PUBLIC RELEASE SUMMARY

INGARD GENE BY MONSANTO

Introduction

The National Registration Authority for Agricultural and Veterinary Chemicals (NRA) has before it an application for registration of a new product which is a genetically modified plant pesticide product, *Bacillus thuringiensis* var. *kurstaki* delta endotoxin as produced by the *Cry1A (c)* gene and its controlling sequences, expressed in cotton (*Gossypium hirsutum* L.). The product is: INGARD GENE BY MONSANTO.

Genetically transformed cotton containing the INGARD gene has been assessed jointly by three separate agencies from different regulatory viewpoints. The Genetic Manipulation Advisory Committee (GMAC) has assessed the implications of the introduction of the product into the Australian environment, including the possibility of outcrossing leading to gene escape into native *Gossypium* species, the development of weediness in cultivated cotton and the development of insect resistance to the delta endotoxin. The National Food Authority (NFA) has carried out an assessment of the safety of the cotton seed oil from genetically modified cotton. The National Registration Authority is currently assessing the potential human health and safety, occupational health and safety, trade, efficacy and environmental effects arising from the use of the product by the Australian cotton industry.

GMAC has approved a number of planned release proposals during the development phase of INGARD cotton in Australia. These releases evaluated efficacy, yield and fibre quality, field performance, and environmental impacts including insect resistance. The releases were also necessary for variety selection and seed increase.

Monsanto submitted a proposal to GMAC in 1995 seeking approval for a general release of INGARD cotton anywhere in Australia, at any time. GMAC has finalised its advice to Monsanto and has recommended a restricted general release with a further recommendation that unrestricted general release should not proceed until further data are available to:

- fully assess the consequences of transfer of the INGARD gene to native Australian *Gossypium* (cotton) species; and

- determine the suitability of current resistance management strategies.

The NFA has determined that the data provided by Monsanto indicate that there are no observable differences between the oil from cotton containing the INGARD gene and the oil from traditional cotton.

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Particulars of Registration Application

Proposed product name:	INGARD GENE BY MONSANTO
Applicant company:	Monsanto Australia Limited 12th Floor, 600 St Killed Road MELBOURNE VIC 3004
Host Organism:	Cultivated cotton (*Gossypium hirsutum)* modified by genetic manipulation to incorporate a gene derived from the soil microbe *Bacillus thuringiensis* variety *kurstaki.*
Name of active constituent:	*Bacillus thuringiensis* variety *kurstaki* delta endotoxin as produced by the *Cry1A(c)* gene and its controlling sequences.
Statement of claims:	It is claimed that the *Bacillus thuringiensis* var. *kurstaki* delta endotoxin as produced by the INGARD gene *(Cry 1A(c)* gene and its controlling sequences*)* in cotton will provide control of Cotton bollworm (*Helicoverpa armigera*) and Native budworm (*Helicoverpa punctigera*).

Background

Australian cotton production has a gross annual value of $750 million and cotton is a major contributor to Australia's export income from primary production. Cotton production in Australia takes place in Queensland and New South Wales, with approximately 250,000 hectares being grown currently.

The cotton industry is a major user of agricultural chemicals, particularly insecticides. The lepidopteran caterpillar pests *Helicoverpa armigera (*cotton bollworm) and *Helicoverpa punctigera* (native budworm) infest up to 100% of the crop and up to $100 million is spent annually for chemical control. Irrigated cotton is currently sprayed with insecticides 10-12 times per season, about 80% of these applications being for control of *Helicoverpa* spp.

Heavy chemical use in the cotton industry is leading to increasing environmental concerns.

Helicoverpa armigera populations have developed resistance to many conventional insecticides, some of which have become relatively ineffective against this pest. Resistance management strategies have been put in place by the cotton industry in order to delay the development of resistance, but there is increasing concern that the industry is running out of options for control of *Helicoverpa*.

The transgenic plants containing the INGARD gene have been modified to express a gene, derived from the soil bacterium *Bacillus thuringiensis,* that produces a highly specific insecticidal protein (Bt delta endotoxin or Bt protein) that is toxic to the major caterpillar pests of cotton. The Bt protein is nontoxic to humans, other animals and most other insects.

The cotton industry sees the introduction of Bt cotton as having the potential to provide a significant reduction in pesticide useage while further increasing the opportunity for integrated pest management approaches in cotton crops.

