FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

6 August 2008
[13-08]

DRAFT ASSESSMENT REPORT

APPLICATION A614

FOOD DERIVED FROM GLYPHOSATE-TOLERANT COTTON LINE GHB614

DEADLINE FOR PUBLIC SUBMISSIONS: 6pm (Canberra time) 17 September 2008
SUBMISSIONS RECEIVED AFTER THIS DEADLINE WILL NOT BE CONSIDERED
(See 'Invitation for Public Submissions' for details)

For information on matters relating to this Assessment Report or the assessment process generally, please refer to http://www.foodstandards.gov.au/standardsdevelopment/

Executive Summary

On 27 September 2007, Food Standards Australia New Zealand (FSANZ) received a paid Application from Bayer CropScience Pty Ltd (the Applicant) seeking approval for food derived from genetically modified (GM) cotton, line GHB614 under Standard 1.5.2 – Food produced using Gene Technology in the *Australia New Zealand Food Standards Code* (the Code). Standard 1.5.2 prohibits a food produced using gene technology from being sold or used as an ingredient or component of any food unless it is listed in the Table to clause 2 of that Standard. To be approved under Standard 1.5.2, FSANZ conducts a pre-market safety assessment on all GM foods before they may be sold in Australia and New Zealand.

The genetic modification in cotton line GHB614 consists of a single herbicide tolerance trait introduced by the transfer of a gene encoding a modified form of the enzyme 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS). This enzyme catalyses a key step in the shikimate pathway for biosynthesis of aromatic amino acids in plants, and is normally inhibited by glyphosate which ultimately leads to the death of the plant. Two simple mutations were introduced into the wild type e*psps* gene derived from corn, using site-directed mutagenesis. The mutations introduced into the 2mEPSPS enzyme significantly reduce its sensitivity to glyphosate, allowing continued function in the presence of the herbicide. Plants expressing 2mEPSPS are therefore able to tolerate treatment with glyphosate-containing herbicides.

Cotton line GHB614 has been developed for cultivation in major cotton producing countries worldwide, including eventually in Australia. Cotton derivatives, such as cottonseed oil and linters, are used in many food products and may enter the Australian and New Zealand food supply via locally produced and imported processed products. Currently, there is no approval to grow cotton line GHB614 in Australia or New Zealand.

Safety Assessment

FSANZ has completed a comprehensive safety assessment of food derived from glyphosate-tolerant cotton line GHB614, which included consideration of (i) the genetic modification introduced into the plant; (ii) the potential toxicity and allergenicity of the novel protein; and (iii) the composition of GHB614 cottonseed, compared with that from conventional cotton varieties.

No public health and safety concerns were identified in the safety assessment. On the basis of the available evidence, including detailed studies provided by the Applicant, food derived from glyphosate-tolerant cotton line GHB614 is considered as safe and wholesome as food derived from other commercial cotton varieties.

Labelling

If approved, food derived from glyphosate-tolerant cotton line GHB614 will be required to be labelled as genetically modified if novel DNA and/or novel protein is present in the final food. Studies undertaken by the Applicant indicate detectable levels of the novel protein, 2mEPSPS, in linters and cottonseed meal, but not in refined cottonseed oil.

Labelling addresses the requirement of section 18(1)(b) of the Act; provision of adequate information relating to food to enable consumers to make informed choices.

Impact of regulatory options

Two regulatory options were considered in the assessment: (1) no approval; or (2) approval of food derived from glyphosate-tolerant cotton line GHB614, based on the conclusions of the safety assessment. Following analysis of the potential costs and benefits of each option on affected parties (consumers, the food industry and government), approval of this Application is the preferred option as the potential benefits to all sectors outweigh the costs associated with the approval.

Purpose

The Applicant seeks approval for glyphosate-tolerant cotton line GHB614 in the Table to clause 2 of Standard 1.5.2 – Food produced using Gene Technology.

Preferred Approach

Amend Standard 1.5.2 – Food produced using Gene Technology, to include food derived from glyphosate-tolerant cotton line GHB614 in the Table to clause 2.

Reasons for Preferred Approach

An amendment to the Code approving food derived from glyphosate-tolerant cotton line GHB614 in Australia and New Zealand is proposed on the basis of the available scientific evidence, for the following reasons:

- the safety assessment did not identify any public health and safety concerns associated with the genetic modification used to produce glyphosate-tolerant cotton line GHB614;
- food derived from glyphosate-tolerant cotton line GHB614 is equivalent to food from the conventional counterpart and other commercially available cotton varieties in terms of its safety for human consumption and nutritional adequacy;
- labelling of certain food commodities derived from glyphosate-tolerant cotton line GHB614 will be required if novel DNA and/or protein is present in the final food; and
- a regulation impact assessment process has been undertaken that also fulfils the requirement in New Zealand for an assessment of compliance costs. The assessment concluded that the preferred option is an amendment to the Code.

Consultation

The Initial Assessment was advertised for public comment between 12 December 2007 and 6 February 2008. Thirteen submissions were received during this period and a summary of these is attached to this report. FSANZ has taken the submitters' comments into account in preparing the Draft Assessment of this application. Specific issues relating to glyphosate-tolerant cotton line GHB614 have been addressed in this report.

Public submissions will be invited on this Draft Assessment Report.

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INVITATION FOR PUBLIC SUBMISSIONS

FSANZ invites public comment on this Initial Assessment Report for the purpose of preparing an amendment to the Code for approval by the FSANZ Board.

Written submissions are invited from interested individuals and organisations to assist FSANZ in preparing the Final Assessment of this Application. Submissions should, where possible, address the objectives of FSANZ as set out in section 18 of the FSANZ Act. Information providing details of potential costs and benefits of the proposed change to the Code from stakeholders is highly desirable. Claims made in submissions should be supported wherever possible by referencing or including relevant studies, research findings, trials, surveys etc. Technical information should be in sufficient detail to allow independent scientific assessment.

The processes of FSANZ are open to public scrutiny, and any submissions received will ordinarily be placed on the public register of FSANZ and made available for inspection. If you wish any information contained in a submission to remain confidential to FSANZ, you should clearly identify the sensitive information, separate it from your submission and provide justification for treating it as confidential commercial material. Section 114 of the FSANZ Act requires FSANZ to treat in-confidence, trade secrets relating to food and any other information relating to food, the commercial value of which would be, or could reasonably be expected to be, destroyed or diminished by disclosure.

Submissions must be made in writing and should clearly be marked with the word 'Submission' and quote the correct project number and name. While FSANZ accepts submissions in hard copy to our offices, it is more convenient and quicker to receive submissions electronically through the FSANZ website using the Standards Development tab and then through Documents for Public Comment. Alternatively, you may email your submission directly to the Standards Management Officer at submissions@foodstandards.gov.au. There is no need to send a hard copy of your submission if you have submitted it by email or the FSANZ website. FSANZ endeavours to formally acknowledge receipt of submissions within 3 business days.

Submissions need to be received by FSANZ by 6pm (Canberra time) 17 September 2008.

Submissions received after this date will only be considered if agreement for an extension has been given prior to this closing date. Agreement to an extension of time will only be given if extraordinary circumstances warrant an extension to the submission period. Any agreed extension will be notified on the FSANZ website and will apply to all submitters.

Questions relating to making submissions or the application process can be directed to the Standards Management Officer at standards.management@foodstandards.gov.au.

If you are unable to submit your submission electronically, hard copy submissions may be sent to one of the following addresses:

Food Standards Australia New Zealand
PO Box 7186
Canberra BC ACT 2610
AUSTRALIA
Tel (02) 6271 2222
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INTRODUCTION

An Application was received from Bayer CropScience Pty Ltd on 27 September 2007 seeking approval in the Code for food derived from genetically modified (GM) cotton, line GHB614 (known commercially as GlyTol^{TM}), under Standard 1.5.2 – Food produced using Gene Technology. Cotton line GHB614 is tolerant to the broad leaf herbicide glyphosate.

This Draft Assessment includes a full scientific evaluation of the food (oil and linters) derived from cotton line GHB614 according to FSANZ guidelines[1], to assess its safety for human consumption. Public comment is now sought on the safety assessment and proposed recommendations prior to a Final Assessment and completion of the Application.

1. Background

1.1 Current Standard

Genetically modified (GM) foods must be approved in Standard 1.5.2 before they may be sold in Australia and New Zealand. If approved, the food is listed in the Table to clause 2 of the Standard. Approval is contingent upon the completion of a pre-market safety assessment undertaken by FSANZ.

1.2 Description and Purpose of the Genetic Modification

The genetic modification in cotton line GHB614 consists of a single herbicide tolerance trait introduced by the transfer of a modified 5-enol-pyruvylshikimate-3-phosphate synthase gene, *2mepsps*, derived from corn. The EPSPS protein is a key enzyme involved in the shikimate pathway for biosynthesis of aromatic amino acids in plants, and is normally inhibited by glyphosate, ultimately leading to the death of the plant. Two simple mutations were introduced into the wild type e*psps* gene from corn, using site-directed mutagenesis. The mutations introduced into the 2mEPSPS enzyme significantly reduce its sensitivity to glyphosate, allowing the enzyme to continue to function in the presence of the herbicide. Plants expressing 2mEPSPS are therefore able to tolerate treatment with herbicides containing glyphosate as the active ingredient.

The Applicant has developed glyphosate-tolerant cotton line GHB614 for cultivation in major cotton producing countries worldwide, including eventually in Australia. While cottonseed oil is used in a large number of food products consumed by humans, the main source in the Australian domestic market is from cotton cropped locally. It is expected therefore that if approved, oil derived from GHB614 cotton will be found mainly in imported foods and will not be present in significant amounts in the Australian or New Zealand markets. To date, the Applicant has not made an application to the Office of the Gene Technology Regulator (OGTR) seeking approval for the commercial cultivation of glyphosate-tolerant cotton line GHB614 in Australia.

1.3 Regulatory status in other countries

The Applicant has sought approval for GHB614 cotton with the United States Food and Drug Administration and the United States Department of Agriculture, and FSANZ is advised that these are likely to be finalised in the near future.

[1] FSANZ (2007) Guidance Document – Safety Assessment of Genetically Modified Foods

Food and feed approval for GHB614 cotton has been recently granted by Health Canada and the Canadian Food Inspection Agency respectively.

2. The Issue / Problem

Before food derived from glyphosate-tolerant cotton line GHB614 can enter the food supply in Australia and New Zealand, it must be assessed for safety and an amendment to the Code must be approved by the FSANZ Board. The Board decision on the draft variation is subsequently notified to the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council). An amendment to the Code may only be gazetted once the Ministerial Council process has been finalised.

Bayer CropScience Pty Ltd has therefore applied to FSANZ for a variation to Standard 1.5.2 to include food derived from glyphosate-tolerant cotton line GHB614.

3. Objectives

The objective of this assessment is to determine whether it would be appropriate to amend the Code to approve the use of food derived from cotton line GHB614 under Standard 1.5.2. In developing or varying a food standard, FSANZ is required by its legislation to meet three primary objectives, which are set out in section 18 of the FSANZ Act. These are:

- the protection of public health and safety;
- the provision of adequate information relating to food to enable consumers to make informed choices; and
- the prevention of misleading or deceptive conduct.

In developing and varying standards, FSANZ must also have regard to:

- the need for standards to be based on risk analysis using the best available scientific evidence;
- the promotion of consistency between domestic and international food standards;
- the desirability of an efficient and internationally competitive food industry;
- the promotion of fair trading in food; and
- any written policy guidelines formulated by the Ministerial Council.

4. Key assessment questions

The following questions were identified at Initial Assessment:

Is food derived from glyphosate-tolerant cotton line GHB614 as safe for human consumption as that derived from conventional varieties of cotton?

Is other information available, including from the scientific literature, general technical information, independent scientists, other regulatory agencies and international bodies, and the general community, that needs to be considered?
Are there any other considerations that would influence the outcome of this assessment?

RISK ASSESSMENT

In addressing the key assessment questions, FSANZ has considered information provided by the Applicant on the nature of the genetic modification, the molecular characterisation, the characterisation of the novel protein, a compositional analysis of GHB614 cotton and any nutritional issues, as well as previously held information relating to the safety of the 2mEPSPS protein which has been previously assessed. FSANZ has also considered resource material including published scientific literature and general technical information available in the public domain. The summary and conclusions from the full safety assessment report (at **Attachment 2**) are presented below.

