOREWORD

The Australian Pesticides and Veterinary Medicines Authority (APVMA) is an independent statutory authority 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), Environment Australia (Risk Assessment and Policy Section), the National Occupational Health and Safety Commission and State departments of agriculture and environment.

In addition, the APVMA is required to request and have regard to the advice of the Gene Technology Regulator, with respect to any application that involves a Genetically Modified Organism (GMO) or the product of a GMO.

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 all products containing new active ingredients for use in food producing crops and animals.

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 or Vet] Manual: The Requirements Manual for [Agricultural/Veterinary] Chemicals* and *[Ag/Vet] Requirements Series*.

This Public Release Summary is intended as a brief overview of the assessment that has been completed by the APVMA and 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.

More detailed technical assessment reports on all aspects of the evaluation of this chemical can be obtained by completing the order form in the back of this publication and submitting with payment to the APVMA. Alternatively, the reports can be viewed at the APVMA Library, 1st Floor, 22 Brisbane Avenue, Barton, ACT.

The APVMA welcomes comment on the usefulness of this publication and suggestions for further improvement. Comments should be submitted to the Program Manager-Pesticides Division, Australian Pesticides and Veterinary Medicines Authority, PO Box E240, Kingston ACT 2604.

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## LIST OF ABBREVIATIONS AND ACRONYMS

<b>AC</b>	Active Constituent
<b>ACGRA</b>	Australian Cotton Growers Research Association
<b>ADI</b>	Acceptable Daily Intake (for humans)
<b>ai</b>	active ingredient
<b>ARfD</b>	Acute Reference Dose (for humans)
<b>Bt</b>	<i>Bacillus thuringiensis</i>
<b>bw</b>	body weight
<b>d</b>	day
<b>DAT</b>	Days After Treatment
<b>DIR</b>	Dealings involving Intentional Release
<b>DNA</b>	deoxyribonucleic acid
<b>DT<sub>50</sub></b>	Time taken for 50% of the concentration to dissipate
<b>DT<sub>90</sub></b>	Time taken for 90% of the concentration to dissipate
<b>D.Wt</b>	Dry Weight
<b>EA</b>	Environment Australia
<b>F.Wt</b>	Fresh Weight
<b>g</b>	gram
<b>GMO</b>	Genetically Modified Organism
<b>h</b>	hour
<b>ha</b>	hectare
<b>kg</b>	kilogram
<b>kt</b>	kilotonne
<b>L</b>	Litre
<b>LC<sub>50</sub></b>	concentration that kills 50% of the test population of organisms
<b>LD<sub>50</sub></b>	dosage of chemical that kills 50% of the test population of organisms
<b>mg</b>	milligram
<b>mL</b>	millilitre
<b>MRL</b>	Maximum Residue Limit
<b>ng</b>	nanogram
<b>NOEC/NOEL</b>	No Observable Effect Concentration Level
<b>OGTR</b>	Office of the Gene Technology Regulator
<b>OCS</b>	Office of Chemical Safety
<b>ppb</b>	parts per billion
<b>ppm</b>	parts per million
<b>s</b>	second
<b>SUSDP</b>	Standard for the Uniform Scheduling of Drugs and Poisons
<b>TGA</b>	Therapeutic Goods Administration
<b>mg</b>	microgram
<b>WHP</b>	Withholding Period

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## INTRODUCTION

This publication provides a summary of the data reviewed and an outline of the regulatory considerations for the proposed registration of Bollgard II Cotton Event 15985, which contains the new active constituent *Bacillus thuringiensis* var. *kurstaki* delta-endotoxins as produced by the *CryIAc* and *Cry2Ab* genes and their controlling sequences. The product is proposed to be used for the control of cotton bollworm and native budworm in cotton.

Responses to this Public Release Summary will be considered by the APVMA in deciding whether the product should be registered and in determining appropriate conditions of registration and product labelling.

Copies of full technical evaluation reports on the active and the product, covering toxicology, occupational health and safety aspects, residues in food and environmental aspects are available from the APVMA on request (see order form on last page). They can also be viewed at the APVMA library located at the APVMA offices, First Floor, 22 Brisbane Avenue, Barton ACT 2604.

Written comments should be received by the APVMA by 10 July 2003. They should be addressed to:

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## EXECUTIVE SUMMARY

### ***The Applicant***

Monsanto Australia Limited (the applicant).

### ***The Application***

The APVMA has assessed an application from the applicant to approve the active *Bacillus thuringiensis* var. *kurstaki* delta-endotoxins as produced by the *CryIAc* and *Cry2Ab* genes and their controlling sequences (the endotoxins), register the product Bollgard II Cotton Event 15985 (Bollgard II) and approve the associated label.

If the application is granted, Monsanto and the cotton industry intend to use the upcoming southern growing season (September start), to phase out the single gene (*CryIAc*) product, Ingard Gene by Monsanto (Ingard) and phase in Bollgard II. The aim for the following season would be to use Bollgard II exclusively.

### ***The Proposed Decision***

The APVMA proposes to approve the endotoxins as the active constituent and register Bollgard II subject to conditions.

### ***The Active Constituent***

The endotoxins are protein insecticides belonging to a group of compounds known as 'microbial disrupters of insect midgut membranes'. Once consumed by susceptible insects, the proteins react with enzymes in the midgut causing them to be 'activated'. The activated toxins bind to the midgut epithelium (membranous tissue lining the gut wall), causing disruption to the cell membrane by altering permeability and pH, cell lysis and disintegration of the epithelium. This, in turn leads to midgut paralysis, body paralysis, starvation, septicaemia and death. Regarding insecticide resistance, the endotoxins are classed as Group 11C Insecticides.

### ***The Product***

Bollgard II is the *CryIAc* and *Cry2Ab* genes and their controlling sequences. The genes and their controlling sequences produce the endotoxins by expression in the cotton plant cells containing Bollgard II. Bollgard II is supplied as the genes and their controlling sequences present in cotton seed germ plasm.

Internationally, Bollgard II is registered or grown commercially in the USA. However, it is under trial or nearing registration in other countries, including Australia.

Monsanto Australia Ltd has already been granted licences by the Gene Technology Regulator, under the *Gene Technology Act 2000*, for commercial release of Bollgard II and Bollgard II/Roundup Ready cotton in current cotton growing areas south of latitude 22°S (Office of the Gene Technology Regulator Application DIR 012/2002). Limited field trials of Bollgard II cotton have been permitted north of 22°S with licence conditions designed to prevent dissemination of the GMO and its transgenes.

### ***The Assessment***

The significant difference between Bollgard II and Ingard is the addition of the second gene, *Cry2Ab* and its controlling sequences. As the APVMA remains satisfied Ingard continues to meet the legislative requirements for registration, the assessments for this application have focused primarily on the potential risks involved with the addition of the *Cry2Ab* gene. Other genes associated with the modification are not discussed in detail as they have no effect on the

pesticidal properties of the endotoxins and are considered to be of low risk to human and environmental safety as advised by the OGTR, Therapeutic Goods Administration (TGA) and Environment Australia (EA).

The proposed findings of the assessment are summarised below.

### **Chemistry and Manufacture**

Adequate information has been provided to demonstrate the method by which the active constituent (the endotoxins) and the product (the genes and their controlling sequences) have been produced. Assessment of the analysis of the genetic modification and the proteins produced in cotton found that no additional genetic information or proteins (e.g. 'impurities') of concern were produced via the introduction of the *CryIAc* and *Cry2Ab* genes and their controlling sequences. The stability of the genetic modification was found to be acceptable, in that the presence and position of the genes were unchanged after five generations. This has continued to be demonstrated via subsequent breeding of Australian varieties.

### **Toxicology**

The TGA recommended approval of the active constituent and registration of the product with respect to human health, noting that the *Cry2Ab* protein was of very low acute oral toxicity and was not similar to any known allergens or gliadins. No Acceptable Daily Intake or Acute Reference Dose is required, as no endotoxins are expected in food commodities. Scheduling, first aid instructions and safety directions are not required because of the low toxicity of and exposure to the endotoxins. The APVMA accepts this advice.

### **Residues**

When deciding to register Ingard, the APVMA also included a Table 5 entry in the MRL Standard for *Bacillus thuringiensis kurstaki* delta endotoxin protein. This entry covers the use requested in this application (both endotoxins). [Note: Table 5 exists for situations where residues do not or should not occur in foods or animal feeds or are otherwise of no toxicological significance.]

In addition to the product's low toxicity, residues are not expected in food commodities. On this basis, there is no need to amend the MRL Standard and the risk to human health from residues in food is considered to be negligible. It should be noted that Food Standards Australia New Zealand has concluded previously that the oil and linters from Bollgard II cotton are as safe for human consumption as those from other conventional varieties of cotton.

### **Occupational Health And Safety**

On the basis of the very low toxicity and likely negligible exposure to humans working with cotton containing the Bollgard II genes, the National Occupational Health and Safety Commission (NOHSC) advised that Bollgard II could be safely used by workers when used as proposed. The APVMA accepts this advice.

### **Environment**

Environment Australia's Biotechnology Branch (EA) provided advice on the risk to the environment from the proposed use. In this document, "the environment" means animals and plants that are not intended to be harmed by the active constituent or the product.

The endotoxins are considered unlikely to bioaccumulate in the environment, based on soil tests demonstrating rapid degradation. The endotoxins are very specific in their modes of action. Toxicity tests on a wide variety of organisms showed toxicity only to Lepidoptera (target pests) and possibly Diptera (e.g., flies and mosquitoes, *Cry2Ab* endotoxin only).

Dipterans are an important part of the freshwater aquatic ecosystems of northern Australia. Therefore the question of toxicity to Dipterans is of concern. Although it is unlikely that dipterans will be exposed to significant concentrations of endotoxin, the currently available data are inconclusive.

Ingard is currently restricted to areas south of 22°S to manage the risk of weediness and outcrossing to feral and native cotton species associated with use in the north. Data provided were considered insufficient to demonstrate that Bollgard II would not become a weed or outcross in areas north of 22°S.

Accordingly, full commercial use of the product south of 22°S is not considered likely to have unintended effects on the environment. (Note: before unrestricted use north of 22°S is considered not to be an undue risk, adequate data on the toxicity to Dipterans, effect of lepidopteran herbivory on weediness potential and the ability to cross pollinate with untested native cotton species will be required).

### **Trade**

Finite residues are not expected to be present in any human food commodities as a result of the use of this product. Therefore, it is not expected that there will be any undue prejudice to trade with other countries with regards to residues in exported food commodities.