Products containing *Bacillus thuringiensis* variety *kurstaki* are currently registered in Australia for control of cotton bollworm *(Helicoverpa armigera)* and native budworm *(Helicoverpa punctigera)* in cotton when applied as a ground or aerial spray. Whilst this is not a new active constituent for use on cotton, the proposed novel means of delivering the pesticidal protein warrants inviting comment on this product.

The INGARD gene, known in the US as the BOLLGARD^{TM} gene, has been given regulatory approval by the US Environment Protection Agency in consultation with other US agencies for an initial period of 5 years.

Summary of nature and effect of the genetic modification

The parent organism is cultivated cotton, *Gossypium hirsutum.* Cotton, which is exotic to Australia, is grown in New South Wales and Queensland as a major agricultural crop. The transgenic plants have been modified to express a gene, derived from the bacterium *Bacillus thuringiensis,* that produces a highly specific insecticidal protein (Bt protein) that is toxic to the major caterpillar pests of cotton. The Bt protein is nontoxic to humans, other animals and most other insects. Insecticidal activity is expressed throughout the plant for the entire season.

A delta-endotoxin gene has been inserted into the cotton plant to produce the CryIA(c) insecticidal protein (also known as *B.t.k.* HD-73 protein). Transgenic plants express this gene in most plant parts, particularly in young leaves and flower buds. When the plants are attacked by insect pests which are susceptible to the toxins, the toxins initially inhibit insect feeding and subsequently result in the death of the insect pests.

In addition to the Bt protein genes, the transgenic cotton plants contain a selectable 'marker' gene, neomycin phosphotransferase *(nptII gene*), that confers resistance to the antibiotics kanamycin and neomycin. This marker gene was inserted to allow selection of the transgenic plants from nonmodified plants during regeneration of the plants in tissue culture. The protein produced by this gene is known as NPTII.

The inserted DNA also contains a bacterial gene, encoding resistance to spectinomycin and streptomycin *(aad gene),* which is not expressed in the transgenic plants. This gene was used as a selectable marker for the genetic manipulations in the bacterial hosts, before the transfer of the gene to the cotton plants.

AGRICULTURAL ASSESSMENT

Effectiveness of Insect Control by INGARD Cotton

Efficacy data in support of the registration of INGARD cotton was reviewed by scientists in NSW Agriculture and the Queensland Department of Primary Industry. Trial data was provided from a number of US and Australian studies.

Reviewers considered that transgenic cotton resistant to *Helicoverpa* species offers the potential for more efficacious deployment of the Bt insecticidal proteins when compared with conventional sprays. The use of INGARD cotton could eliminate, or at least significantly reduce, the number of conventional sprays to control *H. armigera,* with concomitant environmental and other benefits.

INGARD cotton is regarded as compatible with existing integrated pest management systems (IPM).

The use of INGARD cotton is expected to alter the populations of insects present in cotton crops. Beneficial insects are unaffected by the Bt delta endotoxin and may increase in INGARD cotton crops, providing predatory control of other arthropod pests which will not be controlled by the Bt protein, and which would previously have been controlled by broad spectrum insecticides.

During the growing season other pest insects (including mites, thrips, mirids and aphids) are also present. It is not known to what extent members of these other groups of pests may prove problematic in INGARD cotton crops. Monitoring of insect populations (both beneficial and pest species) in both INGARD cotton and conventional cotton crops is currently underway.

Conclusion

The NRA is satisfied that the data from trials supporting the efficacy of INGARD gene by Monsanto demonstrate the efficacy of the product. It is intended that a condition of the proposed registration be that additional efficacy data generated under local Australian conditions in the 1996/7 season is provided to the NRA within 12 months.

The NRA will require Monsanto to keep records of the area planted to INGARD cotton and make this information available to the NRA as required. The applicant will also be required to monitor insect populations in INGARD cotton crops and provide a report to the NRA in the first two years of registration.

The NRA proposes to restrict planting of INGARD cotton to 30,000 hectares in New South Wales and southern Queensland and to review conditions of registration annually for the first two years.

Resistance Management

A major concern identified in the use of the INGARD gene in cotton is the potential for development of resistance in *Helicoverpa armigera* populations to the Bt delta endotoxin. Resistance development could negate the benefits of the new technology, possibly within a short period of time.