5. Safety Assessment

5.1 Safety Assessment Process

The safety assessment applied to food from cotton line GHB614 addresses only food safety and nutritional issues. It therefore does not address: environmental risks related to the environmental release of genetically modified plants used in food production; the safety of animal feed or animals fed with feed derived from GM plants; or the safety of food derived from the non-GM (conventional) plant.

In conducting a safety assessment of food derived from glyphosate-tolerant cotton line GHB614, a number of criteria have been addressed including: a characterisation of the transferred genes, their origin, function and stability in the cotton genome; the changes at the level of DNA, protein and in the whole food; compositional analyses; evaluation of intended and unintended changes; and the potential for the gene expression product, the 2mEPSPS protein, to be either allergenic or toxic in humans.

5.2 Outcomes of the Safety Assessment

Cotton is one of the oldest cultivated crops, providing over 40% of the total fibre used in the world. Cottonseed can be processed into oil, meal, hulls and linters. Only the oil and linters are typically used as human food due to the presence of natural toxicants in the seed, which may cause toxicity if consumed in sufficient amounts. These substances are removed or reduced by the processing of cottonseed into oil and linters.

Cottonseed oil has been in common use as food since the middle of the nineteenth century. It is used in a variety of foods including frying oil, salad and cooking oil, mayonnaise, salad dressing, shortening, margarine and packing oil. Cotton linters are short fibres removed from the cottonseed during processing and are a major source of cellulose for both chemical and food uses. They are used in high fibre dietary products and as a viscosity enhancer (thickener) in ice cream, salad dressings and toothpaste.

The cotton variety, Coker 312, was used as the parental variety for the transformation.

Although no longer widely grown, Coker 312 is still considered a commercially acceptable cultivar with a long history of safe use.

The modified *epsps* gene present in cotton line GHB614 was derived from corn, the world's third leading cereal crop behind wheat and rice. Corn-derived products are routinely used in a large number and diverse range of food products and have a long history of safe use.

5.2.1 Molecular Characterisation

A modified version of the wildtype corn *epsps* gene, designated *2mepsps*, was inserted into the conventional cotton line Coker 312, generating cotton line GHB614. The combined results from the molecular characterization of cotton line GHB614 confirm the presence of one functional, intact copy of the *2mepsps* gene at a single site in the plant genome. The new genetic trait is stably incorporated into the cotton genome and is transferred to subsequent generations in a normal pattern of inheritance. No antibiotic resistance marker genes are present in cotton line GHB614.

5.2.2 Characterisation of Novel Protein

The modified gene encodes the 47 kDa 2mEPSPS protein, characterized by two amino acid substitutions in the naturally occurring corn enzyme: one at position 102 and the other at position 106 of the protein. These specific amino acid changes significantly reduce the binding of glyphosate, allowing the enzyme to function normally in the presence of the herbicide.

The 2mEPSPS protein is expressed at relatively low levels in cottonseed. The average level of the 2mEPSPS protein in fuzzy seed from GHB614 cotton plants, grown under normal field conditions including spraying with glyphosate, was approximately 21 μg/g, on a fresh weight basis, which corresponds to about 0.01% of the total crude protein. The 2mEPSPS protein was not detected in processed oil fractions derived from GHB614 cottonseed.

The potential toxicity and allergenicity of the 2mEPSPS protein has been assessed previously by FSANZ and no safety concerns were identified. The protein is more than 99% identical to the endogenous corn protein, which is a natural component of the food supply. The results from a large number of studies confirm the identity and physicochemical and functional properties of the 2mEPSPS protein expressed in GHB614 cotton. No adverse effects were identified in acute toxicity studies in mice using purified 2mEPSPS protein. The 2mEPSPS protein does not exhibit sequence similarity with known protein toxins or allergens, and is degraded like other dietary proteins in conditions that mimic human digestion. Based on bioinformatic, biochemical and acute animal toxicity studies, 2mEPSPS is considered non-toxic to humans and is unlikely to be allergenic.

5.2.3 Compositional Analyses

Compositional analyses were done to establish the nutritional adequacy of cotton line GHB614, and to compare it to the conventional counterpart when grown under typical cultivation conditions. The components analysed in cottonseed were protein, fat, carbohydrate, amino acids, fatty acids, vitamins, minerals, and the anti-nutrients gossypol, phytic acid and cyclopropenoid fatty acids.

No differences of biological significance were observed between cotton line GHB614 and its conventional counterpart. Some minor differences in key nutrients were noted however these do not raise safety concerns as the observed levels were within the range of values measured for commercial cotton varieties and are considered to reflect normal biological variability. On these grounds, food derived from glyphosate-tolerant cotton line GHB614 is considered to be compositionally equivalent to food from its conventional counterpart.

5.2.5 Nutritional Impact

The detailed compositional studies are considered adequate to establish the nutritional adequacy of food derived from GHB614 cotton. On the basis of the comparative assessment, the introduction of cottonseed oil and linters from GHB614 cotton into the food supply would be expected to have negligible nutritional impact. This was confirmed in a feeding study in rapidly growing broiler chicks, which demonstrated that cotton line GHB614 is equivalent to its conventional counterpart and other commercial varieties of cotton in its ability to support typical growth and well being in animals.

5.3 Conclusion

No potential public health and safety concerns have been identified in the assessment of glyphosate-tolerant cotton line GHB614. On the basis of the data provided in the present Application, and other information available to FSANZ, food derived from glyphosate-tolerant cotton line GHB614 is as safe and wholesome as food derived from conventionally produced cotton varieties.

RISK MANAGEMENT

6. Options

There are no non-regulatory options that could apply to this Application. The two regulatory options available for this Application are:

6.1 Option 1: Prohibit food derived from cotton line GHB614

Maintain the *status quo* by not amending Standard 1.5.2 to approve food derived from glyphosate-tolerant cotton line GHB614.

6.2 Option 2: Approve food derived from cotton line GHB614

Vary Standard 1.5.2 to permit the sale and use of food (oil and linters) derived from cotton line GHB614, with or without specified conditions of use listed in the Table to clause 2 of the Standard.

7. Impact Analysis

7.1 Affected Parties

In considering this Application, affected parties include the following:

- Consumers, particularly those who have concerns about biotechnology;

- Food importers and distributors of wholesale ingredients;

- Manufacturing and retail sectors of the food industry; and

- Government generally, where a regulatory decision may impact on trade or WTO obligations, and enforcement agencies in particular, which will need to ensure that any approved products are correctly labelled.

The cultivation of cotton line GHB614 in Australia or New Zealand could have an impact on the environment, which would need to be formally assessed by the Office of Gene Technology Regulator (OGTR) in Australia, and by various New Zealand government agencies including the Environmental Risk Management Authority (ERMA) and Ministry of Agriculture and Fisheries (MAF) before growing in either country could be permitted. At this stage, no applications concerning cotton line GHB614 have been received by these agencies.

7.2 Benefit Cost Analysis

In the course of developing food regulatory measures suitable for adoption in Australia and New Zealand, FSANZ is required to consider the impact of all options on all sectors of the community, including consumers, the food industry and governments in both countries. The regulatory impact assessment identifies and evaluates, though is not limited to, the costs and benefits of the regulation, and its health, economic and social impacts.

7.2.1 Option 1 – continue to prohibit food from cotton line GHB614

Consumers: Possible restriction in the availability of certain imported food products if they are found to contain ingredients derived from cotton line GHB614.

No impact on consumers wishing to avoid GM foods, as food from GHB614 cotton is not currently permitted in the food supply.

Government: Potential impact if considered inconsistent with WTO obligations but impact would be in terms of trade policy rather than in government revenue.

Likely to be increased costs associated with monitoring required for un-approved GM material derived from GHB614 cotton which may inadvertently be incorporated into imported food products. Costs incurred relate to the use of detection methodology including: labour and reagent costs, methodology validation, and maintenance of methodology consistency and competency.

Industry: Possible restriction on certain imported foods once cotton line GHB614 is commercialised overseas.

Potential longer-term impact - any successful WTO challenge has the potential to impact adversely on food industry.

7.2.2 Option 2 – approve food from cotton line GHB614

Consumers: No restriction on imported food products if containing ingredients derived from GHB614 cotton.

Benefit of lower prices, to the extent that savings from increased or improved production efficiencies are passed on.

Potential impact on consumers wishing to avoid GM foods by a possible restriction of choice of products, or increased prices for non-GM food products.

Government: Benefit in that there would be no potential for trade disruption as a result of the detection of unapproved GHB614 cotton derivatives in imported food products.

Approval of GHB614 cotton on the basis of the risk assessment would ensure no conflict with WTO obligations.

Likely to be increased costs associated with the additional monitoring required to ensure compliance with the labelling provisions of the Code. Costs incurred relate to the use of detection methodology including: labour and reagent costs, methodology validation, and maintenance of methodology consistency and competency.

Industry: Broader market access and increased choice in raw materials for food manufacturing.

Benefit to importers of processed foods containing cottonseed oil and linters as ingredients as foods derived from GHB614 cotton would be compliant with the Code.

Possible cost to food industry as some food ingredients derived from GHB614 cotton may be required to be labelled as genetically modified.

If grown in Australia at a late date, primary producers may benefit from an increased choice of crop lines with potentially lower production costs and higher yields

7.3 Comparison of Options

As the safety assessment of cotton line GHB614 found that food derived from that line is as safe for human consumption as food from conventional cotton varieties, option 1 is likely to be inconsistent with Australia and New Zealand's WTO obligations.

Option 1 would also offer little benefit to consumers wishing to avoid GM foods; a number of GM cottons are already approved for food use in Australia and New Zealand, and maintaining a prohibition on food from GHB614 cotton could potentially limit the availability of imported food products in Australia and New Zealand, once that line is commercialised in other countries.

Under Option 2, if primary producers choose to grow GHB614 cotton because of its particular agronomic characteristics and potential for improved production efficiency, these effects could flow on to other sectors, including consumers, as lower food prices in Australia and New Zealand. Government would also benefit in that potential disruption to trade would be avoided. While there will be costs to government associated with the additional monitoring required to ensure compliance with the Code, similar costs are also likely to be associated with Option 1.

Therefore, the overall impact on monitoring resources would apply whether cotton line GHB614 is approved or not approved. In terms of some consumers wishing to avoid GM foods, there is unlikely to be any additional impact, as a number of GM cotton lines are already approved for food use in Australia and New Zealand. In addition, until the Applicants seek approval for environmental release of GHB614 cotton through the appropriate regulatory channels, it is likely only to be present in very small amounts in imported food products.

As food derived from GHB614 cotton has been found to be safe for human consumption and the potential benefits outweigh the potential costs, Option 2 is therefore the preferred option.

COMMUNICATION

8. Communication and Consultation Strategy

This Application seeks approval of a food under an existing standard. As a result, FSANZ has applied a basic communication strategy to the Application, which involves advertising the availability of assessment reports for public comment in the national press and making the finalized reports available on the FSANZ website. FSANZ will issue a media release drawing journalists' attention to the matter.

As normally applies to all GM food assessments, the Draft Assessment Report for this Application will be available to the public on the FSANZ website and distributed to major stakeholders. Public comment on this Draft Assessment will be sought prior to preparation of the Final Assessment Report.

9. Consultation

9.1 Public consultation

The Initial Assessment was advertised for public comment between 12 December 2007 and 6 February 2008. Thirteen submissions were received during this period and a summary of these is included in **Attachment 3** to this Report.

FSANZ has taken the submitters' comments into account in preparing the Draft Assessment of this Application.

Responses to general issues, such as labelling of GM foods, food allergies, and the safety of recombinant DNA in the food supply, are available from the FSANZ website or from published information[2]. Specific issues relating to food derived from cotton line GHB614 have been addressed in this report. The major issues raised are discussed here.

9.1.1 Use of peer-reviewed studies for the safety assessment

Queensland Health stated in its submission that the data considered by FSANZ will need to include peer-reviewed studies which ascertain the safety of the food. The Country Women's Association expressed a similar view by calling for independent scientific testing of GM foods before FSANZ considers the use of the products.