As Ingard has been registered since 1996, it can be expected that produce from genetically modified (GM) cotton (eg lint, seed and seed oil) has been exported in significant quantities to other countries. In addition, it has been widely documented that Australia's cotton crop is approximately 30% GM cotton. To the APVMA's knowledge, there has been no prejudice to Australia's trade as a result of this use of GM cotton. As Bollgard II is very similar to Ingard with regard to the genetic modification, it is not expected that produce from Bollgard II cotton will result in undue prejudice to Australia's trade with other countries.

### **Efficacy and Crop Safety**

State departments of agriculture and primary industries provided advice regarding matters of efficacy, resistance management and crop safety. Efficacy data collected from two years of trials across all Australian cotton growing regions, along with supporting data from the USA, were provided to demonstrate efficacy. Data included laboratory bioassays using field grown leaf samples, in-field pest monitoring and recording the number of insecticide sprays used. Expression levels in different plant parts were also provided as well as levels of boll damage and fruit retention.

The data provided were considered sufficient to demonstrate that Bollgard II was providing at least equivalent and in most cases superior efficacy to Ingard by all measures. However, the APVMA does not consider that Bollgard II will offer sufficient control of *Helicoverpa* spp. in all situations, for the entire season. Therefore, the label will include instructions requiring users to monitor crop pest levels as required in conventional cotton as well as attempt to manage the crop so it is stressed as little as possible. Should industry set pest thresholds be reached, users are advised to use supplementary control measures.

The application requested that no limitations (cap) be placed on the percentage of Bollgard II cotton grown (compared to the total cotton crop), however a detailed Resistance Management Plan (RMP), requiring measures such as refuge planting and fixed cotton planting windows, would be required of growers by the applicant. The applicant argued that the dual action of the two endotoxins would significantly reduce the chance of resistance to either or both delta-endotoxins and that the resistance management measures required by the RMP would be

sufficient to manage it. In addition, the applicant proposed that yearly monitoring of resistance levels would be undertaken.

This proposal was endorsed by the Transgenic and Insect Management Strategy (TIMS) Committee (of ACGRA) as the best method of managing resistance in the transitional period. The TIMS Committee is comprised of representatives from the farming and chemical industries, as well as government, with expertise in pest management issues.

The State government departments of primary industries and agriculture have also advised that the RMP will be adequate to manage resistance. The APVMA also notes the published literature on the subject, which supports refuges as little as 10% for two-gene cotton.

Based on this information and support, the APVMA can be satisfied that the RMP refuge requirements alone will be sufficient to effectively manage resistance to one or both endotoxins. The data supports the use of Bollgard II without a cap restriction as long as all users comply with the RMP.

### **The Label and Conditions of Registration**

A label is proposed to be attached to the containers of seeds that carry the product (Bollgard II). 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 RMP. It is proposed the TUA and RMP be required as Conditions of Registration (consistent with the current regulation of Ingard), 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 Bollgard II 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 Bollgard II 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. Monsanto Australia Limited (Monsanto) ensure seed containing Bollgard II is only supplied to or used by, persons holding a current TUA with Monsanto. The TUA must legally allow Monsanto 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. Monsanto provide to users of Bollgard II 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 Bollgard II 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, Monsanto must have the RMP approved by the APVMA before providing the amended RMP to users;
4. Monsanto must take all reasonable steps to ensure cotton containing Bollgard II is not grown north of latitude 22° South;
5. Monsanto must ensure that all suppliers of cotton seed containing Bollgard II and agents acting on their behalf, are fully aware of all conditions of registration for Bollgard II;
6. Monsanto must ensure that any containers holding seed containing Bollgard II carry a label approved by the APVMA for Bollgard II;
7. Monsanto must keep a record of or have access to:
  - a. each TUA made, including details of the size and location of cotton containing Bollgard II, 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 Bollgard II, including the details of the purchaser, the relevant TUA, the date of supply and quantity supplied;
8. Monsanto 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. Monsanto must provide reports to the APVMA at defined times throughout the season, which detail:
  - a. the hectares of total cotton and cotton containing Bollgard II planted per Valley;
  - 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.

### Summary and Conclusions

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 Ingard; 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* var. *kurstaki* delta-endotoxins as produced by the *Cry1Ac* and *Cry2Ab* genes and their controlling sequences (the endotoxins), register the product Bollgard II Cotton Event 15985 (Bollgard II) and approve the associated label.

The following sections provide further details of each component assessment.

## CHEMISTRY AND MANUFACTURE

### Summary of the APVMA's Evaluation of *Bacillus thuringiensis* var. *kurstaki* delta-endotoxins as produced by the *CryIAc* and *Cry2Ab* genes and their controlling sequences in the product Bollgard Cotton Event 15985.

The APVMA has evaluated the chemistry aspects of *Bacillus thuringiensis* var. *kurstaki* delta-endotoxins as produced by the *CryIAc* and *Cry2Ab* genes and their controlling sequences (the endotoxins), including the manufacturing/transformation process, quality control procedures and analytical methods, 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 012/2002 involving Bollgard II (see <http://www.ogtr.gov.au/ir/rarmp.htm#final>). A brief summary of the APVMA assessment is provided below.

#### Manufacture

Bollgard II cotton event 15985 was created through the retransformation of Ingard cotton event 531 (registered as Ingard Gene by Monsanto, 'Ingard') with the genetic material in the Bollgard II event 15985. Bollgard II event 15985 contains the following genes and partial gene sequences (additional to Ingard) inserted via genetic engineering techniques (particle acceleration method):

#### *Cry2Ab* gene

The *Cry2Ab* gene encodes for the insecticidal protein, *Cry2Ab*, derived from the common soil bacterium *Bacillus thuringiensis* var. *kurstaki*. *Cry2Ab* protein has insecticidal activity specific to certain species of insect.

#### *b*-glucuronidase (*uidA*) gene

The  $\beta$ -glucuronidase (*uidA*) gene is derived from *Escherichia coli* strain K12 and encodes for the enzyme  $\beta$ -D-glucuronidase (GUS). The GUS protein is used as a scorable marker protein in plant transformation, enabling easier detection of both the *Cry2Ab* and GUS proteins due to their close affinity.

The gene cassette that produces the *Cry2Ab* protein also contains the controlling sequences required to insert the necessary genes into the cotton germ plasm, as well as produce the endotoxin in the necessary concentration to be effective. Disarmed strains of the bacterium *Agrobacterium tumefaciens* (genes responsible for tumour induction in plants were removed) were used to transfer the necessary genes and controlling sequences into the plant cell genome. The DNA sequences which drive expression of the protein originate from the Cauliflower mosaic virus and *Petunia hybrida*.

#### Stability of the Genetic Changes

To determine if the transferred genes are stable across generations, a series of tests were conducted with the progeny. Data from four generations, including two generations backcrossed to commercial cultivars, were analysed, comparing the frequency of observed to expected number of progeny that expressed the *Cry2Ab* protein. Expression of *Cry2Ab* was assessed by enzyme linked immunosorbent assay (ELISA). The results demonstrated the expected segregation ratios for the different crossings with respect to the *Cry2Ab* protein. Genetic stability was also confirmed by Southern and Western blot analysis of the inserted DNA across five plant-breeding generations.

These results demonstrated that the DNA insert is stable in the plant genome across at least five breeding generations. Previously conducted and successful field trials with Bollgard II in different cotton varieties are also evidence of insert stability.

#### Characterisation of the Genes in the Plant

A range of molecular tools, including Southern blot and Polymerase chain reaction (PCR) analyses were used to characterise the inserted DNA in cotton containing event 15985. Genomic plant DNA was analysed using Southern blot analysis to determine the insert and copy number, the integrity of coding regions of both genes and their respective regulatory sequences. Data from the molecular analyses were consistent with a single DNA insertion into the genome of cotton containing event 15985.

#### Molecular Characterisation of Cotton Event 15985

Genomic DNA was analysed by Southern blotting to determine the number of insertion events, the copy number of the inserted DNA and the integrity of the insertions. The submitted data showed that *Cry2Ab* cotton event 15985 contains one DNA insertion, in addition to the insert present in the parental line (i.e. the Ingard event), which was concluded to only be the *Cry2Ab* and GUS protein genes and the related DNA sequences. Therefore only the *Cry2Ab* and GUS proteins, in addition to the genes and sequences already present via Ingard, are expected to be produced in the plant.

# HUMAN TOXICOLOGICAL ASSESSMENT

## Evaluation of Toxicology

The mode of action of the two *Cry* proteins in question is highly specific to certain species of insects due to the number of important factors that must be present before the endotoxins can be effective. These involve the high pH environment of the midgut which enables the protein to be soluble as well as the required midgut binding receptors for the endotoxins. In addition specific gut proteases are required to convert the *CryIAc* protoxin into the active core toxin. In contrast the human gastrointestinal tract has a very low pH (1.2) acidic environment, rendering the protein effectively insoluble and therefore inactive.

The *Cry* class of proteins, including the *CryIAc* protein, are also well represented in many commercial Bt formulations currently used in Australia. The *Cry2Ab* protein however is poorly expressed in Bt bacteria and so is unlikely to be present in high concentrations. The *CryIAc* protein produced in Bollgard II is considered to be 99.4% identical to the naturally occurring Bt toxin and while the *Cry2Ab* protein is not thought to be present in high concentrations, it is highly similar (97% at the amino acid level) to the *Cry2Aa* protein which is present in significant concentrations in commercial Bt insecticide spray products.

The toxicity of the *CryIAc* protein and other compounds expressed by Bollgard II which are also found in Ingard, are considered to be no different to when they are expressed by Ingard. As such the APVMA considers the risk to human health to be no greater from these compounds in Bollgard II and this assessment will focus primarily on the risks associated with the additional compounds from the Bollgard II event.

### Acute Studies

The *Cry2Ab* protein was found to be of low oral toxicity in mice, with an LD<sub>50</sub> of >1450 mg/kg bw in one study and >4000 mg/kg bw in another study. Assessment of the acute oral toxicity of the β-glucuronidase (GUS) protein was limited by the doses used. No abnormal signs or deaths were seen at 100 mg/kg bw (the highest dose tested).

### Other Studies

There were no similarities between the *Cry2Ab* or β-glucuronidase proteins and any known allergens or gliadins. This indicates that the potential for sensitisation by these proteins is low. *Cry2Ab* proteins were similar to *Cry2* toxins, as expected, however no similarities to other toxins were found. Both proteins were rapidly digested in simulated gastric fluid and degraded within 24 h in simulated intestinal fluid. The insecticidal activity of the *Cry2Ab* protein, as measured in insect bioactivity tests, was lost following digestion.