In order to minimise the possibility of resistance development in pest populations, a transgenic cotton pest management strategy has been developed by the cotton industry and will be put in place to manage INGARD cotton. Elements of the resistance management plan include:

- Refugia. Each grower will be required to grow a refuge crop to produce sufficient *Bacillus thuringiensis* susceptible *Helicoverpa* moths to dominate the mating with any survivors from INGARD crops and thus maintain resistance at low levels. Refuges could be cotton crops or other *Helicoverpa* host crops, appropriately managed to produce moths.
- Early planting of crops to limit period of selection for resistant insects.
- Cultivation for destruction of pupae in soil after harvest.
- Late season *Helicoverpa* control when larval numbers exceed a specified threshold as indicated by careful monitoring.
- Adherence to integrated pest management strategies.

Conclusion

The NRA will require adherence to the resistance management strategy endorsed by the Transgenic and Insect Management Strategy (TIMS) committee, as part of the approved use-pattern for the INGARD product. Conditions placed on the registration will be reviewed annually for the first two years, at least.

The NRA will also require annual reports on the effectiveness and compliance with resistance management strategies and monitoring of pest and other insect populations, including susceptibility levels in *Helicoverpa* populations.

ENVIRONMENTAL ASSESSMENT

An environmental assessment of the use of INGARD gene is currently being undertaken by the Environment Protection Agency (EPA). The environmental assessment has not been completed but the following discussion highlights the issues under consideration by the EPA.

Environmental Fate of the Bt Toxin

Expressed protein entering the environment

The maximum amount of expressed protein entering the environment is estimated to be about 335 g endotoxin per hectare in a growing season. This is similar to the rate of application used with conventional Bt products.

During the life of the plant the endotoxin in INGARD cotton is enclosed within plant cells. Bt delta endotoxin will enter the soil environment as cotton plant roots and above-ground plant material after ploughing in. If above ground stubble is burned after harvest, the endotoxin would be combusted, posing no potential hazard *per se,* and reducing the amount of Bt toxin entering the soil.

Degradation of endotoxin in the soil

Bt endotoxin in INGARD cotton is enclosed within plant cells. The main route of degradation is microbial degradation by fungi, bacteria and non-lepidopteran detritus feeders in the soil or in soil surface mulch. Degradation of above-ground biomass by photolysis, as is the case for conventional Bt products, is possible but not likely.

Bt protein will degrade readily when added to soil as a component of post-harvest INGARD cotton plants, and at rates comparable to that reported for microbial formulations where the Bt protein is also intracellular.

Available data suggest that it is likely that biodegradation rates of transgenic plant material will be high enough not to cause a persistence or accumulation problem in soils.

Toxic Effects On Non-Target Organisms

As Bt delta endotoxin in INGARD cotton is enclosed within the plant, exposure of nontarget organisms is restricted to those animals that eat the plant or those that live in the soil (from the roots, and above ground plant parts after ploughing in). Exposure of non-plant-feeding organisms is therefore less acute, and more indirect than that from currently registered insecticides based on the Bt delta endotoxin.

Consumption by birds is expected to be negligible. The presence of gossypol makes cotton seed unpalatable to birds; further to this, cotton seeds are inaccessible to birds due to the cotton lint. After harvest, the number of cotton bolls remaining in the field

is very low, and although there is potential for birds to take seed from the ground, this has not been documented.

Key features of Bt delta endotoxin of relevance to effects on non-target organisms are:

- the Bt protein has to be activated by proteolysis in the digestive system of insects before it is toxic, ie it has to be ingested first;
- activated toxin has then to bind to specific receptors on gut epithelium to have an effect; different Bt toxins require different receptors, conferring specificities of action;
- The CryIA(c) insecticidal protein expressed by the INGARD gene is specific to lepidopteran insects.

Bt endotoxins are well known for their safety to non-target animals, including humans, other mammals, birds, reptiles and fish. The Bt delta endotoxin is not toxic to non-lepidopteran insects.

Testing by the proponent demonstrates that toxicity effects on nontarget species of the Bt protein produced by transgenic plants are no different from those produced by 'natural' forms of Bt protein. Submitted data for INGARD protein fed to honey bees, parasitic wasps, lady birds and lacewing larvae indicate low toxicity to insects from Orders other than Lepidoptera.

In other insect studies, only Lepidoptera were killed by the toxin, and members of Orthoptera, Homoptera, Diptera and Coleoptera were unaffected. For quails (Northern Bobwhite) and mice, oral toxicity was very low.