[2] http://www.foodstandards.gov.au/foodmatters/gmfoods/frequentlyaskedquest3862.cfm; GM Foods – Safety Assessment of Genetically Modified Foods, Food Standards Australia New Zealand (2005)

9.1.1.1 Response

In undertaking a food risk/safety assessment, FSANZ considers the scientific merit of all available data in their various forms, including both published (peer-reviewed) studies and unpublished studies, in order to base its decisions on 'the best available evidence', recognising that both types of data have their strengths and limitations. This same consideration exists for the evaluation of drugs for human or veterinary use or the use of agricultural chemicals. The emphasis or weighting placed on individual studies depends on whether FSANZ has access to all the data or only an abridged summary from which to make an independent evaluation and interpretation. Overall, FSANZ believes that both published and unpublished studies are important in establishing standards to protect public health.

While there is a perception that a peer-reviewed article in a scientific journal has greater authority for a safety assessment, this must be balanced against some of the limitations of published material, due to the level of detail reported by the author and known publication bias. Efforts to minimize journal publication costs through limiting the article size, has the inevitable consequence of data being presented almost exclusively in summary or minimal form. Therefore, many of the important technical details or supporting observations are not included so that the 'pathway' to the conclusions is not always transparent. Often, the paucity of important technical information prevents validation of the conclusions.

Unpublished studies submitted by applicants are normally performed to reporting standards determined by Good Laboratory Practice (GLP) and Quality Assurance and are complete with individual data, summaries and statistical analysis. A major benefit of GLP is to establish minimum standards of documentation, but the extent of documentation that is specified by GLP standards is too voluminous to be included in published studies. The limitation of unpublished studies can be that the results are usually discussed only within the context of that particular study and do not refer to other companion studies. The nature of these studies also means that they are sometimes evaluated as 'commercial-in-confidence', but this does not diminish the quality or validity of the data.

As part of its assessment, FSANZ must consider whether the data package that is submitted is sufficient to establish the safety of the food.

In the case of food derived from cotton line GHB614, the data package considered by FSANZ in undertaking the safety assessment consisted primarily of raw laboratory data to studies undertaken in a number of independent laboratories in addition to unpublished data provided by the Applicant. In this particular case, FSANZ considered the supporting studies were sufficiently detailed to meet requirements.

In undertaking a GM food safety assessment, FSANZ also has regard to any other relevant information, including peer-reviewed published studies, where these are available. FSANZ has noted that many companies eventually go on to publish studies that have been submitted for regulatory assessment. The level of detail in the published versions is usually considerably less than that originally submitted to regulators for assessment. In the main, FSANZ does not consider that the absence of peer-reviewed studies precludes a robust, evidence-based assessment.

9.1.2 Enforcement costs

Queensland Health requested in its submission that FSANZ provide detailed advice in the Draft Assessment Report on the enforcement costs determined by FSANZ in the cost benefit analysis and how those costs were agreed upon by the jurisdictions in regard to this Application. The NSW Food Authority noted in its submission that extensive costs are incurred in monitoring for the presence of GM food, particularly when resources at the government level are limited: labour and reagent costs, methodology validation, methodology consistency and competency maintenance, and stated that these costs should be factored into the benefit cost analysis. This issue has been raised in other current GM food applications.

9.1.2.1 Response

FSANZ is aware of the concerns raised by various jurisdictions in relation to monitoring and enforcement costs associated with GM foods and agrees that, over time, the successive approval of new GM foods may significantly impact on monitoring resources. This is a general issue affecting all GM applications and is not specific to any particular GM application.

Following the receipt of submissions, FSANZ contacted relevant jurisdictions to try and obtain quantitative information regarding the associated costs in order to better reflect this in the benefit cost analysis. The jurisdictions that were contacted indicated they undertake a range of testing and monitoring activities in relation to GM foods as well as other types of foods. Testing for GM foods is significantly more expensive than the cost of other testing.

In 2003, a review of labelling of GM foods was undertaken by FSANZ which included an assessment of compliance and enforcement activities and information of GM food surveys including a previous Implementation Sub-Committee (ISC)[3] Coordinated Survey. The ISC Coordinated Survey included a business record audit and product testing. A total of 51 samples were tested under the survey, costing a total of $AUD33,660 or $AUD660 per sample. FSANZ understands from recent contact with the National Measurements Institute (NMI), that the current cost of testing (per sample) remains approximately the same as in 2003.

At this stage however it is not possible to ascertain the total cost of monitoring activities for GM foods, and this will vary between jurisdictions depending on the amount of monitoring being undertaken. In the absence of detailed quantitative information, it is therefore only possible for the benefit cost analysis to be qualitative in nature.

FSANZ considers it important to recognize however that, because GM foods are continually entering international trade, such costs are largely unavoidable and will arise irrespective of whether or not GM foods are approved in Australia and New Zealand. In the case of approved GM foods, monitoring is required to ensure compliance with labelling requirements; in the case of GM foods that have not been approved, monitoring is required to ensure they are not illegally entering the food supply. The costs of monitoring and enforcement are thus expected to be comparable in either case.

[3] ISC comprises heads of the appropriate Australian (Commonwealth and State/Territory) and New Zealand inspection and enforcement agencies and is responsible, among other things, for overseeing the development and implementation of a consistent approach across jurisdictions to enforcing food regulation and standards.

Any regulatory decision taken by FSANZ is therefore unlikely to significantly affect the cost impact on jurisdictions, in terms of their responsibilities to enforce the Code.

The increased monitoring and enforcement burden being placed on jurisdictions was discussed by ISC at its meeting in April 2008, where it was agreed that a national compliance and monitoring strategy for GM foods is required to assist with consistent implementation of Standard 1.5.2. It was also agreed to hold a workshop to develop a draft strategy for consideration by ISC. FSANZ welcomes this approach to the development of a national strategy to address this issue.

9.2 World Trade Organization (WTO)

As members of the World Trade Organization (WTO), Australia and New Zealand are obliged to notify WTO member nations where proposed mandatory regulatory measures are inconsistent with any existing or imminent international standards and the proposed measure may have a significant effect on trade.

The draft variation to the Code proposed in this report, to allow food derived from cotton line GHB614 in Australia and New Zealand, is likely to have a liberalising effect on international trade, as currently the food is prohibited. FSANZ considers therefore that notification of this Application, under the Sanitary and Phytosanitary Measures (SPS) Agreement is not necessary.

CONCLUSION

10. Conclusion and Preferred Approach

Preferred Approach

Amend Standard 1.5.2 – Food produced using Gene Technology, to include food derived from glyphosate-tolerant cotton line GHB614 in the Table to clause 2.

10.1 Reasons for Preferred Approach

An amendment to the Code to give approval to the sale and use of food derived from cotton line GHB614 in Australia and New Zealand is proposed on the basis of the available scientific evidence, for the following reasons:

- the safety assessment did not identify any public health and safety concerns associated with the genetic modification used to produce glyphosate-tolerant cotton line GHB614;
- food derived from glyphosate-tolerant cotton line GHB614 is equivalent to food from the conventional counterpart and other commercially available cotton varieties in terms of its safety for human consumption and nutritional adequacy;
- labelling of certain food products derived from glyphosate-tolerant cotton line GHB614 will be required if novel DNA and/or protein is present in the final food; and

- a regulation impact assessment process has been undertaken that also fulfils the requirement in New Zealand for an assessment of compliance costs. The assessment concluded that the preferred option is option 2, an amendment to the Code.

11. Implementation and Review

Following the consultation period for this document, a Final Assessment of the Application will be completed and the draft variation considered for approval by the FSANZ Board. The FSANZ Board's decision will then be notified to the Ministerial Council.

ATTACHMENTS

1. Draft variation to the *Australia New Zealand Food Standards Code*
2. Draft safety assessment report
3. Summary of public submissions

Attachment 1

Draft variation to the *Australia New Zealand Food Standards Code*

Standards or variations to standards are considered to be legislative instruments for the purposes of the Legislative Instruments Act (2003) and are not subject to disallowance or sunsetting.

To commence: on gazettal

[1] ***Standard 1.5.2*** *of the Australia New Zealand Food Standards Code is varied by inserting in the* Table to clause 2 –

Food derived from glyphosate-tolerant cotton line GHB614	

Attachment 2

SAFETY ASSESSMENT: FOOD DERIVED FROM GLYPHOSATE-TOLERANT COTTON LINE GHB614

Summary and Conclusions

Background

Glyphosate normally exerts herbicide activity by binding and inactivating EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), an enzyme that is essential for the synthesis of proteins in plants. Cotton line GHB614 has been genetically modified (GM) for tolerance to glyphosate herbicides by expression in the plant of a modified *epsps* gene from corn, *2mepsps*, which introduces two amino acid changes in the enzyme. The amino acid changes in the 2mEPSPS protein significantly lower the sensitivity to glyphosate, allowing the enzyme to continue to function in the presence of the herbicide.

Although no plans currently exist to introduce cotton line GHB614 into the Australian cropping system, the Applicant intends that it will be approved for growing in major cotton producing countries overseas, including eventually Australia. Once approved, food products, such as cottonseed oil and linters, could enter the market via imported foods or from locally produced crops.

In conducting a safety assessment of food derived from glyphosate-tolerant GHB614 cotton, a number of criteria have been addressed including: a characterisation of the transferred gene, its origin, function and stability in the cotton genome; the changes at the level of DNA, protein and in the whole food; compositional analyses; evaluation of intended and unintended changes; and the potential for the newly expressed protein to be either allergenic or toxic in humans.

The safety assessment addresses only food safety and nutritional issues; it does not address environmental risks related to the release of GM food crops into the environment, the safety of animal feed or food products derived from animals fed GM plants, or the safety of food derived from the non-GM (conventional) plant.

History of Use

Cotton is one of the oldest cultivated crops, providing over 40% of the total fibre used in the world. Cottonseed can be processed into oil, meal, hulls and linters. Only the oil and linters are typically used as human food due to the presence of natural toxicants in the seed, which may cause toxicity if consumed in sufficient amounts. These substances are removed or reduced by the processing of cottonseed into oil and linters.

Cottonseed oil has been in common use as food since the middle of the nineteenth century. It is used in a variety of foods including frying oil, salad and cooking oil, mayonnaise, salad dressing, shortening, margarine and packing oil. Cotton linters are short fibres removed from the cottonseed during processing and are a major source of cellulose for both chemical and food uses. They are used in high fibre dietary products and as a viscosity enhancer (thickener) in ice cream, salad dressings and toothpaste.

The cotton variety, Coker 312, was used as the parental variety for the transformation. Although no longer widely grown, Coker 312 is still considered a commercially acceptable cultivar.

The modified *epsps* gene present in cotton line GHB614 was derived from corn, the world's third leading cereal crop behind wheat and rice. Corn-derived products are routinely used in a large number and diverse range of foods and have a long history of safe use.

Molecular Characterisation

A modified version of the wildtype corn *epsps* gene, designated *2mepsps*, was inserted into the conventional cotton line Coker 312, generating cotton line GHB614. The combined results from the molecular characterization of cotton line GHB614 confirm the presence of one functional, intact copy of the *2mepsps* gene inserted at a single site in the plant genome. The new genetic trait is stably incorporated into the cotton genome and is transferred to subsequent generations in a normal pattern of inheritance. No antibiotic resistance marker genes are present in cotton line GHB614.

Characterisation of Novel Protein

The modified gene encodes the 47 kDa 2mEPSPS protein, characterized by two amino acid substitutions in the naturally occurring corn enzyme: one at position 102 and the other at position 106 of the protein. These specific amino acid changes significantly reduce the binding of glyphosate, allowing the enzyme to function normally in the presence of the herbicide.

The 2mEPSPS protein is expressed at relatively low levels in cottonseed. The average level of the 2mEPSPS protein in fuzzy seed from GHB614 cotton plants, grown under normal field conditions including spraying with glyphosate, was approximately 21.2 μg/g, on a fresh weight basis, which corresponds to about 0.01% of the total crude protein. The 2mEPSPS protein was not detected in processed oil fractions derived from GHB614 cottonseed.

The potential toxicity and allergenicity of the 2mEPSPS protein has been assessed previously by FSANZ and no safety concerns have been identified. The protein is more than 99% identical to the endogenous corn protein, which is a natural component of the food supply. The results from a large number of studies confirm the identity and physicochemical and functional properties of the 2mEPSPS protein expressed in GHB614 cotton. No adverse effects were identified in acute toxicity studies in mice using purified 2mEPSPS protein. The 2mEPSPS protein does not exhibit sequence similarity with known protein toxins or allergens, and is degraded in conditions that mimic human digestion, as other dietary proteins. Based on bioinformatic, biochemical and acute toxicity studies, 2mEPSPS is considered non-toxic to humans and is unlikely to be allergenic.