### Discussion

As the toxins are only expressed in the growing cotton plant, there is limited potential for human exposure. While portions of cotton plants are used for human consumption, these either are not expected to contain proteins (such as cotton seed oil), or undergo processing procedures expected to denature proteins. Food for human consumption is therefore not expected to contain any endotoxin. It was also shown that the endotoxins are rapidly digested in the mammalian digestive system. The endotoxins are highly specific for certain species of insects, who have specific receptors in the mid gut which binds the toxin, opening ion-specific pores and disrupting digestive processes.

The acute oral toxicity test on the  $\beta$ -glucuronidase protein was done at low doses (100 mg/kg), however there is limited expression of this protein in cotton tissue (<0.007% dry weight in the seed) and it was felt that dosing at this level gave an adequate margin of safety for expected exposure. There were no abnormal clinical signs at the top dose.  $\beta$ -glucuronidase protein is also a ubiquitous protein, present in many components of the diet.

As the *Bacillus thuringiensis* delta endotoxin is not found in processed cotton components which are likely to be used as food, no Acceptable Daily Intake (ADI) or Acute Reference Dose (ARfD) is required to be set. Scheduling is not required for the *Bacillus thuringiensis* var *kurstaki* delta endotoxins produced by the *Cry 1Ac* and *Cry2Ab* genes in cotton plants. No first aid instructions are required due to the low toxicity of the proteins and because there is negligible user exposure to the endotoxins, safety directions are also not required.

## RESIDUES ASSESSMENT

### Residues in Food

#### Residues in cotton-seed oil and linters

As processing removes or deactivates all proteins from commodities available for human consumption, cotton-seed oil and linters, there are no residues concerns regarding human consumption of the edible commodities produced from Bollgard II Cotton Event 15985. Additionally, the toxicological assessment determined that no ADI or ARfD are necessary, as *Bacillus thuringiensis var. kurstaki* delta endotoxins *CryIAc* and *Cry2Ab* are not found in commodities available for human consumption.

#### Residues in Animal feed

The concentrations of *Bacillus thuringiensis var. kurstaki* delta endotoxins *CryIAc* and *Cry2Ab* were determined in leaf, seed and whole plant. Additionally, levels of the artefact marker and selection proteins; D-glucuronidase (GUS), neomycin phototransferase (NPTII) and 3''(9)-O-aminoglycoside adenylyltransferase II (AAD) were determined in leaf, seed and whole plant. The concentrations of proteins in plant tissues are tabulated following.

Table 1: Levels of *CryIAc* and *Cry2Ab*, GUS, NPTII and AAD in recombinant cotton event 15985 from 16 field locations (1998 and 1999 growing seasons)

	Protein Concentration range (mg/kg fresh weight)		
	Leaf	Seed <sup>1</sup>	Whole Plant
<i>CryIAc</i>	0.39-4.19	1.30-4.84	0.07-0.32
<i>Cry2Ab</i>	4.55-33.3	31.8-85.5	7.3-22.1
GUS	51.7-202	36.3-271	NA <sup>#</sup>
NPTII	2.44-33.7	8.88-18.3	NA
AAD	ND*	ND	NA

<sup>#</sup>Not Analysed

\*Not Detected

<sup>1</sup>including hulls

The results demonstrate that *CryIAc*, GUS and NPTII proteins are expressed at levels comparable to the currently registered Ingard cotton (approval number 48296). The *Cry2Ab* protein is expressed at levels significantly higher than the *CryIAc* protein (up to 85ppm in seed on a fresh weight basis). Toxicological studies have indicated that the oral toxicity of *Cry2Ab* endotoxin to humans and other mammals is low and that the protein is rapidly degraded in gastric fluid. Therefore no finite residues of any proteins expressed by Bollgard II are expected in animal tissues for human consumption from the feeding of cotton containing Bollgard II to animals.

*Bacillus thuringiensis var. kurstaki* delta endotoxin proteins expressed in recombinant cotton are currently exempt from the requirement to establish an Australian MRL. The current proposal is encompassed by this exemption, and as such no amendments to the Australian *MRL Standard* are required.

#### Conclusion

There are no concerns to human health from residues in either cotton or animal commodities resulting from the proposed use of Bollgard II.

## **OCCUPATIONAL HEALTH AND SAFETY ASSESSMENT**

### **Assessment of Occupational Health & Safety**

The human toxicological assessment found that compounds produced by Bollgard II are considered to be of low toxicity and very low risk to human health due to the combination of low toxicity, allergenicity and negligible exposure of people to the compounds. As a result, no safety directions are considered necessary to warn users of any potential toxicological risk.

Therefore, the risk to people working in Bollgard II cotton crops and with any produce or waste from Bollgard II cotton crops is also considered to be very low. As a result, no further safety precautions for workers, such as personal protective equipment or re-entry periods are considered necessary.

## ENVIRONMENTAL ASSESSMENT

### Mode of action and specificity of endotoxins

The mode of action of Cry toxins involves several steps. First, the crystal protoxin is dissolved in the alkaline insect gut, then proteolytically processed to yield a smaller active form of the toxin. This processed proteinaceous toxin is stable in the insect gut and when in contact with the mid-gut epithelium, binds to specific receptors on the cell membrane. Certain regions of the Cry toxins insert into the cell membrane to form specific or non-specific pores or ion channels through manipulation of the membrane receptor. This disrupts the osmotic balance of the endothelial cells, causing them to swell and burst. The insect stops feeding and septicaemia sets in, ultimately leading to death.

The high degree of specificity of toxicity to particular groups of insects is conferred on the Cry toxins by the specific and sequential nature of the steps involved in their mode of action.

### Environmental exposure

Assuming that a 1 kg fresh plant expresses 4 µg/g F.Wt *CryIAc* (a maximum level), with 100 000 plants ha<sup>-1</sup>, the upper limit of *CryIAc* entering the environment is estimated to be about 400g ha<sup>-1</sup>. The maximum environmental exposure to *Cry2Ab* is about 5 kg ha<sup>-1</sup>, using a maximum expression level of 50 µg/g F.Wt for *Cry2Ab* in Bollgard II cotton. These values are likely to be overestimates, but their use allows a cautious approach. The soil concentration of toxins can be calculated to be 400 ng/g (*CryIAc*) and 5 µg/g (*Cry2Ab*) soil, if we assume a soil density of 1 g/cm<sup>3</sup>, no degradation, and that all toxin in a whole plant (including a full root system) enters the soil to a depth of 10 cm in one instant.

However, maximum exposure could be higher than this if we also consider a worst-case scenario where the levels of root 'exudation' determined by Dr Gupta Vadakattu and Dr Stephanie Watson (CSIRO Land and Water, Adelaide, SA) applied. Root 'exudation' probably refers to a situation where Bt toxin may be released into the soil continuously through the growth cycle of the plants through rapid turnover of abundant root hairs and root cap cells at and adjacent to growing root tips.

Assuming release of approximately 4 µg g<sup>-1</sup> F.Wt of root per 24 hours, and that roots comprise 300 g F.Wt per plant and plants reach 160 days of age, an additional 3.84 kg of *CryIAc* ha<sup>-1</sup> would enter the soil over a season, giving an upper limit soil concentration to be 4.24 µg *CryIAc* per g soil. By analogy for *Cry2Ab*, and assuming root 'exudation'/whole plant contributions in the same proportionality for *Cry2Ab* as *CryIAc*, an additional 48 kg ha<sup>-1</sup> of *Cry2Ab* would enter the soil over a season, giving a projected worst-case-scenario maximum *Cry2Ab* soil concentration of 53 µg/g soil.

The above calculations are likely to be overestimates because the highest observed levels of endotoxin expression are used, the calculations assume that all toxin produced over a growing season enters the environment at once, and toxin degradation is not taken into account (this is usually rapid in soils).

### Environmental fate

Studies by the proponent on soil degradation show 50% and 90% degradation times for leaf tissue (DT<sub>50</sub> and DT<sub>90</sub>) for *Cry2Ab* in Bollgard II cotton of 2.3 days and 15 days respectively using an insect bioassay and based on non-linear decay. Soil cores collected three months post-tillage from a range of Ingard crop sites in the USA contained no detectable toxin, using ELISA and bioassays with a detection limit of 3.68 ng and 8 ng of toxin per g of soil respectively.

Similarly, an Australian soil study by Dr Gupta Vadakattu and Dr Stephanie Watson (CSIRO Land and Water, Adelaide, SA) on degradation of *CryIAc* in buried Ingard and Ingard/RoundupReady (RR) cotton tissue at an Australian field site (Narrabri), found that levels of endotoxin in Ingard and Ingard/RR cotton leaves in litter bags declined from 1.14

and 0.98 µg/g soil D.Wt respectively at week 0, to 0.12 and 0.25 µg/g D.Wt after 2 weeks, and to levels that were not quantifiable thereafter due to soil inhibition of the ELISA. While no soil degradation data are available for *Cry2Ab* in Australian soils, similar rates to that for *Cry1Ac* would be expected.

These experiments indicate a rapid initial degradation rate of *Cry1Ac* once leaf material is incorporated into the soil, with remaining minor amounts degrading more slowly, and that this occurs in a typical Australian soil. High rates of degradation would suggest that the maximum endotoxin levels predicted (above) are likely to be overestimates in many cases and that any continual input of toxin from roots is unlikely to result in accumulation of toxins in the field.

Interpretation of data on mobility of endotoxins in soil suggests that while free toxin may be partially mobile, this would be offset by the likely low *rate* of input of free endotoxin (from gradual degradation of cotton trash or release from roots during growth) and by relatively rapid degradation rates. It is unlikely that any endotoxin would move beyond the immediate site of cropping due to these factors, except in unusual circumstances of extreme or flash flooding. In this case, however, there would likely be a considerable dilution of any toxin present in soils.

In summary, there is no evidence from a variety of studies that endotoxins from Bollgard II cotton accumulate or persist in soils, and therefore exposure levels *in situ* are unlikely to exceed those calculated above.

### **Environmental toxicity**

#### *Cry1Ac*

Toxicity testing of *Cry1Ac* has been previously evaluated for Ingard Cotton. There is no new information that would suggest that *Cry1Ac* is toxic to organisms other than to insects in the Order Lepidoptera (moths and butterflies).

#### *UidA*

*UidA* encodes the β-glucuronidase (GUS) enzyme, which catalyses the cleavage of a range of β-glucuronides. GUS is used widely as a marker gene, and poses no environmental risks.

#### *Cry2Ab*

The high degree of specificity of *B. thuringiensis* (Bt) Cry toxins is well known and the *Cry2A* toxin class is no exception, exhibiting insecticidal activity toward lepidopteran and some dipteran species only. Tests for *Cry2Aa* toxins have covered species from the insect Orders Lepidoptera, Diptera, Coleoptera, Orthoptera, Hymenoptera, Homoptera, Neuroptera, Hemiptera, and Isoptera, the insect relatives Collembola, and the Crustacean order Isopoda.