The only non-target organisms likely to experience the toxic effects of Bt cotton are non-target lepidopterans. In current pest management regimes, with heavy insecticide use, these lepidopterans are already killed or otherwise affected. In addition, many beneficial and other non-target non-lepidopteran insects are much more numerous in transgenic cotton crops due to the reduced number of applications of *Helicoverpa* control sprays. Net adverse effects of Bt cotton on non-target organisms are minimal, and much less than for conventional cotton crops.

Prediction Of Environmental Hazard

From the foregoing considerations, the EPA predicts that there will be no significant direct hazards as consequences of the environmental fate of Bt delta endotoxin and toxicity to non-target organisms.

The potential environmental hazard of the transgenic plant itself must also be considered. Potential hazards include the possibility of gene escape into native cotton species or other plants, the possibility of cultivated transgenic cotton becoming an environmental weed and the possibility of insect populations developing resistance to the Bt delta endotoxin.

Unintended INGARD gene transfer (or escape) to native cotton and other species

The potential for unintended gene transfer to native *Gossypium* and other species has been assessed by GMAC. The EPA has provided input to this Committee in its assessment of this issue, and supports its recommendations.

Barriers to such gene transfer in a general release will comprise only natural reproductive barriers, including geographic separation (disjunction). The only native *Gossypium* species known to occur in the current major cotton-growing areas of New South Wales and southern Queensland is *G. sturtianum*, and this species is uncommon in the areas where cotton is grown. Most Australian native *Gossypium* species are endemic in north-western WA, with other species occurring in the Northern Territory and western and northern Queensland.

Other factors, apart from geographic separation, constitute strong reproductive barriers to gene transfer to native species. The assessment concludes, therefore, that the risk of gene transfer to native cotton and other species is low. Nevertheless, the EPA considers that further information should be presented and gathered by the proponent on the sterility and viabililty of hybrids between native diploid cottons and transgenic cotton (and of artificially doubled triploid hybrids), the potential for introgression of the Bt gene into native cottons, and the likelihood of emergence of fertile hybrids, before general commercial release in north-western Australia, the Northern Territory and central and northern Queensland can be recommended.

The consequences of Bt gene transfer to a native species, should it ever occur, are unknown. Fitness of native species could be increased through increased fecundity and survival due to less herbivory by insects. Gene transfer involving transgenes that enjoy a strong selective advantage, such as that from a synthetic insect-resistance gene of bacterial origin, is likely to be irreversible. Such a gene transfer would constitute an unprecedented jump across species barriers.

Potential adverse impacts could therefore be: (i) ecosystem imbalance; (ii) 'pollution' of natural gene pools; with no control of the evolutionary fate of the transgene; and (iii) the possibility that any native lepidopteran species, especially any specific to the native *Gossypium* species, may be reduced in abundance.

Gene transfer (escape) to cultivated cotton and likelihood of INGARD cotton becoming a weed

Transfer of the INGARD gene to non-transgenic cultivated cotton or to naturalised cotton subsequent to commercialisation seems likely and is generally regarded as inevitable. Cotton is not known to be a weed in any part of the world. In the major cotton growing regions of Australia, New South Wales, and southern and central Queensland, cotton does not persist naturally because the climate and habitat are unsuitable. Therefore it seems very unlikely that transgenic cotton will become a weed problem in these areas.

In northern Australia, where the climate and habitat are more appropriate for cotton to become naturalised, and where insect resistance may confer some advantage, more data should be collected and presented to demonstrate lack of survival of cotton in natural habitats, before general release in north-western Australia, the Northern Territory and northern Queensland can be recommended.

Insect Resistance Management Strategy

Emergence of insect resistance in target species *(Helicoverpa armigera* and *H. punctigera) is* a possibility well recognised by industry and the proponent. EPA considers that the adverse impacts of the emergence of insect resistance primarily impact on the cotton industry itself. Adverse effects for the environment are the foregone benefits of Bt cotton and other Bt products, should insect resistance emerge; the benefits to the environment of reduced pesticide usage will be attenuated if insect resistance emerges prematurely.

The main concern from an environmental perspective is, therefore, that transgenic cotton will be released with only one Bt gene, when it has been modelled that pyramiding two genes in the same plant, in conjunction with an appropriate refuge, is predicted to delay resistance many fold.