Compositional Analyses

Compositional analyses were done to establish the nutritional adequacy of cotton line GHB614, and to compare it to the conventional counterpart when grown under typical cultivation conditions. The components analysed in cottonseed were protein, fat, carbohydrate, amino acids, fatty acids, vitamins, minerals, and the anti-nutrients gossypol, phytic acid and cyclopropenoid fatty acids.

No differences of biological significance were observed between cotton line GHB614 and its conventional counterpart. Some minor differences in key nutrients were noted however the levels observed were within the range of values measured for commercial cotton varieties and therefore most likely reflect normal biological variability. Food derived from glyphosate-tolerant cotton line GHB614 is considered to be compositionally equivalent to food from the conventional counterpart.

Nutritional Impact

Detailed compositional studies are considered adequate to establish the nutritional adequacy of food derived from GHB614 cotton. The introduction of cottonseed oil and linters from GHB614 cotton into the food supply would therefore be expected to have little nutritional impact. This was also demonstrated in a feeding study in rapidly growing broiler chicks, which demonstrated that cottonseed from GHB614 cotton is equivalent to its conventional counterpart and cottonseed from other commercial varieties of cotton in its ability to support typical growth and well being.

Conclusion

No potential public health and safety concerns have been identified in the assessment of glyphosate-tolerant cotton line GHB614. On the basis of the data provided in the present Application, and other information available to FSANZ, food derived from glyphosate-tolerant cotton line GHB614 is as safe and wholesome as food derived from conventionally produced cotton varieties.

1. BACKGROUND

A safety assessment has been conducted on food (cottonseed oil and linters) derived from cotton that has been genetically modified (GM) for tolerance to herbicides containing glyphosate as the active ingredient. The GM cotton is referred to as line GHB614.

Glyphosate (N-phosphonomethylglycine) is a non-selective, broad spectrum herbicide. The mode of action of glyphosate is to specifically bind to, and block, the activity of a native plant enzyme, 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS). EPSPS is a key enzyme in the shikimate pathway in plants which links the metabolism of carbohydrates to the biosynthesis of ring-containing compounds including aromatic amino acids. Plant EPSPS enzymes are normally inactivated by glyphosate which leads to cellular deficiencies in certain amino acids resulting ultimately in the death of the plant.

In cotton line GHB614, tolerance to glyphosate is achieved through expression in the plant of a modified form of the EPSPS enzyme, 2mEPSPS, derived from corn. Two point (single nucleotide) mutations were introduced to the corn *epsps* gene to generate *2mepsps*, using site-directed mutagenesis. These changes significantly reduce the sensitivity of the 2mEPSPS enzyme to glyphosate, allowing it to continue to function in the presence of the herbicide.

Cotton line GHB614 has been developed for agriculture in major cotton producing countries worldwide, including Australia.

2. HISTORY OF USE

2.1 Host organism

The host organism is cotton (*Gossypium hirsutum* L.), grown extensively for its fibre. Cotton is one of the oldest cultivated crops, providing over 40% of the total fibre used in the world (OECD 2004). Only the cotton boll, which develops from the plant ovary, is used for either textile fibre or food/feed. The cotton boll, once harvested, is processed ("ginned") to separate the cottonseed from the cotton fibre. Cottonseed can be processed into four major by-products: oil, meal, hulls and linters. Only the oil and linters are typically used as human food.

Food products from cottonseed are limited to highly processed products due to the presence of the natural toxicants, gossypol and cyclopropenoid fatty acids in the seed, which may cause toxicity if consumed in sufficient amounts. These substances are removed or reduced by the processing of the cottonseed into oil and linters.

Cottonseed oil is regarded as premium quality oil and has a long history of safe food use. It is used in a variety of foods including frying oil, salad and cooking oil, mayonnaise, salad dressing, shortening, margarine and packing oil. It contains predominantly unsaturated fatty acids. Cottonseed oil has been in common use since the middle of the nineteenth century and achieved GRAS (Generally Recognised As Safe) status under the United States Federal Food Drug and Cosmetic Act because of its common use prior to 1958. In the USA, it ranks third in volume behind soybean and corn oil, representing about 5-6% of the total domestic fat and oil supply.

Cotton linters are short fibres removed from the cottonseed during processing and are a major source of cellulose for both chemical and food uses.
They are used as a cellulose base in products such as high fibre dietary products as well as a viscosity enhancer (thickener) in ice cream, salad dressings and toothpaste.

The other major by-products – meal and hulls – are used as stock feed. Cottonseed meal is not used for human consumption in Australia or New Zealand. Although it has permission to be used for human food (after processing) in the USA and other countries, it is primarily sold for stock feed. Human consumption of cottonseed flour has been reported, particularly in Central American countries and India where it is used as a low cost, high quality protein ingredient in special products to help ease malnutrition. In these instances, cottonseed meal is inexpensive and readily available. Cottonseed flour is also permitted for human consumption in the USA, provided it meets certain specifications for gossypol content, although no products are currently being produced.

Australia crushes around 150-200,000 tonnes of cottonseed annually, producing about 30-40,000 tonnes of oil. Cotton is not grown in New Zealand. Cottonseed oil makes up around 15% of the total domestic fat and oil supply and is primarily used in the food service/food manufacturing sector.

The cotton variety Coker 312 was used as the parental variety for the transformation. Coker 312 is a United States Protected Variety of SEEDCO Corporation which has been shown to respond favourably to tissue culture and transformation techniques. Although no longer widely grown, Coker 312 is still considered a commercially acceptable cultivar.

2.2 Donor Organisms

Corn, *Zea mays*, is the source of the *epsps* gene that was modified to produce the *2mepsps* gene in cotton line GHB614. Corn is the world's third leading cereal crop, behind wheat and rice, and is grown in over 25 countries (OECD 2002). Also known as maize, corn has been grown in Mexico and Central America for some 8000 years and in Europe for 500 years and can thus be said to have a long history of safe use as a human food. The majority of corn that is grown however is destined for use as animal feed. In 2005, worldwide production of corn was over 700 million tonnes, with the United States and China being the major producers (FAOSTAT 2005).

The *epsps* gene was isolated from a cell suspension of Black Mexican Sweet (BMS) maize (Lebrun *et al.*1997). Black Mexican is a cultivar of New England (USA) sweet corn originally introduced to the food supply in 1864. Sweet corn is categorized as a vegetable and is mainly used for human consumption.

Corn was also the source of some of the regulatory gene elements. Other plants used as a source of regulatory elements include *Arabidopsis thaliana* and sunflower (*Helianthus annuus*). Arabidopsis is not consumed as food however is not considered to be harmful in humans or other animals. Sunflowers have a safe history of human consumption; both the whole seed and extracted oil are readily consumed.

3. MOLECULAR CHARACTERISATION

Molecular characterisation is necessary to provide an understanding of the genetic material introduced into the host genome and helps to frame the subsequent parts of the safety assessment. The molecular characterisation addresses three main aspects: the transformation method together with a detailed description of the DNA sequences introduced to the host genome; a characterisation of the inserted DNA including any rearrangements that may have occurred as a consequence of the transformation; and the genetic stability of the inserted DNA and any accompanying expressed traits.

Studies submitted:

1. Description of vector pTEM2, M. Lecleir; completed July 2007. Report ID: BIO2-004_VectDescript_029
2. Detailed insert characterization of *Gossypium hirsutum* transformation event GHB614, V. Habex and M. Lecleir; completed October 2006. Study No: BBS06-001
3. Full DNA sequence of event insert and integration site of *Gossypium hirsutum* transformation event GHB614, V. Habex; completed September 2006. Study No: BBS06-004. Amendment completed January 2007; Report No: BBS06-004-F1
4. Demonstration of the nature of the flanking sequences of *Gossypium hirsutum* transformation event GHB614, V. Habex and M. Lecleir; completed October 2006. Study No: BBS06-005

3.1 Transformation method

Cotton line GHB614 was developed through *Agrobacterium*-mediated transformation of the cotton variety Coker 312, using the transformation vector pTEM2 (see following sections).

Cotton explants were exposed to a culture of disarmed *Agrobacterium tumefaciens* containing plasmid p-TEM2. After co-culture, the cotton cells were regenerated to whole plants using the appropriate regeneration media with 500 mg/L claforan to eliminate residual *Agrobacterium*, and then selected with glyphosate. The shoots that developed were transferred to the greenhouse, further tested for tolerance to glyphosate, and allowed to flower and set seed.

The transformation was confirmed by 2mEPSPS enzyme activity assay, by glyphosate application to leaves, and by polymerase chain reaction (PCR) and Southern blot analyses.

3.2 Description of the breeding process

The primary transformant (R_0) was crossed with its isogenic non-transgenic parental line. The progeny of this backcross effectively becomes a transgenic parental line which can then be used in conventional breeding programs to cross with other non-transgenic cotton lines to develop a number of new glyphosate-tolerant cotton varieties. This process is depicted in Figure 1 below.

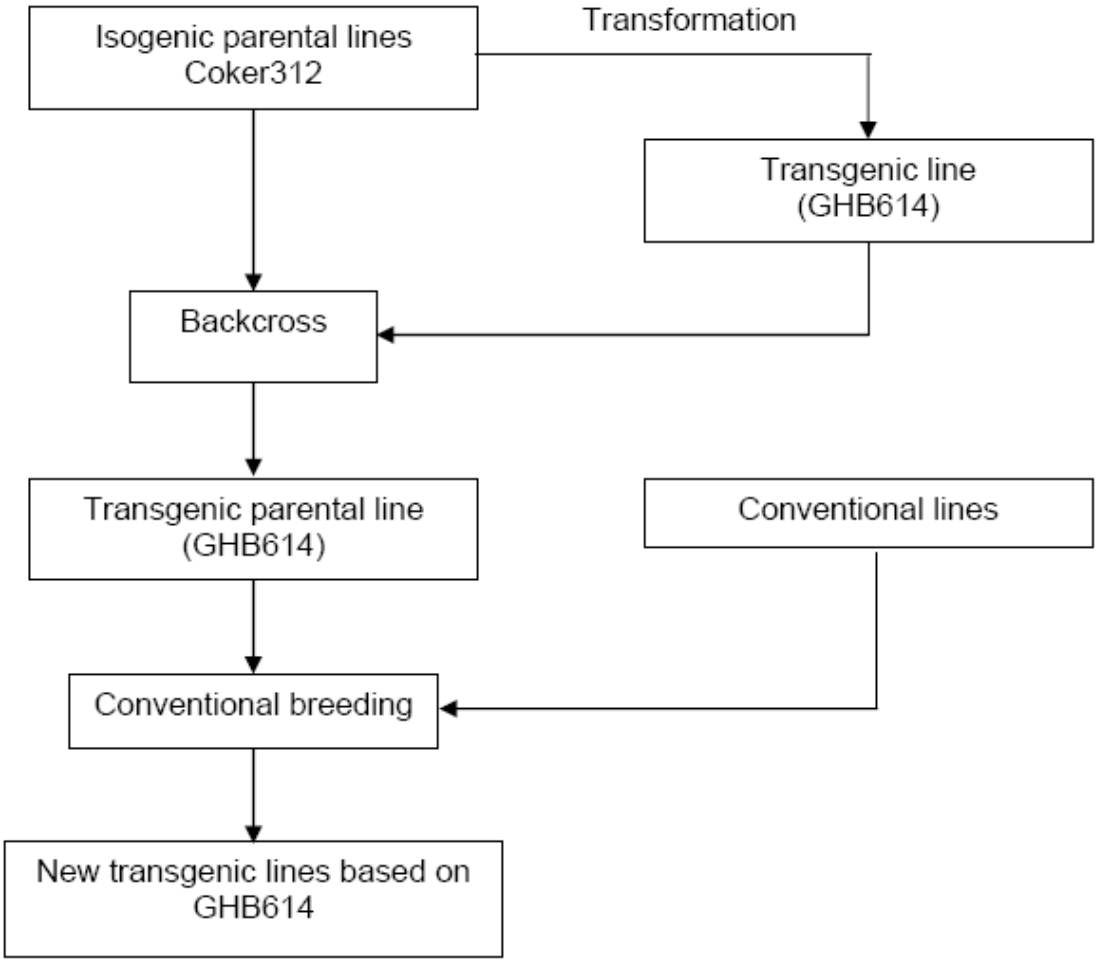

Figure 1: Schematic diagram of the breeding to develop new lines of cotton based on transformation event GHB614 cotton.