*Cry2Ab* toxin has a similar high degree of specificity, however there are contrasting reports of toxicity of this toxin class to Diptera. One study reports toxicity while four other studies report no toxicity (eg LC<sub>50</sub> of >100ppm). These different results may be due to differences in testing protocols or to slight differences in amino acid composition of the toxin.

Toxicity tests by the proponent for a number of representative species, used either cottonseed meal (for bobwhite quail and catfish), lyophilised and ground leaf tissue (for Collembola), or purified *Cry2Ab* protein (for the remaining tests) as test substances. Minimum No Observable Effect Concentration (NOEC) values for *Cry2Ab* were achieved for bobwhite quail, channel catfish, adult honey bee, larval honey bee, ladybird beetle, Collembola, green lacewing larvae, parasitic Hymenoptera and earthworm.

Toxicity studies with three susceptible insect species using three different cotton tissues (containing either *Cry1Ac*, *Cry2Ab*, or both) showed that toxicity is additive. Therefore it is appropriate to consider NOEC values of each toxin to different insect species additively when predicting potential ecotoxicity.

The maximum concentrations of *CryIAc* and *Cry2Ab* toxins possible in soils are estimated (above) to be approximately 400 ng/g soil and 5 µg/g soil respectively or, if worst-case scenarios of maximum root 'exudation' and zero degradation apply, 4.24 µg/g soil and 53 µg/g soil respectively. Reported NOEC values for *CryIAc* are 20 ppm for most species tested by the proponent, and for *Cry2Ab* 68 ppm (adult honey bee), 69.5 ppm (Collembola), and substantially higher values (above) for other species tested.

For *CryIAc* toxin, the worst-case scenario maximum soil concentration (4.24 µg/g soil) is about five times less than the established NOECs. It is therefore unlikely that there will be any toxic effects on non-target organisms except Lepidoptera. For *Cry2Ab*, in the worst-case scenario (no degradation of toxin, either within a plant or the soil), some reported NOECs are similar to the estimated soil concentration. The estimate is highly unlikely to be realised, however. Furthermore, reported NOECs are the maximum test concentrations used in the studies and, in the context of the wide congruence of data on specificity of *Cry* toxins in general, actual NOECs are probably much higher. The reported NOEC values for *CryIAc* and *Cry2Ab* are also higher than the maximum toxin levels measured in leaves (4.19 µg/g F.Wt. and 49.4 µg/g F.Wt. respectively), so direct feeding on leaf matter is unlikely to produce toxic effects in non-lepidopteran non-target animals.

In studies that report no dipteran toxicity, cited LD<sub>50</sub> values for *Cry2Ab* are >20ppm and >100 ppm. Our estimated, worst-case scenario maximum level of *Cry2Ab* in soil after cropping (53 µg/g soil) and the maximum level of *Cry2Ab* expressed in leaves (49.4 µg/g F.Wt) lie between these values. Although our estimate of the maximum level of *Cry2Ab* in soil after cropping is almost certainly a considerable overestimate, there is also a study that reports toxicity of *Cry2Ab* to Diptera, therefore toxicity to Diptera in some conceivable scenarios is not entirely ruled out. Even if *Cry2Ab* is toxic to Diptera, further circumstances would need to apply for there to be any impacts on dipteran species. Consideration of potential exposure routes and of species potentially exposed, suggest that many well-known dipteran species in cotton crops are unlikely to be significantly exposed.

While on-site exposure could be above-ground or below-ground, potential adverse effects on Diptera would be unlikely to be any greater than those of existing synthetic insecticide applications where cotton cropping already occurs. On balance, dipteran exposure off-site is not likely to be significant in current cropping areas where tail-water return systems are in place, and impacts on Diptera (if *Cry2Ab* is toxic to Diptera) would probably be less than under current pesticide use practices. Exposure on-site would be limited also by the requirement under an Insect Resistance Management Plan to limit the area sown to Bt cotton.

In a commercial Bollgard II cropping scenario, exposure of Diptera to *Cry2Ab* toxin could be more significant in northern Australia than in current cotton-growing areas, because Diptera may be a more significant component of aquatic systems in the north, a high percentage of Bollgard II is most likely required in the north (due to previous resistance problems to synthetic pesticides), and tail water recycling systems may not apply.

In a previous evaluation of potential indirect ecological effects ('tri-trophic' effects) of Ingard cotton, prey-mediated toxicity impacts on predators of target lepidopteran species, such as ladybeetles and lacewings, and on hymenopteran parasitoids of target species were concluded to be minimal. A more recent study found that aphids fed on a diet with *CryIAb* contained 250–500 times less toxin than was in the diet, and when fed on *CryIAb* corn contained no detectable toxin.

In a recent Australian study (by Dr Gupta Vadakattu and Dr Stephanie Watson, CSIRO Land and Water, Adelaide, SA) differences in microbial growth indicators ('respiratory quotient' – a measure of microbial activity per unit microbial biomass - and 'substrate induced respiration' - respiration rate resulting from the substrate material) suggest that microbial population growth on Ingard cotton leaf litter might be different than for non-Bt varieties. Microscopic examination revealed an apparent increase in fungi and fungal spores on the Bt-cotton residues compared to the non-Bt residues. Experiments did not indicate whether these

changes were likely to be detrimental, neutral or beneficial in an agricultural situation. By contrast, a US study found no apparent effects of Cry1Ab of Bt corn roots or biomass on earthworms, nematodes, protozoa, bacteria or fungi.

Three studies submitted by the proponent examined the abundance of non-target arthropod species above-ground on plots of conventional, Ingard or Bollgard II cotton in Queensland and the East Kimberley region of Western Australia. In two studies, trial plots were “unsprayed” and in the third they were “unsprayed with pesticides for Lepidoptera”. Results suggest that there are no major impacts of Bollgard II cotton, above ground, on insects other than Lepidoptera (and possibly Diptera), including secondary ecological impacts.

An independent study examined overall arthropod community composition in fields of Bt cotton (Ingard and some Bollgard II cotton) compared to conventional cotton in the western USA. Natural enemy abundance and overall arthropod diversity was not adversely affected by Bt cotton compared to non-transgenic cotton, whereas adverse impacts did arise from insecticide applications. Comparison of single gene cotton with Bollgard II cotton showed similar predator abundance and arthropod richness, diversity and evenness. Results support the conclusion that Bt cropping does not impact adversely upon farming ecosystem function and diversity compared to pest management regimes for non-transgenic varieties.

### **Unintended gene transfer to native and feral cotton south of 22° S**

It is extremely unlikely that Cry toxin transgenes will be transferred from Ingard cotton to native species (*Gossypium sturtianum* or *G. australe*) in NSW and Southern and Central Queensland south of 22°S. In addition to significant plant species reproductive barriers, there are few populations of native cottons close to commercial cotton crops in the approved area south of 22°S. In respect of transgene transfer to feral cotton, this will occur where and if feral populations occur close to cotton crops.

### **Potential invasiveness of cotton conferred by Cry genes south of 22° S**

Bollgard cotton is no more likely than conventional cotton to become a weed in New South Wales or southern Queensland cotton growing regions. A number of factors other than lepidopteran insect herbivory (low temperature and reliable water availability) limit growth, survival and persistence. Transgene transfer to feral *G. hirsutum* in areas south of latitude 22°S is not an issue given the rarity of persistent feral cotton populations there.

### **Occurrence of feral cotton in Northern Australia**

The distribution of feral cotton in the Northern Territory (NT) was studied during the 1998 dry season (May and June) by the NT Department of Primary Industries and Fisheries. There are a considerable number of persistent populations of feral cotton distributed throughout the NT, often in quite isolated locations. Additionally, some populations examined in the survey occur in or near proposed cotton growing regions in the NT. Existing populations are believed to be primarily derived from varieties and cultivars introduced early in the NT’s history of European settlement. The findings suggest that habitats favourable for feral cotton growth are not uncommon in this region.

There is a potential risk that Bt transgenes could increase the weediness of cultivated cotton or of feral cotton after transgene transfer from Bt cotton in northern Australia, through providing increased protection against lepidopteran herbivores. This could occur if lepidopteran herbivory currently limits survival and persistence of feral cotton.

### **Likelihood of transgene transfer to feral cotton in northern Australia**

Gene flow via pollen dispersal can occur between sexually compatible species, including cultivated or feral cotton (*G. hirsutum* and *G. barbadense*), certainly at low frequencies and over short distances. Such gene transfer has been experimentally demonstrated over 45–60 m in the USA and over a 50 m bare ground isolation zone in Central Queensland.

Frequencies of transgene transfer to feral cotton populations would depend on a multitude of factors, including the occurrence of feral cotton, survival and reproduction rates of Bollgard II

volunteers, and abundance and behaviour of insect pollen vectors. Whatever the frequency, the key information required to evaluate the significance of gene transfer is information on whether or not the traits confer a significantly altered selective advantage to feral cotton, hence the need for 'exclusion studies' (below).

### **Potential for invasiveness conferred by transgenes**

In proposed cotton growing areas of northern Australia (ie. north of 22°S) there may be habitats that favour growth and persistence of cotton away from agricultural sites due to a reliable water supply, high nutrient soils and low fire frequency. In these habitats at least, lepidopteran insect feeding pressure may limit survival and persistence (and hence population growth) of feral cotton to a greater extent than elsewhere. Consequently, there is a risk that the *Cry* genes from Bt cottons may confer a selective advantage to cotton in these habitats, allowing feral populations to become more invasive or weedy.

A CSIRO study to research these concerns about potential weediness of Bt cottons in northern Australia examined the influence of the *Cry* transgenes on factors including germination, survivorship, fecundity and invasiveness over two seasons. The report concluded that Bt genes would not confer increased weediness on feral cotton. However, elsewhere the report states that current opinion in the literature is that it is difficult to interpret longer-term ecological trends (such as invasiveness of an alien species) using data collected over a short period of time, especially when assessing a perennial species such as cotton.

Transgene transfer could be most significant if transfer to existing feral populations occurred. These are apparently well-adapted to the habitats where they are found, as self-sustaining populations exist. It is commonly thought that existing feral populations are primarily derived from previous cultivars of cultivated cotton or ornamental cottons; these may have become very well adapted to environmental conditions in northern Australia over many years of persistence. Further information on the impacts and importance of Lepidoptera exclusion on existing cotton populations will be required before a decision is made about commercial release of Bollgard cotton in this region. If such studies demonstrate conferral of selective advantage, further information on feral cotton distribution should also be collected.