Conclusion

It is expected that the use of INGARD should significantly reduce the number of conventional chemical insecticide sprays per season to control *Helicoverpa* spp, with significant benefits to the environment.

The Bt delta endotoxin protein is not expected to accumulate in soil and there are likely to be no toxic effects, or no unacceptable toxic effects, on non-target organisms.

As far as available data demonstrate, the EPA is satisfied that the risk of gene escape into native *Gossypium* species appears to be minimal. The concerns raised are not sufficient to delay large scale seed increases or commercial release in the current major cotton growing areas.

The EPA recommends that the two-gene Bt cotton needs to be developed as soon as possible OR the staging of the increase in planting area be suitably protracted, to minimise emergence of insect resistance.

The NRA intends to limit the use of INGARD cotton to 30,000 hectares in the current cotton growing areas of NSW and southern Queensland pending further environmental data.

PUBLIC HEALTH AND SAFETY ASSESSMENT

Toxicology

The Environmental Health and Safety Unit of the Department of Health and Family Services (EHSU) has assessed toxicology data provided in support of the registration of the INGARD gene.

The EHSU has previously assessed *Bacillus thuringiensis* var. *kurstaki* in regard to its use as a traditional insecticidal spray for the control of *Helicoverpa armigera* and *Helicoverpa punctigera* in cotton. The assessment in regard to INGARD has focused on toxicological implications arising from its incorporation into cotton by gene technology.

Studies with this protein have revealed very low acute toxicity when administered to rodents via the oral, dermal, and inhalation routes. No skin sensitisation or irritation have been reported in guinea pigs and rabbits, whilst slight eye irritation in rabbits has been noted. As this protein was found not to be glycosylated it is thought to be non-allergenic.

The Bt delta endotoxin protein is highly selective for lepidopteran caterpillars. When insects ingest this protein it binds to specific receptors in the mid-gut, gets incorporated into the membrane and forms ion-specific pores. These events disrupt digestive processes and lead to death. Birds, fish and mammals lack the receptors on the surface of the gastro-intestinal tissues necessary for binding of the protein toxin to the cell surface. Therefore the Bt delta endotoxin is not active in the gut of birds, fish or mammals. Any Bt delta endotoxin protein that enters the mammalian gut is rapidly denatured by gastric juices.

The inserted gene sequence was found to be stable over a number of generations. Studies on the levels of expression of the transferred genes have revealed that the product of the *aad* gene (AAD protein) is not expressed in cotton plants. The levels of CryIA(c) and NPTII proteins expressed in cotton tissues were very low and relatively consistent. NPTII is ubiquitous in the environment and found in our food and bodies.

Conclusion

In view of the low toxicological hazard posed by *Bacillus thuringiensis* var. *kurstaki* delta endotoxin, poisons scheduling is not considered necessary, and as the end-use cotton seed product is marketed and used in a manner which precludes exposure, safety directions and first aid instructions are also not considered necessary.

Residues In Food Commodities

The use of INGARD gene was evaluated by the Chemical Residues Section of the NRA.

a) Residues in cotton seed oil

Cotton seed oil is the only cotton commodity that is used directly for human consumption. The proteins are not expected to readily dissolve in oil. Data supplied by the applicant indicated that the Bt endotoxin and NPTII proteins, expressed by the Bt gene and the neomycin phosphotransferase marker gene respectively, were not found in cotton seed oil produced from INGARD cotton.

b) Residues in livestock

Data supplied indicated that the maximum level of CryIA(c) protein seen in plant tissue was in young leaves and ranged from 1.5 to 62 ppm. Mature cotton plants contained up to 2 ppm CryIA(c) proteins. The maximum level of NPTII was 20 ppm in leaves. Data supplied on processed cotton seed meal indicated that no CryIA(c) protein was present after processing and a maximum of 1 ppm of NPTII was present. Thus at the time of harvesting and after cotton processing, commodities that could be fed to livestock are not expected to contain large amounts of Bt or marker gene proteins. Both the proteins are very labile to digestion by proteases in livestock digestive tracts, thereby minimising the potential for absorption if consumed.