3.3 Description of the gene construct

3.3.1 Coding gene

The transformation vector used to generate cotton line GHB614, p-TEM2, contains one gene expression cassette within the left and right border segments (T-DNA). The sequence of the *2mepsps* gene is derived from the wildtype *epsps* gene from corn (*Zea mays*) with two single nucleotide mutations introduced by site directed mutagenesis. A methionine codon has been added to the N-terminal end of the 2mEPSPS protein sequence in order to restore the cleavage site of the optimized plastid transit peptide. The double mutant produces a 47 kDa protein with normal enzyme function and reduced affinity for glyphosate.

3.3.2 Other elements

The Ph4a748At promoter and h3At intron are regulatory elements used to control expression of the *2mepsps* gene in cotton and are derived from the histone H4 gene of the plant *Arabidopsis thaliana*. The use of these elements directs high level constitutive expression, particularly in rapidly growing plant tissues.

TPotp C, encodes the optimized transit peptide derived from genes of corn and sunflower and targets the mature protein to the plastids where it is normally located in the cell. The 3'histonAt terminator from *Arabidopsis thaliana* corresponds to the polyadenylation signal which is essential to end transcription of the introduced gene.

A full description of the genetic elements within the T-DNA of the transformation vector is provided below.

Symbol	Definition	Source	Size	Reference	Function
LB	Left border repeat	*Agrobacterium tumefaciens*	25	Zambrysky, 1988	Cis-acting element for T-DNA transfer
Ph4a748At	Promoter	*Arabidopsis thaliana*	1011	Chaboute *et al.*, 1987	High level constitutive expression
intron1 h3At	Intron	*Arabidopsis thaliana*	517	Chaubet *et al.*, 1992	High level constitutive expression
TPotp C	Optimized transit peptide	*Zea mays, Helianthus annuus*	373	Lebrun *et al.*, 1996	Targets the mature protein to the plastids, where the wild-type protein is located
2mepsps	Glyphosate tolerance gene	*Zea mays*	1338	Lebrun *et al.*, 1997	Herbicide tolerance and selectable marker
3'histonAt	Terminating signal	*Arabidopsis thaliana*	743	Chaboute *et al.*, 1987	Stop signal
RB	Right border repeat	*Agrobacterium tumefaciens*	25	Zambrysky, 1988	*Cis*-acting element for T-DNA transfer

3.4 Characterisation of the genes in the plant

A number of molecular analyses were conducted to determine the number of insertions and characterise the inserted DNA in GHB614 cotton. One copy of the introduced gene expression cassette is present in cotton line GHB614. A summary of each of the molecular analyses and the findings are given below.

3.4.1 Southern blot analyses

Genomic DNA from leaf tissue of GHB614 cotton plants (identity confirmed by PCR) was analysed using Southern blot analysis to determine the insert number, the copy number, the integrity of the inserted *2mepsps* gene cassette, and evaluate the presence or absence of plasmid backbone sequences. Conventional wildtype cotton variety Coker312 (used in the transformation) was used as the negative control for these analyses. The transformation vector, p-TEM2, and a wildtype control with 1 copy of digested pTEM2 were used as positive hybridisation controls. The latter reconstituted sample served to show that the hybridizations were performed under conditions allowing detection of target sequences.

Isolated genomic DNA samples from GHB614 cotton and conventional cotton were digested with nine different restriction enzymes, separated on agarose gels and then subjected to Southern blot analysis. To determine the insert and copy number of the introduced DNA, the separated DNA fragments were transferred to a membrane and sequentially hybridized with different radioactively labelled probes: four probes containing each single genetic element present in the p-TEM2 vector used for the transformation, and the complete T-DNA probe. The number of hybridising fragments detected indicates the number of inserts present in GHB614 cotton.

The hybridisation results obtained with the DNA positive and negative controls demonstrate that the Southern blot analysis was performed under conditions allowing hybridization of the specific probes with the target sequences. Based on a comparison of the size and pattern of observed fragments with the expected fragment sizes from digestion of genomic DNA, a single and unique site of insertion of the transgenic sequences is present in cotton line GHB614.

3.4.2 Polymerase chain reaction and DNA sequence analyses

The organisation of the genetic elements within the insert in GHB614 cotton was further characterised using PCR analysis by amplifying three overlapping regions of DNA spanning the entire length of the insert. The PCR products generated, following PCR of genomic DNA from GHB614 cotton, were all of the expected size.

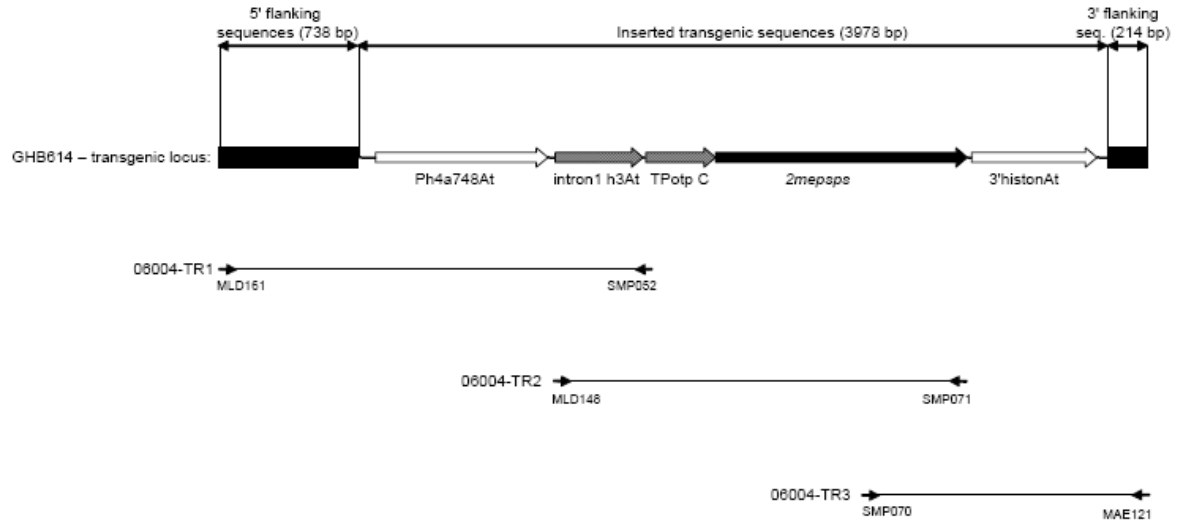

Figure 2: Overlapping PCR products generated across the insert in GHB614 cotton

The PCR products generated from GHB614 cotton genomic DNA were subject to DNA sequencing to further confirm the organisation of genetic elements within the insert, as well as to determine the 5' and 3' insert-to-genomic DNA junctions, and the complete DNA sequence of the inserted DNA and adjacent genomic DNA regions. A consensus sequence of the inserted DNA was generated by compiling the results of numerous sequencing reactions performed on each of the overlapping PCR products. This consensus sequence was then aligned to the DNA sequence of the corresponding T-DNA to determine if any changes had occurred during the transformation process.

The sequence determination indicated that the size of the inserted DNA in GHB614 cotton is 3978 base pairs (bp) and the arrangement of the genetic elements within the insert is identical to the corresponding transformation vector, pTEM2. In addition to the insert sequence, 214 bp of Right Border flanking sequence (3' end of insert) and 738 bp of Left Border flanking sequence (5' end of insert) were found to be completely identical to the cotton genomic sequences present at the integration site before transformation.

Determination of the wildtype target locus sequence was performed using DNA isolated from homozygous (BC_2F_5) transgenic and control cotton DNA (see Figure 3). A flanking DNA specific primer upstream of the T-DNA insert was used together with a flanking DNA specific primer downstream of the T-DNA insert to amplify the target (insertion) site in the non-transgenic cotton. A 947 bp segment was generated and sequenced. The obtained sequence was aligned with the 5' and 3' flanking sequences in cotton even GHB614. A fragment of 17 bp is present in the non-transgenic cotton but not in GHB614 at the transgene locus. Therefore, this short fragment was deleted at the insertion site upon integration of the T-DNA from pTEM2.

3.4.3 Bioinformatic analysis of the 5' and 3' junction regions

Bioinformatic analyses were performed on the junction regions between plant genomic and inserted DNA in GHB614 cotton, to ascertain whether any known cotton genes were interrupted by insertion of the transgene and whether putative polypeptides encoded by the 5' and 3' junction regions were likely to be expressed. These analyses were entirely theoretical, but were conducted to exclude the possibility that chimeric proteins would be produced in GHB614 cotton as a result of the transformation.

Studies submitted:
1. Bioinformatics analysis of newly created ORFs from GlyTol cotton transformation event GHB614, N. Vandermarliere and K. De Pestel; completed August 2007.
Report No: 2006-GHB614-EPC-018
2. Bioinformatics analysis of the pre-insertion locus of *Gossypium hirsutum* transformation event GHB614, V. Habex and S. Tanghe; completed May 2007.
Report No: 2006-GHB614-NAC004
3. GlyTol Cotton Elite Event GHB614 (Glyphosate-tolerant cotton) *In silico* analysis of putative Open Reading Frame (ORF) sequences for identifying potential homologies to known toxins and allergens, Junguo Zhou and C. Herouet-Guicheney; completed November 2006.

To identify the presence of endogenous genes located near the 5' and 3' junction regions in GHB614 cotton, a BLASTn similarity search was performed (version 2.0, National Centre for Biotechnology Information, NCIB). The BLASTn similarity search compares a specific query nucleotide sequence with sequences in nucleotide databases.

The results show no homology of the flanking DNA regions in GHB614 cotton with known cotton genes, mRNA, cDNA or ESTs present in the databases used.

Open reading frame (ORF) analysis and gene search tools were applied to predict the presence of any newly created coding sequences in the 5' flanking genomic/insert DNA junction region and in the 3' flanking insert/genomic DNA junction region. The ORFs were defined as regions between start (ATG) and stop (TAA, TAG, TGA) translation codons with a minimum size of eight amino acids (corresponding to twenty-four nucleotides, not including stop codon). In all cases, all six reading frames were examined.

Two ORFs were found across the 5' region. Several bioinformatics tools were applied to look for regulatory elements such as core promoters, polyadenylation (polyA) signals and ribosome binding sites (RBS) to gauge whether these identified ORFs could be putatively active. No ORFs or genes were found across the 3' region.

The findings relating to the deduced ORFs at the 5' junction region were[4]:

- ORF-1 (sense strand): a CAAT-box, a potential polyA signal and RBS were found. No homology was found with a TATA-box.

- ORF-2 (anti-sense strand): homology was found with a CAAT-box and a polyA signal. No homology was found with the TATA-box and RBS.

The Applicant claims that the sequence similarities with certain regulatory elements are not sufficient indication of newly created, functional ORFs. The absence of a TATA-box in ORFs 1 and 2 and no ribosome binding site in ORF-2 would indicate that these ORFs are not active either at the level of transcription or translation and therefore the probability of expression of a newly created peptide due to insertion of the transgene is remote.

The putative polypeptides from each ORF identified in GHB614 cotton were subjected to detailed *in silico* analysis to evaluate homology with known toxins or allergens contained in a number of large, publicly available databases, including the updated Uniprot-Swissprot, Uniprot-trEMBL, PIR, DAD, Nrl-3d, GenPept and Allergen databases, using FindPatterns or BLASTP algorithms. The overall structural similarity of the putative polypeptides to sequences in each database was assessed. The extent of structural relatedness was evaluated using visual inspection of the aligned sequences, the calculated percent identity over a linear contiguous eight amino acid segment, and *E* (expectation) score. The *E* score is a statistical measure of the likelihood that the observed similarity could have occurred by chance. A larger *E* score indicates a lower degree of similarity. Typically, alignments between two sequences will need to have an *E* score of less than 1×10^{-5} to be considered to have significant homology. Based on this global analysis, the putative ORF-1 and ORF-2 amino acid sequences showed no biologically relevant identities with known toxins or allergens.

3.4.4 Conclusion

Detailed molecular analyses indicate that one functional copy of the *2mepsps* gene expression cassette has been inserted at a single genomic locus in GHB614 cotton.

[4] The transcription complex recognises the CAAT-box and initiation of transcription starts at the TATA-box. The presence of a polyA signal sequence at the 3' end of an ORF results in the addition of a polyA tail which protects the mRNA from degradation. Ribosome binding at the RBS is necessary for initiation of translation.