### **Likelihood of transgene transfer to native cotton species in northern Australia**

Most Australian native *Gossypium* species occur in north western Western Australia (WA), with a concentration of species in the monsoonal Kimberley region. *G. cunninghamii* is restricted to the Coburg Peninsula (NT), and *G. robinsonii* is found in WA west of the Great Sandy and Gibson deserts.

New analyses of the risk of transgene transfer from cultivated cottons to native cotton species in Australia conclude that the likelihood of Bt transgenes escaping to native *Gossypium* populations is "functionally zero". This conclusion is based primarily on the distribution of native species relative to proposed cotton growing areas, the likely limited pollen transfer, and very low probability of producing hybrid seed and establishing sexually reproducing hybrid plants.

The studies convincingly argue that only one K-genome species (most likely to successfully hybridise) "occurs close enough to any of the [proposed] cotton-growing regions in northern Australia or the main roads servicing the region to be at risk" (i.e. *G. rotundifolium* in the Broome growing area). The argument would apply where Bt cotton cultivation was restricted to the proposed cotton growing regions in northern Australia, but would not perhaps fully apply where unrestricted Bt cotton release north of latitude of 22°S was permitted.

Considering proposed Bt cotton-growing in the Ord/West Kimberley region and the geographical distribution overlap of *G. rotundifolium* with the proposed Broome growing area, other factors would limit the potential for successful hybridisation. These include cross pollination only over short distances, an extremely low or effectively zero rate of hybrid seed formation (where *G. rotundifolium* is used as a female plant), and predicted low or zero rates of seed viability, hybrid establishment and fertility.

Fifty seven attempts were made to cross *G. hirsutum* with *G. rotundifolium* using the K genome species as female. In no case was seed set, and a possible explanation is that the differences in chromosomal number and composition of these reciprocal hybrids may result in failure in development of hybrid endosperm and its normal functions. It is not as clear whether other K genome species have rates of seed set in such crosses as low as those of *G. rotundifolium* because fewer attempts at crossing these species were made.

The likelihood of transgene transfer from cultivated Bt cotton to native cotton species in proposed cotton-growing regions (ie Ord River Irrigation Area, and circumscribed areas in the West Kimberley, Katherine, Darwin, Richmond and Weipa regions), including to *G. rotundifolium* in the proposed Broome growing area, is extremely low. Therefore, there is no unacceptable risk from this hazard.

## **Conclusions**

In conclusion, the use of Bollgard II south of latitude 22°S is not likely to have an unintended effect that is harmful to animals, plants or the environment. This conclusion is consistent with the licence granted by the OGTR (DIR012/2002), which allows the release of Bollgard II cotton south of latitude 22°S. The uncertainty about environmental risks north of latitude 22°S, primarily relating to feral cotton weediness and but also in respect of *Cry2Ab* toxicity to Diptera, will require that more information be provided before extension of unrestricted commercial use of Bollgard II north of latitude 22°S can be considered.

## **ASSESSMENT OF OVERSEAS TRADE ASPECTS OF RESIDUES IN FOOD**

### **Relevant Export Commodities**

Cottonseed and meal are significant export commodities. In 2000-1 Australia exported 657 kt of cottonseed, 0.64 kt cottonseed oil and 59 kt of cottonseed and sunflowerseed oil (Australian Commodity Statistics 2001, 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 Bollgard II. .

### **Overseas Registration Status and MRLs**

Both the United States and Japan have assessed Bollgard II cotton with respect to the risks from consuming the resultant food commodities. The United States determined that the *Cry2Ab* 'protein and the genetic material necessary for its production in corn or cotton are exempt from the requirement of a tolerance when used as plant-pesticides in the food and feed commodities of field corn, sweet corn, popcorn, cotton seed, cotton oil, cotton meal, cotton hay, cotton hulls, cotton forage, and cotton gin by-products'. Japan has accepted Bollgard II cotton as safe for human consumption.

In addition, Taiwan has omitted *Bacillus thuringiensis* from their MRL standard.

### **Potential for Undue Prejudice to Australia's Export Trade**

As all major importing countries have established exemptions for commodities originating from recombinant cotton event 15985, no undue prejudice to trade exists from the export of commodities incorporating cotton event 15985.

In addition, as Australia has been exporting food commodities resultant from the use of Ingard 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 Bollgard II in cotton.

## EFFICACY AND CROP SAFETY ASSESSMENT

### Justification and Proposed Use Pattern

Data was provided to demonstrate that Bollgard II will provide equivalent or better efficacy against *Helicoverpa armigera* (*Ha*) and *Helicoverpa punctigera* (*Hp*) than Ingard.

It is claimed that the benefits of replacing Ingard with Bollgard II will not only be more effective control of *Helicoverpa spp.* but reduce the risk of resistance developing to either endotoxin (*Cry1Ac* or *Cry2Ab*).

The primary issues under consideration are efficacy, crop safety and resistance management.

### Evaluation of Efficacy and Crop Safety

Efficacy data collected from two years of trials from across all Australian cotton growing regions, along with supporting data from the USA was provided as demonstration of efficacy. Data included both laboratory bioassays using field grown leaf samples, in-field pest monitoring and recording the number of insecticide sprays used. Expression levels in different plant parts were also provided as well as levels of boll damage and fruit retention.

Laboratory bioassays were conducted on collected leaf samples from Bollgard II, Ingard and unmodified conventional cotton, and assessed against larval survival and larval development rates throughout the cotton season. The trial protocols involved using standard leaf samples (the upper, most fully expanded leaf), and recording the fate and development of neonate and second instar larvae on the leaves.

This methodology was considered satisfactory for the purposes of a controlled comparison of Bollgard II with Ingard and conventional cotton. The bioassay data did provide clear evidence that Bollgard II in most cases offers superior control of *Helicoverpa* (*Ha* & *Hp*) compared to Ingard with respect to protein expression in new leaves. However, in-field checks were required to satisfy that *Helicoverpa* were not surviving in areas of lower protein expression, high pest pressure did not affect efficacy or if efficacy was sustained all season.

Field monitoring trials were conducted in 2001/2002 to demonstrate the efficacy of Bollgard II and Ingard in the cotton crop as a whole rather than only the efficacy of new leaves. Trials were undertaken in 4 areas from across the main cotton growing areas of NSW and Queensland. Trials involved recording the larval survival and development throughout the season to determine when larvae begin to survive as well as if numbers reach commercial threshold levels (ie where a *Helicoverpa* targeted spray would be required).

Results demonstrated that survival and threshold levels were reached in Ingard cotton as early as 58 days after planting (DAP), but ranged up to 99 days. Bollgard II crops did not start surviving until at least 88 days, however in most cases, threshold levels were not reached all season, demonstrating season long protection. Where thresholds were reached it was close to the end of the season (124 and 141 DAP). In all cases, Bollgard II provided superior efficacy to Ingard.

Although it could be argued the general pest pressure for this season was generally low, evidence of suitable efficacy in high pest pressure situations was still provided. One site did face very high pest pressures, measured by the levels of egg infestation, where up to 800% *Helicoverpa* egg threshold levels was recorded (80 eggs a metre). Despite this pressure, the Bollgard II crop did not have any significant levels of larvae survival for the entire season.

As a supplement to the leaf bioassays, data on expression levels throughout the season in terminals and squares, two plant features important to crop yields, were also provided to demonstrate that larvae would not be able to survive in areas of low protein expression. The results demonstrated that expression levels in terminals and squares in Bollgard II cotton at 108 DAP were considerably greater (x2-x4) than in Ingard even at 80 days DAP and would still be sufficient to provide adequate efficacy.

Data demonstrating the number of insecticide sprays required was also argued as a means of demonstrating a benefit and therefore efficacy to the grower. Thirteen commercially run trials were used to demonstrate the difference in spray requirements for Bollgard II, Ingard and conventional (sprayed) cotton.

Results demonstrated that Bollgard was far superior to both Ingard and conventional cotton. Of the thirteen sites, the Bollgard II crops received only two *Helicoverpa* specific sprays compared to the 21 for Ingard and 100 for conventional, or an average per crop of 0.2, 1.6 and 8.3 respectively. It was also noted that although two sprays were made to Bollgard II crops, *Helicoverpa* threshold levels were never reached.

When all spray applications were included, the total numbers (and averages) were 32 (2.5), 52 (4.0) and 109 (9.1) respectively, indicating that the additional *Helicoverpa* sprays provided to the conventional crop were also having an impact on the non-*Helicoverpa* pests which were not controlled by either Bollgard II or Ingard. Irrespective on this, is that while Ingard has managed a credible 39% to 62% total spray reduction since registration in 1996 (56% for 2001/02), Bollgard was able to achieve a 73% total spray reduction for 2001/02. In addition, it was noted (see below) that fruit retention and first position bolls were also greater in Bollgard II than both Ingard and conventional cotton.

Two additional measurements of efficacy with respect to cotton crop protection are the final percentage of first position retention of all fruiting branches developed prior to cut out and actual levels of boll damage. All other agronomic inputs being equal, these measurements should also demonstrate the effectiveness of Bollgard II in controlling *Helicoverpa*.

Mean first position fruit retention percentages were calculated from Bollgard II, Ingard and conventional cotton from 8 separate sites. The resultant means for Bollgard II, Ingard and conventional cotton was 80%, 68% and 61% respectively. It was also noted that even with an average of 8.3 *Helicoverpa* sprays for the conventional crop, it was not able to yield as great as either Bollgard II or Ingard.

Although the trials were conducted in the USA, data from 13 trials did indicate a considerable decrease in percent square damage from conventional cotton to both Bollgard II and Ingard.

Although the trial results have demonstrated that Bollgard II is able to provide considerably greater levels of protection to both Ingard and sprayed conventional cotton, it is recognised that under high pest pressure, economic thresholds for *Helicoverpa* may still be reached towards the end of the season. Therefore, the product label includes instructions requiring crop monitoring for *Helicoverpa* and that should industry thresholds be reached, it may be necessary to use alternative control measures such as *Helicoverpa* insecticidal sprays.

### **Evaluation of Resistance Management**

It was proposed that due to the presence of two endotoxins with sufficiently different modes of action, that a cap on the total amount of Bollgard II grown per grower, valley or total crop was not required, other than what would be imposed by the Resistance Management Plan

(RMP) for Bollgard II. In addition, the monitoring of resistance levels in *Helicoverpa* would continue in a similar manner as has occurred for Ingard.