Therefore, due to the small amount of proteins expected to be present in livestock feed, and the rapid degradation by the livestock digestive system, the CryIA(c) and NPTII proteins are not expected to appear in animal commodities derived from animals fed cotton fodder, seed, forage, trash or meal from INGARD cotton.

c) *Recommendations*

The following consequential additions have been recommended for the MRL Standard:

TABLE 5 (Uses of substances where maximum residue limits are not necessary)

Compound	**Use**
Bacillus thuringiensis kurstaki Delta endotoxin protein	**Insecticide expressed in recombinant cotton**

Conclusion

The NRA is satisfied that the incorporation of the INGARD gene into cotton plants is not likely to be harmful to human beings as a result of either consuming cotton oil or consuming animal commodities derived from animals which would have fed on recombinant cotton containing the INGARD gene.

TRADE ASSESSMENT

The applicant was asked to address possible implications of the use of Bt cotton on Australia's trade with other countries. Commodities which are exported include cotton lint and mote and cotton seed for stock feed or oil extraction. There are no detectable residues in lint or mote, but residues could be detected in cotton seed.

The main export markets for Australian cottonseed are Japan (96%), Korea (3.4%) and Taiwan (0.16%). No maximum residue limits have been set for Delta endotoxin of *Bacillus thuringiensis* in countries to which export of cotton oil or animal feed commodities currently occurs.

Some countries have in place or are establishing guidelines relating to transgenic technology. Although no regulations apply, guidelines for transgenic organisms and commodities are currently being put in place in Japan. No regulation or guidelines apply, presently, in Korea or Taiwan. Monsanto states that they are taking steps to gain regulatory approval for export of Bt cotton seed for food and feed use to Japan. Approvals are expected to be in place before the first commercial crop is harvested in Australia in April 1997.

Cotton by-products such as gin trash, cotton stubble, hulls and meal could potentially be used for stock feed. Delta endotoxin residues in animal feed substances are digested by the animal and no residues are detectable in meat or milk from animals fed on cotton by-products. Therefore Australia's meat exports are not expected to be affected by the use of Bt cotton.

The INGARD product, known in the US as BOLLGARD™, has been given regulatory approval there for an initial period of 5 years.

Conclusion

The NRA is satisfied that the proposed use of INGARD Gene by Monsanto would not adversely affect trade between Australia and places outside Australia.

OCCUPATIONAL HEALTH AND SAFETY ASSESSMENT

Occupational health and safety information provided by the applicant was reviewed by Worksafe Australia.

Occupational exposure to Bt cotton plants for workers will occur during cotton cropping and cotton processing. The proteins B.t.k.HD-73 and NPTII are expressed at very low levels in the cotton plant tissues. They are present as intracellular components and exposure will be negligible or non-existent.

Humans working with the plants or seed are not expected to be exposed to the protein under normal circumstances if the plant or seed remain in a whole condition. The Cry1A(c) protein is produced within the double walled cell of the cotton plant and is not expected to be found outside the cell. Human exposure would only be possible if the seeds or tissue rupture. Even if the plant cells rupture, the amount of protein found in any one cell is extremely small, as the protein is expressed at maximum level of approximately 17 ten thousandths of a per cent of the fresh tissue of the plant and 13 thousandths of a per cent of the cotton seed tissue. Therefore for workers handling seeds or tissue in which the cells have ruptured, exposure would be extremely low and immeasurable.

Data presented in the submission indicate that the delta endotoxin protein is nearly identical (>99.4% amino acid sequence homology) to that found in nature and in commercial formulations containing Bt endotoxins. *Bacillus thuringiensis* formulations have been registered for many years and information submitted earlier for registration of insecticidal sprays indicated that Bt endotoxin can be used safely by workers.

Conclusion

The NRA is satisfied that incorporation of the INGARD gene into cotton would not be an undue hazard to the safety of people exposed to it during its handling and use.

GMAC ASSESSMENT

The Genetic Manipulation Advisory Committee (GMAC) assessed the implications of the release and commercialisation of insect-resistant cotton.

GMAC considered the possibility that the transgenic cotton could out-cross with native cotton related *(Gossypium)* species found in Australia, resulting in transfer of the Bt gene to these species. Expert advice provided to the Committee suggested that this was unlikely to occur and GMAC considered that the risk of transfer of the introduced genes to other species related to cotton is low. Cotton is largely self-pollinated and cross-pollination is rare. Gene transfer in the wild is unlikely due to genome incompatibility, the relatively isolated distribution of Australian native *Gossypium* species and different breeding systems. Hybrids resulting from artificial crosses between cotton and wild Australian species are generally sterile, unstable and of poor fitness, and are difficult to maintain, even under glasshouse conditions. Vegetative propagation of cotton does not occur in the field. Nevertheless, the Committee's view was that the possibility of gene transfer to native *Gossypium* species could not be eliminated if Bt cotton were to be planted on a large commercial scale.