The regulatory elements and coding region of the inserted gene are intact and no additions, deletions or gene rearrangements within the T-DNA are present in GHB614 cotton.

3.5 Stability of the genetic changes

A number of analyses were done to demonstrate the stability of the genetic changes in GHB614 cotton. Segregation analysis over multiple generations was done to determine the heritability and stability of the new trait (the *2mepsps* gene) and Southern blot analysis over multiple generations was done to determine the stability of the inserted DNA. Polymerase chain reaction (PCR) testing was used to verify the event.

3.5.1 Segregation analyses

Following the transformation, T_1 seed harvested from self-pollinated T_0 plants surviving a glyphosate herbicide greenhouse screen were planted in the greenhouse for seed increase and evaluation. Resistance screenings were done on subsequent generations in the greenhouse using glyphosate at the 1X rate, to identify segregating seed lots. PCR based analysis was also performed as a secondary means of identifying homozygous plants with the *2mepsps* trait. Selfed T_3 homozygous seed was used to produce homozygous T_4 seed which was the source of the lines used in early event-specific agronomic and stability studies.

For breeding purposes and further evaluation of inheritance, various backcrosses were performed and evaluated in the greenhouse for segregation and glyphosate resistance. Mendelian inheritance for a single gene locus would predict one resistant plant for every one susceptible plant within BC_2F_1 progenies. Furthermore, BC_2F_2 progeny would be expected to show three resistant plants for every one susceptible plant (see Figure 3 and Table 1).

For the segregation analysis, data from a Chi-square test of inheritance were used to determine the heritability and stability of the new trait. The Chi-square test is based on testing the observed segregation ratio of glyphosate resistant plants to the ratio that is expected according to Mendelian principles. All Chi-square values indicate no significant differences between observed and expected genetic ratios across all tested generations of GHB614 cotton. These results are consistent with a single site of insertion for the *2mepsps* gene expression cassette.

Table 1: Segregation Analysis of GHB614 Cotton

Parents and zygosity for the *2mepsps* locus	Generation	Ratio	Observed		Expected		χ^2 calculated[a]
		R:S	R	S	R	S	
Hemizygous "F_1" plant crossed with conventional line B (*2mepsps*/-)x(-/-)	BC_1F_1	1:1	9	12	10.5	10.5	0.43
Hemizygous BC_1F_1 plant crossed with conventional line B (*2mepsps*/-)x(-/-)	BC_2F_1	1:1	11	6	8.5	8.5	1.47
Hemizygous BC_2F_1 plant (conventional line A), self-pollinated (*2mepsps*/-)x(*2mepsps*/-)	BC_2F_2	3:1	28[b]	8	27	9	0.15
Hemizygous BC_2F_2 plant crossed with conventional line B (*2mepsps*/-)x(-/-)	"F_1" population[c]	1:1	7	9	8	8	0.25
Self-pollinated hemizygous "F_1" plants (*2mepsps*/-)x(*2mepsps*/-)	"F_2" populations (pooled)	3:1	113	43	117	39	0.60

[a] assumes a one locus model. There was no significant difference for the χ square goodness-of-fit test for the hypothesis of one locus. To reject the null hypothesis, the χ square value must be greater than 3.84, with one degree of freedom.

[b] tested by homozygosity PCR (19 heterozygous and 9 homozygous plants).

[c] all F1 population material was generated using a hemizygous transgene donor source (BC2F1).

S = susceptible; R = resistant to glyphosate

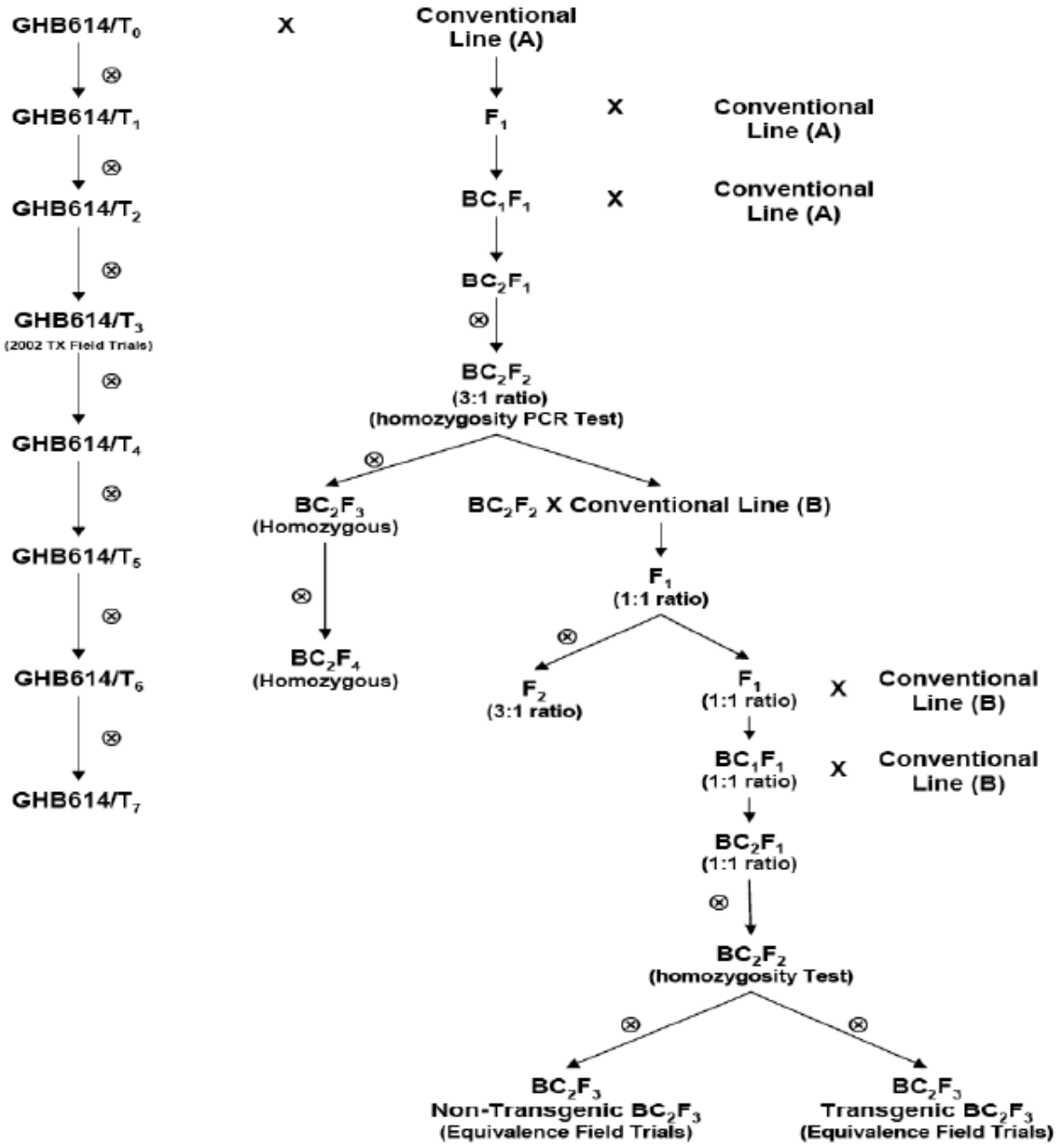

Figure 3: Breeding tree for the development and evaluation of GHB614 cotton

- At each generation, plants were sprayed with glyphosate to eliminate those not expressing the 2mepsps gene
- ⊗ = self cross
- Generation BC_2F_4 (homozygous) was used for detailed insert characterization and protein expression levels.
- Generations T_3, T_4, T_5, T_6 and BC_2F_2 were used for molecular stability analysis.
- Generation T_5 was used for seed composition analysis.
- Generations T_5 and BC_2F_3 were used for replicated agronomic field tests.
- Generation T_7 was used for analyses on absence/presence of vector backbone sequences.

3.5.2 Stability of the inserted DNA

Study submitted:
1. Structural stability analysis of *Gossypium hirsutum* transformation event GHB614, V. Habex; completed October 2006. Report No: 2006-GHB614-NAC005

To determine the stability of the inserted DNA, Southern blot analyses were done using genomic DNA isolated from multiple generations of GHB614 cotton (T3, T4, T5 and T6, see Figure 3 for the specific generations used). For these analyses, DNA samples from leaf tissue representing each generation were digested and probed to detect two integration fragments corresponding to ~4850 bp and ~9100 bp. In all tested samples, the expected 5' and 3' integration fragments were present. These results are consistent with bands detected in other Southern analyses of GHB614 cotton and confirm the stability of the insert across multiple generations of breeding.

3.5.3 Conclusion

The results of the segregation analysis are consistent with a single site of insertion for the *2mepsps* gene expression cassette and the results of the molecular characterization studies. Phenotypic and molecular analyses of breeding lines over multiple generations indicate that the inserted DNA is stably transformed and inherited as a single locus from one generation to the next.

3.6 Presence of antibiotic resistance genes

No genes that encode resistance to antibiotics are present in the genome of GHB614 cotton. The molecular characterisation confirmed the absence of both the *aad* gene and *nptI* fragment, which were present in the plasmid backbone outside of the T-DNA (region between the Left and Right border sequences).

4. CHARACTERISATION OF THE NOVEL PROTEIN

In considering the safety of novel proteins it is important to consider that a large and diverse range of proteins are ingested as part of the normal human diet without any adverse effects, although a small number have the potential to impair health, e.g. because they are allergens or anti-nutrients. As proteins perform a wide variety of functions, different possible effects have to be considered during the safety assessment including potential toxic, anti-nutritional and allergenic effects. To effectively identify any potential hazards requires knowledge of the characteristics, concentration and localisation of all novel proteins expressed in the organism as well as a detailed understanding of their biochemical function and phenotypic effects. It is also important to determine if the novel protein is expressed as expected, including whether any post-translational modifications have occurred.

4.1 Description and function of novel protein

Studies submitted:
1. The double mutant 5-enolpyruvylshikimate-3-phosphate synthase gene product: 2mEPSPS Description and Characterisation, R-J. van der Klis, K. Hendrickx, C. Herouet-Guicheney and D. Rouan; completed September 2006. Report ID: 2006-2mEPSPS-EPC002

Cotton line GHB614 expresses one novel protein, 2mEPSPS, a modified form of the EPSPS protein naturally occurring in corn. A number of different analyses were done to determine the identity, physiochemical properties, *in planta* expression, bioactivity and potential toxicity and allergenicity of the modified protein. Because the expression of a novel protein *in planta* is usually too low to allow purification of sufficient quantities for use in safety assessment studies, a bacterial expression system was used to generate larger quantities of the 2MEPSPS protein for safety assessment.

The 2mEPSPS protein produced in *E. coli* was engineered so its amino acid sequence matched that of the plant-produced 2mEPSPS protein. The equivalence of the bacterial-produced protein to the plant-produced protein was determined as part of the protein characterisation.

4.1.1 Mode of action of glyphosate on EPSPS proteins

Glyphosate acts as a herbicide by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). This endogenous enzyme is involved in the shikimate pathway for aromatic amino acid biosynthesis which occurs exclusively in plants and microorganisms, including fungi. Inhibition of the wildtype EPSPS enzyme by glyphosate leads to deficiencies in aromatic amino acids in plant cells and eventually to the death of the whole plant. The shikimate biochemical pathway is not present in animals. For this reason, enzymes of the shikimate pathway have been considered as potential targets for essentially non-toxic herbicides (such as glyphosate) and antimicrobial compounds.

Naturally occurring EPSPS proteins are widespread in nature and have been extensively studied over a period of more than thirty years. The *epsps* gene from maize has been completely sequenced and it encodes a 47 kDa protein consisting of 445 amino acids. The modified 2mEPSPS protein present in GHB614 cotton differs from the wildtype maize enzyme by two amino acid substitutions – threonine replaced by isoleucine at position 102, and proline replaced by serine at position 106. These two amino acid changes result in a protein with greater than 99.5% identity to the native maize EPSPS protein, however the modified protein is highly tolerant to glyphosate. Plants expressing the modified maize enzyme therefore are able to continue to function adequately in the presence of the herbicide.