The Bollgard II RMP was produced by the cotton industry's Transgenic and Insect Management Strategy (TIMS) Committee as the best method of managing resistance. The TIMS Committee is comprised of representatives from a wide range of organisations and groups, including the Australian Cotton Growers Research Association, QDPI, NSW Agriculture, CSIRO, Avcare and the Cotton CRC. The APVMA recognises the expertise of the committee members representing the member organisations.

The RMP requires various resistance mitigation measures by each grower to ensure resistance to the endotoxins can be effectively managed. These measures include requiring the grower to plant refuge crops of minimum sizes, types and distances from the Bollgard II crop, fixed planting windows, post harvest crop destruction, control of volunteer and ratoon cotton, pupae destruction and trap cropping.

The purpose of refuges is to generate *Helicoverpa* individuals that have not been exposed to the Bollgard II endotoxins (including via any Bt spray toxins), and which will also ensure that any individuals carrying genes encoding for a endotoxin resistance mechanism will have no greater selection advantage over those who do not. Therefore should any individual *Helicoverpa* survive from the Bollgard II crop, they are most likely to reproduce with one of the greater number of individuals produced from the refuge crop (without any resistance gene/s), thereby reducing the chance of the gene/s encoding for resistance to the endotoxins, increasing in prevalence in the resulting generation.

Refuges will vary in size dependant on the type of refuge being grown (eg sprayed or unsprayed cotton, pigeon pea, sorghum or corn) as each crop has different abilities to host *Helicoverpa* and subsequent production of the next generation. This allows flexibility for the grower with respect to the different farm management options a grower may wish to use, but at the same time still producing sufficient numbers of *Helicoverpa* which have not been exposed to Bt toxins.

The other mechanisms listed are all used as a means of either maximising effect of the refuge crops and minimising the chance of any individuals which may have survived the cropping season, due to the presence of an inherent resistance mechanism, surviving to pass on the resistance ability.

Results from trials using individuals known to be resistant to the *CryIAc* endotoxin exposed to *Cry2Ab* endotoxins, has demonstrated that the two endotoxins are sufficiently different in structure and their mode of action, that cross-resistance is not expected. Therefore should an insect pest develop resistance to one of the endotoxins, it is likely that the other endotoxin will still be efficacious, thereby limiting the potential for that resistant individual to pass the trait on to subsequent generations.

Although there are different views on whether there is an additive toxicity effect with the endotoxins, data has been provided to demonstrate that the potency of the *Cry2Ab* endotoxin levels present is significantly higher than that produced by the *CryIAc* endotoxins. This is important with respect to the reduction of *CryIAc* delta-endotoxin expression level in late season crops. Concerns have been raised regarding the possible increased risk to resistance evolving to the *Cry2Ab* delta-endotoxin due to the reduced levels of *CryIAc* delta-endotoxin remaining towards the end of the season.

If additive toxicity is occurring, the sub-lethal effects of the *CryIAc* delta-endotoxin remaining will still add to the potency of the *Cry2Ab* endotoxin, thereby reducing the risk of

insects with a *Cry2Ab* resistance ability surviving. If the claim of additive toxicity is not valid, the high levels of *Cry2Ab* endotoxin remaining, along with the other resistance management measures required by the RMP, still provide additional insurance should resistance mechanisms evolve to sub-lethal *Cry2Ab* endotoxin levels.

In coming to an agreement that the RMP would be sufficient to effectively manage resistance to both endotoxins, the TIMS committee used modelling exercises undertaken, which calculated theoretical numbers of generations until certain levels of resistance emerged in different scenarios (eg Roush 1998). Roush calculated that for single toxin Bt cultivars, the best method to delay resistance is with very large refuges (thus the current 30% cap on Ingard). However, gene pyramids, or stacking, provides a means to delay resistance with much smaller refuges. The modelling demonstrated that using two toxins providing 70% mortality and a 10% refuge, the likely number of generations required to develop resistance to both endotoxins is far greater (more than 10 fold) when applied simultaneously (Bollgard II) than sequentially (Ingard *CryIAc* crop then a *Cry2Ab* crop). This difference increased exponentially with an increase in refuge size. Therefore the difference between Bollgard and Ingard would be greater again (2x) due to the model taking into account two genes sequentially rather than *CryIAc* only.

The RMP will effectively enforce a 90% Bollgard II cap due to the minimum refuge requirement of 10% for unsprayed cotton. Other refuge crops will require refuges greater than 10%.

The applicant has notified the APVMA that should the application be successful, it intends to impose a 40% cap (Ingard + Bollgard II – see RMP p24) on growers for the first year, rather than allow growers to have the maximum Bollgard II possible under the proposed Bollgard II only RMP. Should a grower also be growing Ingard, a maximum of 25% Ingard could be grown. This is intended to be a transitional arrangement for the coming season only, after which Ingard will not be grown commercially.

Based on this information and the support from the States and TIMS, the APVMA is satisfied the RMP refuge requirements alone will be sufficient to effectively manage resistance to one or both endotoxins. The data supports the use of Bollgard II without a cap restriction as long as all users are compliant with the RMP.

The applicant is proposing to have agreements (legal contracts) with the users of Bollgard II to ensure users will carry out the actions required by the RMP, as well as allowing the applicant and their agents access to farms to conduct audits to monitor and if necessary enforce compliance with the RMP. To enable the APVMA to be satisfied the RMP will be followed, the APVMA is intending to require The applicant (via appropriate conditions of registration – see Attachment 2) have such an agreement with all users of Bollgard II. In addition, should The applicant intend to modify the agreement document in any manner, the APVMA must first sight and approve those amendments before it is used.

# RESISTANCE MANAGEMENT PLAN FOR INGARD® and Bollgard II™ COTTON 2003-2004

Developed by Monsanto Australia Limited and the Transgenic and Insect Management Strategy (TIMS) Committee of the Australian Cotton Growers Research Association

## *NSW/SOUTHERN QUEENSLAND SECTION*

Growers of INGARD® cotton are required to practise preventative resistance management. The requirements for resistance management for the 2003-2004 season are set out below. Adherence to the Resistance Management Plan is required under the terms of the INGARD® and Bollgard II™ Technology User Agreement and under the conditions of registration (Agricultural and Veterinary Chemicals Act 1994).

### **1. Combined area of INGARD® / Bollgard II™ cotton planted**

a) The total amount of INGARD® cotton to be grown by one grower (as defined by the growers trading name) in a valley must not exceed 25% of the total cotton being grown by that grower in that valley. The total amount of combined INGARD® and Bollgard II™ cotton to be grown by one grower (as defined by the growers trading name) in a valley must not exceed 40% of the total cotton being grown by that grower in that valley. (A grower may choose to plant up to 40% Bollgard II™ and no INGARD®.)

b) Combined Farm Units. Provided they comply with 1 a), growers with more than one farm unit in a valley may be able to seek approval to combine more than one farm unit and grow a total area of INGARD® cotton in excess of 25%, and combined INGARD®/Bollgard II™ in total excess of 40% of the total cotton area on a single farm unit. Note that as the combined percentage of INGARD®/Bollgard II™ cotton increases above 30% it must be accompanied by an increase in area of effective unsprayed refuge (see Box 2 under item 2. Refuges). Eligible growers who wish to choose this option may apply to a Monsanto Business Manager by submitting a plan detailing: proposed fields and areas of INGARD® cotton, Bollgard II™, conventional cotton and refuges (cotton and/or non-cotton). Only growers who receive approval of their plan by Monsanto will be eligible to proceed with this option.

c) Total cotton is defined as all cotton being grown on a farm unit and includes all INGARD®, Bollgard II™, Roundup Ready and conventional varieties including sprayed and unsprayed cotton refuges. All cotton must be managed as a viable crop and taken through to harvest. Dryland cotton is measured as green hectares.

### **2. Refuges**

Each grower will be required to grow a refuge crop capable of producing large numbers of *Helicoverpa armigera* moths which have not been exposed to selection with Bt, sufficient to dominate the matings with any survivors from INGARD® and Bollgard II™ crops and thus help to maintain Bt resistance at low levels.

The following refuge options are based on the combined area of INGARD®/Bollgard II™ cotton and different refuge options are required as the combined INGARD® / Bollgard II™ percentage increases.

**Box 1 – Irrigated INGARD®/Bollgard II™ cotton combined, not greater than 30%  
(For greater than 30% see box 4)**

For each area of irrigated INGARD®/Bollgard II™ cotton planted, a grower is required to plant a minimum of one of the following:

- (i) Irrigated cotton, which will not be treated** for any reason with products that control *H. armigera*. (If the viability of the refuge is at risk, with prior approval from the Monsanto Compliance Manager a non-Bt pesticide can be applied up to the 4<sup>th</sup> true leaf stage). The area of this cotton refuge should be at least 10% of the combined area of irrigated INGARD®/Bollgard II™ cotton planted.
- (ii) Irrigated cotton, which can be conventionally managed** for *H. armigera* and other pests, however, the use of Bt preparations throughout the season is prohibited. The area of this cotton refuge should be at least equal (100%) to the area of irrigated INGARD®/Bollgard II™ cotton planted.
- (iii) Irrigated pigeon pea which will not be treated** for any reason with products that control *H. armigera*, and managed with water and nutrients to ensure several cycles of flowering throughout the cotton season. The area of this pigeon pea refuge should be at least 5% of the area of irrigated INGARD®/Bollgard II™ cotton planted. Pigeon pea should be planted within two weeks of planting the INGARD cotton or as soon as the soil temperature reaches 17°C (requirement for germination).

**Box 2 – Irrigated Refuge Options, requiring pre-approval by Monsanto**

**iv. Irrigated sorghum or corn.**

An irrigated grower wishing to grow sorghum or corn as a refuge must submit a plan to the local Monsanto Business Manager and receive approval **before** these options can be planted. A plan for a sorghum or a corn refuge must include: a farm map showing proposed location, size, indicative planting dates and varieties for sorghum or corn refuge areas in relation to the INGARD®/Bollgard II™ planted areas. Sorghum or corn refuges must comply with the following:

- (a) Irrigated sorghum, which will not be treated** for any reason with products that control *H. armigera*, and managed to flower from at least 15 January to 28 February. The area of this sorghum refuge should be at least 15% of the combined area of irrigated INGARD®/Bollgard II™ cotton planted. See special conditions below.
- (b) Irrigated, corn which will not be treated** for any reason with products that control *H. armigera*, and managed to flower from at least 15 January to 28 February. The area of this corn refuge should be at least 20% of the combined area of irrigated INGARD®/Bollgard II™ cotton planted.