Use of Bt cotton on a large scale has the potential to lead to development of resistance to the Bt toxin in insect pests. GMAC considered that resistance management strategies, including planting of non-transgenic cotton as a "refuge" for susceptible pests, would be required if Bt cotton were to be planted on a commercial scale. Studies on resistance management strategies are continuing under planned release proposals previously assessed by GMAC.

GMAC considered that unrestricted commercial release of the Bt cotton should not proceed until further data are available to I) fully assess the consequences of transfer of the *CryIA(c)* gene to native Australian *Gossypium* species, and 2) determine the suitability of current resistance management strategies. However, GMAC recognised that large scale planting of the Bt cotton may provide data that would help in making a decision on whether full commercial release should proceed. Specific recommendations for the conduct of the release included the following:

• The release was confined to the cotton-growing areas of southern Queensland and New South Wales because there are relatively few native *Gossypium* species in these areas.

• The release was limited to 5 years, with further review after this period. During this time, GMAC will require annual reports on further work conducted to provide data on: efficacy of insect control; evidence for emergence of resistance in insect pests; and management strategies to delay emergence of insect resistance.

The scale of the release should be staged, with possible increases in the area sown to Bt cotton each season. The appropriate scale at each stage, and the appropriate resistance management strategies, will depend on the data accumulated from further research as the release progresses.

GMAC considered that a limited commercial release of the Bt cotton, under the conditions recommended above, would not pose a significant biosafety risk. Monsanto was advised to seek the advice of the National Registration Authority for Agricultural and Veterinary Chemicals as to the details of the area to be planted at each stage of the release and resistance management strategies to be used. During the 5 years for which this advice remains current, it was recommended that further research be conducted into resistance management strategies and the possible ecological consequences of gene transfer to wild *Gossypium* species.

The NRA will apply appropriate conditions to the registration of the INGARD gene in line with GMAC's recommendations.

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Glossary of terms:

***aad* gene**	The bacterial gene which codes for the proteins which confer resistance to the antibiotics spectinomycin and streptomycin.
Active constituent	The component of a treatment which is responsible for its biological effect.
Acute toxicity -	Immediately measurable effects of a toxin on an organism.
Bt	*Bacillus thuringiensis* var. *kurstaki,* the organism from which the *Cry1A*(c) gene was taken.
Bt cotton	refers to the recombinant cotton containing the INGARD gene (also called INGARD cotton).
Bt delta endotoxin	The protein which is produced by the Cry 1 A (c) gene and its controlling sequences. This is one of the proteins which confer activity against caterpillars. "Bt protein", "Bt delta endotoxin" and "CryIA(c)" and "*B.t.k.* HD-73 protein" all refer to the insecticidal protein produced by *Bacillus thuringiensis* var. *kurstaki* and as expressed in cotton by the INGARD gene. This protein confers insecticidal properties to INGARD cotton against the feeding of immature Lepidoptera.
Bacillus thuringiensis* var. *kurstaki	The micro organism which produces the delta endotoxins which kill the larval stages of lepidoptera (moths and butterflies).
Cotton	*Gossypium hirsutum* L.
Cotton bollworm	*Helicoverpa armigera* Hubner (Noctuidae)
***Cry I A* (c)**	A gene in *Bacillus thuringiensis* var. *kurstaki* which produces one of the toxic proteins which kill the larvae of Lepidoptera.
Cry I A (c) protein	The protein formed by the actions of *Cry I A* (c) gene and its controlling sequences. This protein confers insecticidal properties to INGARD cotton against the feeding of immature Lepidoptera.
Cry II A	A gene in *Bacillus thuringiensis* var. *kurstaki* which produces one of the toxic proteins which kill the larvae of lepidoptera.
Delta-endotoxin(s)	The endotoxin(s) of *Bacillus thuringiensis* var. *kurstaki* which have insecticidal properties against larvae of moths and butterflies.