4.1.2 2mEPSPS activity

For the purposes of conferring tolerance to glyphosate, variants of the naturally occurring EPSPS enzyme would ideally exhibit no alteration in affinity for natural substrates (K_{cat} and K_m unchanged) but would have at least 10-fold enhancement of the K_i for glyphosate. That is, the aim was to identify a modification in the enzyme that would result in a significantly reduced affinity for glyphosate (an amino acid analog) while retaining affinity for the cellular substrates (shikimate-3-phosphate and phosphoenolpyruvate, PEP). In addition, to obtain adequate tolerance levels to glyphosate in plants, the modified protein should be targeted to the chloroplasts where the shikimate pathway is normally functional.

Site directed mutagenesis of the wildtype *epsps* gene from maize produced the double mutant enzyme 2mEPSPS which carries two amino acid changes. When fused to a chimeric optimized chloroplast transit peptide, the 2mEPSPS enzyme is reported to generate optimal glyphosate tolerance in crops (Lebrun *et al.* 1997a). A methionine codon was added to the amino-terminal end of the mature 2mEPSPS protein sequence to restore the cleavage site of

the transit peptide. With the addition of the methionine residue, the mutations are at positions 103 (Thr to Ile) and 107 (Pro to Ser) of the mature protein (445 amino acids).

Kinetic and enzyme activity analyses indicate that the 2mEPSPS enzyme interacts with the normal EPSPS substrates, shikimate-3-phosphate and phosphoenolpyruvate, similarly to the wildtype corn EPSPS enzyme. Biochemical analyses comparing the 2mEPSPS and wildtype EPSPS enzymes also show that:

(i) the specific activities of the enzymes at 25º C and pH 7 are 5.2 and 11.8 U/mg respectively;
(ii) optimal pH activities of the enzymes are between pH 5.5 to 7.5 and pH 7 to 7.5 respectively;
(iii) the activities of both enzymes increase linearly to approximately 60º C, then decrease sharply and at 75º C appear to be inactive;
(iv) the double mutant form of the enzyme appears to be significantly more active at elevated temperatures compared to the wildtype, however 2mEPSPS is inactivated after 10 minutes at 60º C; and
(v) cations and anions have minor but comparable effects on respective enzyme activity.

On sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), the 2mEPSPS and wildtype enzymes co-migrated with the same apparent molecular mass of 47 kDa. Western blot analysis of wildtype and modified proteins revealed a single cross-reacting polypeptide corresponding to the same molecular mass for both enzymes.

4.2 Protein characterisation

Study submitted:
1. Structural and Functional Equivalence of 2mEPSPS protein produced in *Escherichia coli* and GHB614 cotton, *Gossypium hirsutum*, T. Currier and K. Hendrickx; completed November 2006. Study ID: DQ06Q003

The 2mEPSPS protein and wildtype protein were both expressed in *E. coli* and purified to allow a direct comparison of their enzymatic properties (section *4.1.2* above). A range of analytical techniques was then used to determine the identity as well as the physicochemical and functional properties of the plant-produced 2mEPSPS protein isolated from cotton line GHB614 compared with the *E. coli*-produced form (Table 2). The *E. coli*-produced protein was used as a reference standard for these analyses.

Table 2: Criteria and methodologies for demonstrating equivalence between the microbially-produced and plant-produced protein in GHB614 cotton

Equivalence criteria	Methodology
Confirm identity of 2mEPSPS protein	Edman degradation
Comparable immunoreactivity	Western blot analysis
Comparable molecular mass	Mobility in SDS-PAGE
Comparable peptide masses	HPLC/Electrospray Mass Spectrometry (LC/MS) of peptides
Glycosylation profile	Staining SDS-PAGE for glycoproteins
Comparable biological activity	Enzyme activity assay

4.2.1 Characterisation of plant-produced 2mEPSPS

The 2mEPSPS protein was purified from frozen leaves of cotton line GHB614 using a combination of filtration and immunoaffinity chromatography with a covalently attached monoclonal antibody to 2mEPSPS.

Protein identity

The identity of the plant-produced 2mEPSPS was confirmed by Western blot analysis, N-terminal peptide sequencing, SDS-PAGE analysis and HPLC/Electrospray Mass Spectrometry:

(i) Western blot analysis used a monoclonal antibody to the 2mEPSPS protein. The results showed that the electrophoretic mobilities and immunoreactivities of the 2mEPSPS protein produced in *E. coli* and GHB614 cotton plants are indistinguishable.

(ii) The N-terminal amino acid sequence of the 2mEPSPS protein isolated from GHB614 cotton leaves was determined by Edman degradation. The theoretical N-terminal sequence of the 2mEPSPS protein deduced from the gene sequence is: methionine, alanine, glycine, alanine, glutamic acid, glutamic acid and isoleucine. Apart from the terminal methionine residue, the primary N-terminal amino acid residues of the 2mEPSPS from GHB614 cotton exactly matched the theoretical sequence. This result also shows that the N-terminal methionine is missing from the plant-produced 2mEPSPS protein, however this is a common finding in protein sequencing.

(iii) The microbially-produced and GHB614 cotton-produced 2mEPSPS proteins were analysed by SDS-PAGE. Following staining of the gel, the electrophoretic mobility of the protein from the two different sources was the same, and indicated an equivalent molecular weight of approximately 42 kDa (compared with the theoretical molecular weight of 47 kDa calculated from the amino acid sequence). The SDS-PAGE also showed that the protein preparations were highly pure.

(iv) Peptides from a tryptic digest of the microbially-produced 2mEPSPS protein were separated by HPLC and subsequently analysed by electrospray mass spectrometry. Expected peptides from the microbially produced 2mEPSPS protein were identified by SIM with 93% coverage of the 445 amino acids comprising the protein. The ability to identify a protein using this method is dependent on matching a sufficient number of observed tryptic mass fragments to expected (theoretical) mass fragments. The most abundant ion for each peptide from the *E. coli* 2mEPSPS protein was chosen for selected ion monitoring of the peptides produced by tryptic digestion of the 2mEPSPS protein isolated from GHB614 cotton. Peptides from the microbially-produced 2mEPSPS protein were identified in the 2mEPSPS protein from GHB614 cotton with coverage of 91.5% of the protein. The data showed that the calculated masses for the detected peptides from both proteins were identical, which confirms the equivalence of the microbially-produced and plant-produced 2mEPSPS proteins.

Glycosylation analysis

Glycoprotein staining was used to assess whether post-translational glycosylation of the plant-produced 2mEPSPS protein was present. As prokaryotic organisms lack the capacity for protein glycosylation, the *E. coli*-produced 2mEPSPS protein would not be expected to yield a positive result in this analysis. Standard control proteins consisted of a mixture of glycosylated and non-glycosylated proteins. Only the glycosylated standard proteins showed a strong signal with the glycoprotein stain; both the *E. coli*- and plant-produced 2mEPSPS were barely detectable. This analysis indicates the absence of glycosylation in the 2mEPSPS protein from GHB614 cotton and confirms that it is equivalent to the *E. coli*-produced protein in terms of its lack of glycosylation.

Enzyme assay

In the shikimate pathway in plants, chorismate is formed via seven enzymatic steps. The reaction catalysed by EPSPS is the reversible transfer of the enolpyruvyl moiety of phosphoenolpyruvate (PEP) to shikimate-3-phosphate, leading to formation of 5-enolpyruvyl-3-shikimate phosphate (EPSP) and the release of inorganic phosphate. The EPSPS activity assay can be measured according to a published colorimetric method described in Forlani *et al* (1994).

The enzymatic activity of the purified 2mEPSPS protein preparations from *E. coli* and GHB614 cotton leaves was measured in the forward direction using shikimate-3-phosphate and PEP as substrates. The amount of inorganic phosphate released during the reaction was determined using the malachite green dye method, with minor modifications. Both protein preparations generated free phosphate, indicating that 2mEPSPS from either the microbial or plant source showed the expected biological activity. This result confirms that the proteins from the two sources were present in the correct conformation.

4.2.3 Conclusion

Numerous studies have been conducted on the 2mEPSPS protein to confirm its identity and physicochemical and functional properties as well as to determine its equivalence to *E. coli*-produced 2mEPSPS. These studies have demonstrated that the novel protein expressed in GHB614 cotton conforms in size and amino acid sequence to that expected and also exhibits the expected enzymatic activity. The *E. coli*-produced protein was also shown to be equivalent to the plant-produced protein in terms of size, amino acid sequence, physicochemical properties, and enzyme activity. The *E. coli*-produced 2mEPSPS protein was therefore a valid substitute for the plant-produced protein for safety assessment purposes.

4.3 Protein expression levels

4.3.1 Protein expression in greenhouse grown cotton

Studies submitted:
1. 2mEPSPS protein content in leaf, stem, root, square, apex and pollen tissues during the life cycle of the glyphosate-tolerant cotton event GHB614, R-J van der Klis and K. De Pestel; completed October 2006. Report No. 2006-GHB614-EPC-017

A validated Enzyme Linked Immunosorbent Assay (ELISA) method was used to quantify the levels of the 2mEPSPS protein in tissues from GHB614 cotton grown in the greenhouses of Bayer BioScience N.V. (Astene, Belgium). Seeds were planted and at the 1-2 leaf stage (V1-V2), the transgenic plants were sprayed with glyphosate herbicide (0.7% glyphosate and 50 ml/square metre). The non-transgenic parental line was grown in the greenhouses at the same time as the test plants.

Samples from 6 different tissues of cotton line GHB614 were harvested separately covering four different growth stages of the plant (samples collected at 16, 33, 51 and 68 days after planting). Transgenic and non-transgenic plants were chosen randomly out of a starting population of 240 plants. In the first growth stage (V2-V3) leaf specimens from 15 plants were taken; in the other growth stages separate specimens per tissue were harvested from 10 plants. In the first growth stage (V2-V3) and the third growth stage (pre-flowering), young leaf tissue was sampled.

In the second and fourth growth stages (V4-V6 and flowering) stem, root and young leaf tissues were sampled. In the fourth growth stage also square, apex and pollen tissues were sampled. Identical samples were taken from the control cotton line.

In order to analyse the presence of 2mEPSPS protein in these tissues, samples were crushed, extracted and the Total Extractable Protein (TEP) content was determined using the Bradford method (Bradford, 1976). The amount of 2mEPSPS in the total protein extracts was measured using a quantitative ELISA developed by Strategic Diagnostics Inc. (SDI, Newark, DE, USA). However, this 2mEPSPS ELISA also detects traces of endogenous EPSPS protein in plants. To measure the sensitivity of the ELISA, the limit of detection (LOD) was determined for each tissue before the analysis was performed (Table 3). The LOD was defined per tissue as the concentration of (2m)EPSPS protein producing an absorbance that is statistically different from the background absorbance of the non-transgenic cotton line. The LOD was expressed as the concentration of 2mEPSPS per unit of fresh weight (µg/g).

Monoclonal antibodies to 2mEPSPS (produced in bacteria) were used as the capture antibodies; polyclonal detection antibodies were linked to a horseradish peroxidase conjugate. All samples were analysed in duplicate.

Table 3: LOD for the 2mEPSPS protein ELISA in different cotton plant tissues

Tissue	LOD $x10^{-3}$ µg/g
Leaf	4.47
Stem	8.34
Root	27.3
Square	27.3
Apex	8.10
Pollen	16.1

The levels of 2mEPSPS protein in the various tissues obtained from glasshouse-grown GHB614 cotton plants are summarised in Table 4. The protein was detected in all plant tissues. In leaf tissue, the levels of 2mEPSPS decreased over time, whereas the levels in stem tissue remained constant.