NB: Special conditions apply to growers who wish to grow options **iv. (a)** sorghum or **iv. (b)** corn. These conditions are:

- Either refuge will require a minimum of three planting dates. First planting date will be determined by the time to flower for the variety chosen for use in your area and the need for the refuge to be in flower by January 15. Subsequent plantings should then follow at 2 week intervals.
- A single planting of mixed maturity varieties is not acceptable.
- The minimum area of each planting should be at least one third of the total amount of this refuge type required.
- A plan indicating how either of these refuges will be managed must be submitted to and approved by the local Monsanto Business Manager before planting either of these two options.
- These refuge options will be closely monitored during the season to ensure refuge is managed appropriately to provide an effective, attractive refuge from 15 January to 28 February.

### **Box 3 – Dryland INGARD®/Bollgard II™ cotton**

For dryland INGARD®/Bollgard II™ cotton crops, options 2 (i) unsprayed conventional cotton and 2 (ii) sprayed conventional cotton are the only refuge options available, but these can be planted to dryland cotton. All refuges in dryland situations must be planted in fields with the same cropping history as that of the INGARD®/Bollgard II™ fields and must be managed in the same way as the INGARD®/Bollgard II™ fields.

### **Box 4 – Additional Unsprayed Refuge (combined INGARD®/Bollgard II™ greater than 30%)**

It is possible for growers to grow more than 30% of combined INGARD®/Bollgard II™ (eg INGARD® at 25% and Bollgard II™ at 15%). For these situations, when the combined INGARD®/Bollgard II™ percentage increases above 30%, it must be accompanied by an increase in effective unsprayed refuge as follows:

- ❖ 30 – 50% combined INGARD®/Bollgard II™ cotton. The grower will plant an additional area of:
  - Unsprayed cotton refuge amounting to 5% of the combined total area of the INGARD®/Bollgard II™ cotton or alternatively,
  - Unsprayed pigeon pea refuge amounting to 2.5% of the combined total area of INGARD®/Bollgard II™ cotton.
- ❖ 51-100% combined INGARD®/Bollgard II™ cotton. The grower will plant an additional area of:
  - Unsprayed cotton refuge amounting to 10% of the combined total area of INGARD®/Bollgard II™ or alternatively,
  - Unsprayed pigeon pea refuge amounting to 5% of combined total area of INGARD®/Bollgard II™ cotton.

The general refuge requirements set out below also apply to additional refuge areas.

#### **General conditions for all refuges are:**

(a) Refuge crops are to be planted and managed so that the refuge is attractive to *H. armigera* during the growing period of the INGARD®/Bollgard II™ cotton varieties. All cotton refuges should be planted within 2 weeks of the INGARD®/Bollgard II™ cotton on farm.

(b) When the cultivation of a refuge is required the corresponding INGARD®/Bollgard II™ cotton crop should also be cultivated at the same time.

(c) Preparations containing *Bacillus thuringiensis* may be used on INGARD®/Bollgard II™ cotton throughout the season BUT NOT on sprayed refuge crops.

(d) All refuges are to be planted within the farm unit growing INGARD®/Bollgard II™ cotton, preferably on one side of, or adjacent to, the INGARD®/Bollgard II™ cotton fields, but with a separation of no more than 2 km from the INGARD®/Bollgard II™ crops.

(e) All refuges should be at least 2ha in size and no dimension should be less than 48 meters.

(f) All refuge crops should be left undisturbed at least until pupae busting in INGARD®/Bollgard II™ cotton crop is complete [to ensure maximum emergence and effect of late pupae from refuges]. Defoliation or destruction of aerial parts of a refuge should only be carried out after INGARD®/Bollgard II™ cotton removal.

### **3. Planting windows**

All INGARD<sup>®</sup>/Bollgard II<sup>™</sup> crops and cotton refuges are to be planted into moisture or watered up by 15 November 2003.

### **4. Post-harvest crop destruction**

As soon as practical after harvest, INGARD<sup>®</sup>/Bollgard II<sup>™</sup> cotton crops must be destroyed by cultivation or herbicide so that they do not continue to host heliothis.

### **5. Control of Volunteer and Ratoon cotton**

Volunteer and ratoon cotton, within back-to-back fields (either INGARD<sup>®</sup>/Bollgard II<sup>™</sup> plants within conventional cotton fields or conventional plants within INGARD<sup>®</sup>/Bollgard II<sup>™</sup> fields) may impose additional selection pressure on *H. armigera* to develop resistance to the Bt protein produced by INGARD<sup>®</sup>/Bollgard II<sup>™</sup> cotton. Growers must make all reasonable effort to remove volunteer and ratoon plants as soon as possible from all fields being planted with INGARD<sup>®</sup>/Bollgard II<sup>™</sup> cotton following conventional cotton. All reasonable efforts must also be made to remove volunteer and ratoon plants from all fallowed and conventional fields following INGARD<sup>®</sup>/Bollgard II<sup>™</sup> cotton.

### **6. Pupae destruction**

In INGARD<sup>®</sup>/Bollgard II<sup>™</sup> cotton fields, each grower will be required to undertake *H. armigera* pupae destruction after harvest according to the following key guidelines:

- Slash or mulch crop residues and cultivate as soon as possible after harvest, but ensure all cultivation for pupae control is complete before 31 August.
- Ensure disturbance of the whole soil surface to a depth of 10 cm using implements and techniques outlined in the CRDC/Monsanto brochure, 'INGARD<sup>®</sup>/Bollgard II<sup>™</sup> Cotton Resistance Management Requirements'

In Refuge crops:

- It is recommended that conventionally managed refuge crop [i.e. Option 2 (ii)] should be similarly cultivated to manage resistance to conventional insecticides.
- Refuges which have not been treated for *H. armigera* [i.e. Options 2 (i) and 2 (iii)] should preferably be left uncultivated until the following October if possible.

**NB:** If any grower encounters problems in complying with the resistance management plan please contact your Monsanto Business Manager.

## ***CENTRAL QUEENSLAND SECTION***

Growers of INGARD® cotton are required to practice preventative resistance management. The requirements for resistance management for the 2003-2004 season in central Queensland are set out below. Adherence to the Resistance Management Plan is required under the terms of the INGARD® and Bollgard II™ Technology User Agreement and under the conditions of registration (Agricultural and Veterinary Chemicals Act 1994).

### **1. Combined area of INGARD®/Bollgard II™ cotton planted**

a) The total amount of INGARD cotton to be grown by one grower (as defined by the growers trading name) in a valley must not exceed 25% of the total cotton being grown by that grower in that valley. The total amount of combined INGARD® and Bollgard II™ cotton to be grown by one grower (as defined by the growers trading name) in a valley must not exceed 40% of the total cotton being grown by that grower in that valley. (A grower may choose to plant up to 40% Bollgard II™ and no INGARD®.)

b) Combined Farm Units. Provided they comply with 1 a), growers with more than one farm unit in a valley may be able to seek approval to combine more than one farm unit and grow a total area of INGARD® cotton in excess of 25%, and combined INGARD®/Bollgard II™ in total exceeding 40% of the total cotton area on a single farm unit. Note that as the percentage of INGARD®/Bollgard II™ cotton increases above 30% it must be accompanied by an increase in area of effective unsprayed refuge (see Box 2 under item 2. Refuges). Eligible growers who wish to choose this option may apply to a Monsanto Business Manager by submitting a plan detailing: proposed fields and areas of INGARD® cotton, Bollgard II™, conventional cotton and refuges (cotton and/or non-cotton). Only growers who receive approval of their plan by Monsanto will be eligible to proceed with this option.

c) Total cotton is defined as all cotton being grown on a farm unit and includes all INGARD®, Bollgard II™, Roundup Ready and conventional varieties including sprayed and unsprayed cotton refuges. All cotton must be managed as a viable crop and taken through to harvest. Dryland cotton is measured as green hectares.

### **2. Refuges**

Each grower will be required to grow a refuge crop capable of producing large numbers of *Helicoverpa armigera* moths which have not been exposed to selection with Bt, sufficient to dominate the mating with any survivors from INGARD® and Bollgard II™ crops and thus help to maintain Bt resistance at low levels.

The following refuge options are based on the combined area of INGARD®/Bollgard II™ cotton and different refuge options are required as the combined INGARD®/Bollgard II™ percentage increases.

**Box 1 – Irrigated INGARD®/Bollgard II™ cotton combined, not greater than 30%**  
(For greater than 30% see box 4)

For each area of irrigated INGARD®/Bollgard II™ cotton planted, a grower is required to plant a minimum of one of the following:

**(i) Irrigated cotton, which will not be treated** for any reason with products that control *H. armigera*. (If the viability of the refuge is at risk, with prior approval from the Monsanto Compliance Manager a non-Bt pesticide can be applied up to the 4<sup>th</sup> true leaf stage) The area of this cotton refuge should be at least 10% of the combined area of irrigated INGARD®/Bollgard II™ cotton planted.

**(ii) Irrigated cotton, which can be conventionally managed** for *H. armigera* and other pests, however, the use of Bt preparations throughout the season is prohibited. The area of this cotton refuge should be at least equal (100%) to the area of irrigated INGARD®/Bollgard II™ cotton planted.

**(iii) Irrigated pigeon pea which will not be treated** for any reason with products that control *H. armigera*, and managed with water and nutrients to ensure several cycles of flowering throughout the cotton season. The area of this pigeon pea refuge should be at least 5% of the area of irrigated INGARD®/Bollgard II™ cotton planted. Pigeon pea should be planted within two weeks of planting the INGARD cotton or as soon as the soil temperature reaches 17°C (requirement for germination).

**Box 2 – Irrigated Refuge Options, requiring pre -approval by Monsanto**

**iv. Irrigated sorghum or corn.**

An irrigated grower wishing to grow sorghum or corn as a refuge must submit a plan to the local Monsanto Business Manager and receive approval **before** these options can be planted. A plan for a sorghum or a corn refuge must include: a farm map showing proposed location, size, indicative planting dates and varieties for sorghum or corn refuge areas in relation to the INGARD®/Bollgard II™ planted areas. Sorghum or corn refuges must comply with the following:

- a) **Irrigated sorghum, which will not be treated** for any reason with products that control *H. armigera*, and managed to flower from at least 1 January to 14 February. The area of this sorghum refuge should be at least 15% of the combined area of irrigated INGARD®/Bollgard II™ cotton planted. See special conditions below.
- b) **Irrigated, corn which will not be treated** for any reason with products that control *H. armigera*, and managed to flower from at least 1 January to 14 February. The area of this corn refuge should be at least 20% of the combined area of irrigated INGARD®/Bollgard II™ cotton planted.