Denatured	Broken down
Detritus	rotting vegetable material
Diploid	Having two sets of chromosomes
DNA	deoxyribonucleic acid the generic component of the chromosomes which support the gene sequences.
Endotoxin	A toxin produced within the protoplasm (inside) of a bacterium.
Exotoxin	A soluble toxin produced by bacteria
Expressed protein	The protein which is the ultimate agent of the effects of a gene. This may involve some modifications to the peptide chain which was originally coded for by the gene.
Fecundity	The power of a species to rapidly reproduce.
GMAC	Genetic Manipulation Advisory Committee
Gene	A length of the DNA which holds the base sequences which code for the formation of a polypeptide chain (protein).
Genetic transformation	Modification to the total DNA complement of an organism to cause the organism to express new characteristics.
Gossypium hirsutum	Cotton plant
Gossypium sturtianum	A species of native cotton plant found in the cotton growing regions of New South Wales and Queensland
Helicoverpa armigera	A primary lepidopteran pests of cotton and target pest of INGARD cotton. Also known as cotton bollworm, corn earworm, tomato grub, tobacco budworm. Previously known as *Heliothis armigera.*
Helicoverpa punctigera	A primary lepidopteran pest of cotton and target pest of INGARD cotton. Also known as native budworm, previously known as *Heliothis punctigera.*

IPM	Integrated Pest Management. The combination of chemical and biological aspects of pest control to achieve pest management.
INGARD gene-	The *Cry I A* (c) gene and its controlling sequences which has been implanted in cotton (*Gossypium hirsutum*).
Insecticide resistance	The development of a gene, or set of genes, in an insect population which confer immunity to the effects of insecticidal treatments.
Lepidoptera	The order which is entirely composed of the moths and butterflies.
Marker gene	An easily identifiable gene which can be linked to a second gene which is the primary subject of a study. The marker gene can then be used to identify the presence of the study gene.
Monsanto	Monsanto Australia Limited, 12th Floor, 600 St Kilda Road, Melbourne Victoria, 3004, who have applied for the registration of INGARD gene in cotton.
Neomycin phosphotransferase	The protein which detoxifies the effects of the antibiotics kanomycin and neomycin in bacteria.
Neomycin phosphotransferase gene	The gene which codes for the protein neomycin phosphotransferase which detoxifies the effects of the antibiotics kanomycin and neomycin in bacteria.
NPTII	The protein neomycin phosphotransferase.
***nptII* gene**	see Neomycin phosphotransferase gene
ppm	Parts per million
Protease	Enzymes which break down proteins.
Proteolysis	The process in which proteins chains are lysed (cut) as part of their digestion.
Refugia -	Areas, spaces or locations where an organism can avoid the detrimental effects of disease, predation etc. Such areas are thought to reduce the selective pressure for insect populations to develop resistance.

Resistance	-	see Insecticide resistance
Resistance management strategy		A strategy or series of strategies based on the manner in which insecticidal treatments are applied to reduce the likelihood of insect resistance developing. Usually integrate physical, environmental, and ecological aspects of pest control.
Schedule		The category into which a chemical is placed according to its human toxicity.
Transgene		A gene which is moved from one species to another species
Transgenic		Composed of DNA or RNA of more than one species.
Triploid		Having three times the haploid number of chromosomes, 3n.
TIMS committee		Transgenic and Insect Management Strategy committee.

Further Reading

Cotton Pesticides Guide 1995-6. AJ Shaw, NSW Agriculture, Agdex 151/680.

Insect Management Plan for INGARD Cotton - 1996-7 Season. TIMS Committee, Australian Cotton Growers Research Association. April 1996.

Interim Requirements for the Registration of Agricultural and Veterinary Chemical Products. National Registration Authority, February 1995 (available from the NRA).

MRL Standard - Maximum residue limits in food and animal feedstuffs. Commonwealth Department of Human Services and Health. AGPS, Canberra.

The Australian Cotton Industry: *An Economic Assessment.* Cotton Research and Development Corporation. Prepared by: Centre for International Economics Canberra, Sydney. Cameron Agriculture Pty Ltd, Sydney, December 1995.

Transgenic Cottons in Australia GP Fitt *et al. Biocontrol Science and Technology* 4:535-548 1994.

Invitation for submissions

In accordance with sections 12 and 13 of the Agvet Chemicals Code, the NRA invites any person to submit a relevant written submission as to whether the application for registration of INGARD GENE BY MONSANTO should be granted. Such submissions should state the grounds on which the submission is based. Such grounds should relate only to matters that the NRA is required to take into account in deciding whether to grant registration.

Written submissions should be provided by 14 June 1996 and be addressed to:

[redacted]

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