NB: Special conditions apply to growers who wish to grow options **iv (a)** sorghum or **iv (b)** corn. These conditions are:

- Either refuge will require a minimum of three planting dates. First planting date will be determined by the time to flower for the variety chosen for use in your area and the need for the refuge to be in flower by January 1. Subsequent plantings should then follow at 2 week intervals.
- A single planting of mixed maturity varieties is not acceptable.
- The minimum area of each planting should be at least one third of the total amount of this refuge type required.
- A plan indicating how either of these refuges will be managed must be submitted to and approved by the local Monsanto Business Manager before planting either of these two options.
- These refuge options will be closely monitored during the season to ensure refuge is managed appropriately to provide an effective, attractive refuge from 1 January to 14 February.

### **Box 3 – Dryland INGARD®/Bollgard II™ cotton less than 30%**

For dryland INGARD®/Bollgard II™ cotton crops, options 2 (i) unsprayed conventional cotton and 2 (ii) sprayed conventional cotton are the only refuge options available, but these can be planted to dryland cotton. All refuges in dryland situations must be planted in fields with the same cropping history as that of the INGARD®/Bollgard II™ fields and must be managed in the same way as the INGARD®/Bollgard II™ fields.

### **Box 4 – Additional Unsprayed Refuge (combined INGARD®/Bollgard II™ greater than 30%)**

It is possible for growers to grow more than 30% of combined INGARD®/Bollgard II™ (eg INGARD® at 25% and Bollgard II™ at 15%). For these situations, when the combined INGARD®/Bollgard II™ percentage increases above 30%, it must be accompanied by an increase in effective unsprayed refuge as follows:

- ❖ 30 – 50% combined INGARD®/Bollgard II™ cotton. The grower will plant an additional area of:
  - Unsprayed cotton refuge amounting to 5% of the combined total area of the INGARD®/Bollgard II™ cotton or alternatively,
  - Unsprayed pigeon pea refuge amounting to 2.5% of the combined total area of INGARD®/Bollgard II™ cotton.
- ❖ 51-100% combined INGARD®/Bollgard II™ cotton. The grower will plant an additional area of:
  - Unsprayed cotton refuge amounting to 10% of the combined total area of INGARD®/Bollgard II™ or alternatively,
  - Unsprayed pigeon pea refuge amounting to 5% of combined total area of INGARD®/Bollgard II™ cotton.

The general refuge requirements set out below also apply to additional refuge areas.

#### **General conditions for all refuges are:**

(a) Refuge crops are to be planted and managed so that the refuge is attractive to *H. armigera* during the growing period of the INGARD®/Bollgard II™ cotton varieties. All cotton refuges should be planted within 2 weeks of the INGARD®/Bollgard II™ cotton on farm.

(b) When the cultivation of a refuge is required the corresponding INGARD®/Bollgard II™ cotton crop should also be cultivated at the same time.

(c) Preparations containing *Bacillus thuringiensis* may be used on INGARD®/Bollgard II™ cotton throughout the season BUT NOT on sprayed refuge crops.

(d) All refuges are to be planted within the farm unit growing INGARD®/Bollgard II™ cotton, preferably on one side of, or adjacent to, the INGARD®/Bollgard II™ cotton fields, but with a separation of no more than 2 km from the INGARD®/Bollgard II™ crops.

(e) All refuges should be at least 2ha in size and no dimension should be less than 48 meters.

(f) All refuge crops should be left undisturbed at least until pupae busting in INGARD®/Bollgard II™ cotton crop is complete [to ensure maximum emergence and effect of late pupae from refuges]. Defoliation or destruction of aerial parts of a refuge should only be carried out after INGARD®/Bollgard II™ cotton removal.

### **3. Planting Windows**

**Emerald:** All INGARD®/Bollgard II™ crops and cotton refuges are to be planted into moisture or watered up in the period between September 15 and October 31, 2003.

**Dawson Callide Valleys:** All INGARD®/Bollgard II™ crops and cotton refuges are to be planted into moisture or watered up in the period between September 15 and October 31, 2003.

**Belyando:** All INGARD®/Bollgard II™ crops and cotton refuges are to be planted into moisture or watered up in the period between October 10 and November 25, 2003.

#### 4. Post-harvest Crop Destruction

As soon as practical after harvest, INGARD®/Bollgard II™ cotton crops must be destroyed by cultivation or herbicide so that they do not continue to host heliothis.

#### 5. Control of Volunteer and Ratoon Cotton

Volunteer and ratoon cotton, within back-to-back fields (either INGARD®/Bollgard II™ plants within conventional cotton fields or conventional plants within INGARD®/Bollgard II™ fields) may impose additional selection pressure on *H. armigera* to develop resistance to the Bt protein produced by INGARD®/Bollgard II™ cotton. Volunteer and ratoon plants must be removed as soon as possible from all fields being planted with INGARD®/Bollgard II™ cotton following conventional cotton. Volunteer and ratoon plants must also be removed from all fallowed and conventional fields following INGARD®/Bollgard II™ cotton.

#### 6. Late Summer Trap Crop.

The following table shows management recommendations for pigeon pea, when used as a trap crop for INGARD®/Bollgard II™ in CQ. (Note that refuge and late summer trap crops have different purposes and, if pigeon pea is selected for both, two separate plantings will be required. For guidelines on using pigeon pea as a refuge crop see point 2.iii. of the CQ Plan for guidelines).

Criterion	Trap Crop**
Area of pigeon pea	1% of combined INGARD®/Bollgard II™ cotton area, min. 2 ha.
Planting time	After chickpeas destroyed (but not before November 1 or after November 30)
Planting rate ***	35kg/ha
Positioning in field	Away from INGARD®/Bollgard II™ Not in INGARD®/Bollgard II™ field or adjacent
Insect control	Can be sprayed with virus after flowering; avoid pyrethroid spray/drift
Irrigation	Same as cotton + 1 irrigation after cotton is finished
Weed control	Keep free of weeds
Crop destruction	1 <sup>st</sup> to 21 <sup>st</sup> March or within 2 to 4 weeks after defoliation of INGARD®/Bollgard II™ cotton (slash and pupae bust – full disturbance to 10cm)

\*\* Pigeon Pea trap crop is to be planted such that it is attractive (flowering) to *Helicoverpa spp.* after the cotton crop has cut out and as any survivors from the INGARD®/Bollgard II™ crop emerge. Planting Pigeon Pea too early (e.g. before November) or too late (e.g. mid December) is not recommended for cotton crops planted September – October.

\*\*\* Planting rate based on minimum 85% seed germination.

**NB:** If any grower encounters problems in complying with the resistance management plan, please contact your Monsanto Business Manager.

## **LABELLING REQUIREMENTS**

The applicant has provided a label that is intended to be attached to the containers of seed that hold the genes and their controlling sequences that comprise Bollgard II. The APVMA considers the label carries all the necessary information.

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# **Bollgard II**

## **Cotton Event 15985**

**ACTIVE CONSTITUENT:**  
*Bacillus thuringiensis* var. *kurstaki* delta endotoxins  
as produced by the *CryIAc* and *Cry2Ab* genes and their controlling sequences

GROUP	<b>11C</b>	INSECTICIDE
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For in-built protection of cotton against the Cotton Bollworm and Native Budworm

APVMA Approval No. 55786/0703

**READ LABEL BEFORE OPENING THIS BAG**

Cottonseed in this bag contains the Bollgard II technology by Monsanto

## **DIRECTIONS FOR USE**

For use in New South Wales and in Queensland south of Latitude 22° South.  
Cotton containing the Bollgard II technology must be grown in accordance with these directions and the conditions set out in the current Bollgard II Technology User Agreement. Read before planting.

### **Control of Cotton Bollworm and Native Budworm (Heliothis pests)**

Cotton containing the Bollgard II technology expresses *Bacillus thuringiensis* var. *kurstaki* delta endotoxin proteins for the control of the following Lepidopteran cotton insect pests:

Cotton Bollworm *Helicoverpa armigera*

Native Budworm *Helicoverpa punctigera*

Cotton containing the Bollgard II 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 *Heliothis* 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 Bollgard II technology.

The Bollgard II 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 Bollgard II cotton into the future. Growers must practise preventative resistance management as detailed in the 'Resistance Management Plan for Bollgard II Cotton' (the Plan) **for the current year**, as recommended by the TIMS Committee and included in the Bollgard II Technology User Agreement. Note that separate Plans apply in different geographic regions. Additional copies of the current year Plan may be obtained from any Bollgard II Agent or Monsanto.

### **Crop management**

Always grow a Bollgard II cotton variety that is appropriate for the local area. Use the best agronomic and management practices 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 that minimise pest incidence.

## **IMPORTANT NOTICE**

The Bollgard II technology by Monsanto is registered by the Australian Pesticides and Veterinary Medicines Authority (APVMA) under the Agricultural and Veterinary Chemicals Code. The INGARD gene in this seed is protected under Australian Patent Number 638438. This seed may only be used by growers who have a current Bollgard II Technology User Agreement with Monsanto Australia Limited governing the use of the Bollgard II technology. Any use of the Bollgard II technology that contravenes the Agreement will be subject to claims of patent infringement and/or breach of Agreement.

## **LIMIT OF WARRANTY AND LIABILITY**

Buyers and all users are deemed to have accepted the terms set out in the Technology User Agreement upon opening this bag of cottonseed containing the Bollgard II technology.

### **Monsanto Australia Limited**

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## GLOSSARY

<b>Active constituent</b>	The substance that is primarily responsible for the effect produced by a chemical product.
<b>Acute</b>	Having rapid onset and of short duration.
<b>Efficacy</b>	Production of the desired effect.
<b>Epithelium</b>	Membranous tissue covering internal organs and other internal surfaces of the body.
<b>Enzymes</b>	Proteins produced by living organisms which initiate or increase the rate of biochemical reactions.
<b>Formulation</b>	A combination of both active and inactive constituents to form the end use product.
<b>Gliadins</b>	Proteins capable of inducing a toxic response among individuals who lack the enzyme necessary for their digestion.
<b>Insecticide Resistance</b>	The capacity to withstand the effects of an insecticide (eg by the presence of an inbuilt mechanism to negate the insecticide's particular mode of action).
<b>Refuge</b>	A separate crop able to provide a source of <i>Helicoverpa</i> individuals which have not been exposed to the endotoxins.
<b>Toxicology</b>	The study of the nature and effects of poisons.

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<sup>1</sup> Footnote:

Updated versions of these documents are available on the APVMA website <http://www.apvma.gov.au>.

## APVMA PUBLICATIONS ORDER FORM

To receive a copy of the full technical reports for the evaluation of the endotoxins in the product *Bollgard II Cotton Event 15985*, please fill in this form and send it, along with payment of \$30 to:

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Pesticides Division  
Australian Pesticides and Veterinary Medicines Authority  
PO Box E240  
Kingston ACT 2604

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