Table 4: Average 2mEPSPS protein content in different plant tissues of GHB614 cotton grown in the glasshouse

	Average 2mEPSPS protein levels in GHB614 cotton tissues µg/g fresh weight ± SD			
Tissue Type	**Growth Stage 1**	**Growth Stage 2**	**Growth Stage 3**	**Growth Stage 4**
Leaf	11.16 ± 3.73	7.94 ± 2.87	6.52 ± 7.20	0.45 ± 0.22
Stem	ND	1.94 ± 0.61	ND	1.58 ± 0.96
Root	ND	0.99 ± 1.00	ND	4.04 ± 1.71
Square	NA	NA	NA	5.35 ± 0.25
Apex	ND	ND	ND	5.47 ± 0.22

Tissue Type	Average 2mEPSPS protein levels in GHB614 cotton tissues µg/g fresh weight ± SD			
	Growth Stage 1	Growth Stage 2	Growth Stage 3	Growth Stage 4
Pollen	NA	NA	NA	0.16 ± 0.01

ND: not determined; NA: not applicable

4.3.2 Protein expression in field-grown cotton

Studies submitted:
1. Production of RAC (Fuzzy Seed) Samples of GlyTol Cotton and the Non-transgenic Counterpart, USA, 2005, W.J. Kowite; completed October 2006. Study No. DQ05B001
2. Analyses of Raw Agricultural Commodity (Fuzzy Seed) of Cotton GHB614 for 2mEPSPS Protein, USA, 2005, T.C. Currier; completed October 2006. Study No. DQ06Q002
3. Residue Analysis of GlyTol Cotton Processed Fractions, USA, 2006, W.J. Kowite; completed November 2006. Study No. DQ06Q005

The purpose of these studies was to determine the amounts of 2mEPSPS protein in fuzzy cottonseed of transgenic line GHB614, grown in field trials under agricultural conditions typical of the commercial cultivation of cotton. GHB614 cotton and its non-transgenic parental line (Coker 312) were grown within individual plots established at each of nine field trial sites in southern USA. At each site, six plots were planted with transgenic cotton and three plots planted with the non-transgenic control. Three of the transgenic plots were sprayed three times with glyphosate herbicide at the level of 840 g/hectare, and three transgenic plots were untreated.

Because the cottonseed (fuzzy seed) had been ginned but not delinted, it could not be ground into a homogeneous material. A procedure was developed to effectively remove the lint and the associated seed coat. This created two fractions, which were designated ‘kernel’ and ‘lint coat’. The kernel could be easily ground to homogeneity; the lint coat fraction was a relatively homogeneous matrix of intertwined cotton fibres and broken fragments of seed coat. These fractions were analysed separately for 2mEPSPS protein and total extractable protein and the respective values added to give values for the fuzzy seed as received from the field.

The 2mEPSPS protein was found in all fractions of transgenic fuzzy seed (kernel and lint coat). As expected, more than 99.5% of the novel protein was found in the kernel samples. The lint coat generally contained less than 0.5% of the 2mEPSPS protein, and some samples were below the limit of detection. The levels of 2mEPSPS protein varied between different trial sites and between treatments with glyphosate. On a fresh weight basis, the 2mEPSPS protein content in fuzzy seed of GHB614 cotton, not sprayed with glyphosate, ranged from about 15.8 µg/g to 25.5 µg/g fresh weight, with an overall average value of 19.2 ±3.1 µg/g. On a fresh weight basis, the fuzzy seed from GHB614 cotton plants, sprayed with a conventional herbicide regime, contained 2mEPSPS protein in the range 16.2 µg/g to 30.5 µg/g, with an overall average value of 21.2 ± 4.0 µg/g. Using the average values for the amount of novel protein in unsprayed and sprayed fuzzy seed relative to the amount of crude protein, the 2mEPSPS protein comprised an average of 0.0093% ± 0.0018% and 0.0100% ± 0.0019% of the total crude protein respectively.

In a separate field study, cotton line GHB614 and the conventional line Coker 312 were grown under typical agricultural conditions to evaluate the levels of the novel protein 2mEPSPS in eight fractionated agricultural products of cottonseed. The transgenic plot was sprayed three times with glyphosate herbicide equivalent to 0.75 pounds active ingredient per acre. The results of these analyses are presented in Table 5.

Table 5: Levels of 2mEPSPS protein in fuzzy seed and processed fractions of cotton line GHB614 as detected by ELISA

Sample	Average 2mEPSPS protein levels in tissues µg/g fresh weight ± SD	
	GHB614 treated with glyphosate	Conventional Coker 312
Kernel	16.4 ± 3.1	ND
Lint coat	0.67 ± 0.24	ND
Fuzzy seed	6.99	ND
Lint	ND	ND
Linters	ND	ND
Delinted seed	102 ± 2	ND
Seed hulls	6.93 ± 0.40	ND
Meal	0.26 ± 0.10	ND
Toasted meal	ND	ND
Crude oil	ND	ND
Refined, bleached, deodorized oil	ND	ND

ND: not detected

These results show that 2mEPSPS protein was not at detectable levels in cottonseed lint, toasted meal, crude oil and refined/bleached/ deodorised oil. The highest levels of 2mEPSPS occurred in delinted seeds. The amount of 2mEPSPS protein was greatly reduced by processing delinted seeds into meal and toasted meal.

4.4 Potential toxicity of novel proteins

While the vast majority of proteins ingested as part of the diet are not typically associated with toxic effects, a small number may be harmful to health. Therefore, if a GM food differs from its conventional counterpart by the presence of one or more novel proteins, these proteins should be assessed for their potential toxicity. The main purpose of an assessment of potential toxicity is to establish whether the novel protein will behave like any other dietary protein, based on a weight of evidence approach. The assessment focuses on: whether the novel protein has a prior history of safe human consumption, or is sufficiently similar to proteins that have been safely consumed in food; amino acid sequence similarity with known protein toxins and anti-nutrients; and structural properties of the novel protein including whether it is resistant to heat or processing and/or digestion.

Appropriate oral toxicity studies in animals may also be considered, particularly where results from the biochemical, bioinformatic, digestibility or stability studies indicate a reason for further investigation.

4.4.1 History of use

EPSPS enzymes

EPSPS is the sixth enzyme in the shikimate pathway, the metabolic pathway for the biosynthesis of aromatic compounds found in plants and in microorganisms (bacteria and fungi). As such, EPSPS enzymes are ubiquitous in nature and are present in foods derived from all plant and microbial sources. Although differences in amino acid sequence occur naturally, depending on the source of the enzyme, it is apparent that this family of proteins has a long history of safe use as normal constituents of human food and animal feed.

The 2mEPSPS enzyme shows a high amino acid sequence identity to the naturally occurring EPSPS from maize (>99.5%), and to other EPSPS enzymes found in crops with a similarly long history of human consumption (e.g. rice 86%, grape 79%, lettuce 77%, tomato 75% and oilseed rape 75%) or in microbial food sources such as baker's yeast. The EPSPS enzymes present in these and other plant- or microbially-derived foods are all commonly consumed proteins within a normal human diet, and are not associated with any adverse health effects.

The 2mEPSPS has been used in other crops previously assessed by FSANZ. Food derived from glyphosate-tolerant corn line GA21 was approved in Australia and New Zealand in 2000 (Application A362). Corn line GA21 expressing the 2mEPSPS protein was developed in the 1990's and has been assessed and approved in other countries including Japan, Canada, European Union, Korea, Mexico, Argentina, South Africa, China, Taiwan and the USA. It is therefore likely to have been widely distributed in corn based foods.

4.4.2 Similarities with known protein toxins

The complete amino acid sequence of the 2mEPSPS protein expressed in GHB614 cotton is known from the molecular characterization studies. Bioinformatic analyses were done to assess the 2mEPSPS enzyme for any amino acid sequence similarity with known protein

toxins. This *in silico* study was carried out by comparing the complete sequence of 445 amino acids of the 2mEPSPS protein with all protein sequences present in the following large reference databases: Uniprot_Swissprot, Uniprot_ TrEMBL, PIR, NRL-3D, DAD and GenPept. Using the BLASTP (Standard Protein-protein Basic Local Alignment Search Tool) program, the key indicator for this study was a 35% identity with a known protein toxin over a window of 80 amino acids.

The extent of similarity was evaluated using visual inspection of the aligned sequences, the calculated percent identity, and *E* score. The *E* score reflects the degree of amino acid similarity between a pair of sequences and can be used to evaluate the significance of the alignment. A larger *E* score indicates a lower degree of similarity. Typically, alignments between two sequences will need to have an *E* score of less than 1×10^{-5} to be considered as significant homology.

The results of the overall homology search with the 2mEPSPS protein showed no amino acid identity with known toxins. As expected, the search revealed homology only with other EPSPS proteins from various sources. These analyses did not demonstrate any significant similarity between the 2mEPSPS protein and other proteins that may potentially be toxic to humans or other animals.

4.4.3 Acute oral toxicity studies

To examine the potential toxicity of the 2mEPSPS protein expressed in GHB614 cotton, several acute oral toxicity studies using purified 2mEPSPS protein have been conducted.

In a previously evaluated study, the modified EPSPS (2mEPSPS) protein was administered by a single oral gavage dose to ten male and ten female CD-1 mice, at target doses of 5, 15 and 50 mg/kg bodyweight. In this study, this corresponded to actual doses of 3.7, 11.8 and 45.6 mg/kg respectively. A control group of ten mice/sex was administered only the carrier substance without 2mEPSPS. An additional control group of ten mice/sex was administered Bovine Serum Albumin (BSA) in the same carrier substance at the highest target dose (50 mg/kg). At defined stages throughout the duration of the study, clinical observations were performed for mortality and signs of toxicity, and body weights and food consumption measured. At the termination of the study (day 13-14), animals were sacrificed, examined for gross pathology and numerous tissues were collected.

The results of the study showed no statistically significant differences in group mean body weights, cumulative weight gains or food consumption in either males or females at any level of either the BSA control or test material, when compared with the respective carrier control group. All animals survived to the end of the study, and there were no clinical signs observed that could be related to the test material. A unilateral corneal opacity was noted in one male mouse at the high dose level of the test material, but this finding was not considered to be treatment related. The study concluded that there was no evidence of toxicity in mice following a single oral dose of 45.6 mg/kg modified EPSPS (2mEPSPS) protein.

In a more recent study, the 2mEPSPS protein was administered by a single oral gavage dose of 2000 mg protein/kg bodyweight to 5 female OF1 mice. A second group of female mice received the same dose of bovine serum albumin as a negative control. All animals were observed for clinical signs daily for fifteen days and body weights were measured weekly. At termination, all animals were subjected to necropsy including macroscopic examination.

There were no clinical signs, mortalities or treatment related effects on bodyweight in female OF1 mice observed during this study. Based on these findings, it was concluded that no oral toxicity was demonstrated in mice at a very high dose of 2000 mg/kg bodyweight.

4.5 Potential allergenicity of novel proteins

The potential allergenicity of novel proteins is evaluated using an integrated, step-wise, case-by-case approach relying on various criteria used in combination, since no single criterion is sufficiently predictive of either allergenicity or non-allergenicity.

The assessment focuses on: the source of the novel protein; any significant amino acid sequence similarity between the novel protein and known allergens; the structural properties of the novel protein, including susceptibility to digestion, heat stability and/or enzymatic treatment; and specific serum screening if the novel protein is derived from a source known to be allergenic or has amino acid sequence similarity with a known allergen. Applying this approach systematically provides reasonable evidence about the potential of the novel protein to act as an allergen.

4.5.1 Source of novel protein

The source of the 2mEPSPS enzyme present in GHB614 cotton is the maize EPSPS enzyme with two defined amino acid changes that reduce the binding and inactivation of the enzyme by glyphosate. Corn is not regarded as a major food allergen. In addition, humans have been exposed to a suite of EPSPS enzymes through a normal diet containing plants and edible microorganisms. Consumption of a large number of EPSPS enzymes with similar function but different degrees of amino acid homology is therefore usual for humans, and has never been identified with food allergenicity.

4.5.2 Similarity to known allergens

The bioinformatic analyses described above in *4.4.2* also assessed whether the 2mEPSPS protein demonstrated any overall amino acid sequence similarity with known allergens, gliadins or glutenins. Further bioinformatic analysis was applied to the sequence to identify the presence of potential epitope homology by comparing the amino acid sequence of the 2mEPSPS protein, subdivided into 8 amino acid blocks, with known allergens compiled in a large reference database.

The Allergen database of 1433 sequences (release 3.2, 19 April 2006) was built by assembling relevant allergens described in the five large protein databases used in the previous bioinformatic study. The criterion indicating potential allergenicity was a 100% identity with an allergenic protein using a sliding window of eight contiguous amino acids. Segments of eight amino acids were chosen because this is considered to be the smallest number of amino acids that will identify immunologically relevant matches. Searches using smaller segments (e.g. 6 or 7 amino acids) lead to high rates of false positive matches and therefore have little predictive value. In this study, the algorithm used for identifying epitope homology was FindPatterns (GCG package).

No identity between the 2mEPSPS protein analysed in this way and known allergens was identified. These sequence homology searches establish that the 2mEPSPS protein does not share any theoretical structural similarity with known allergenic proteins.

4.5.3 Potential glycosylation sites

Glycosylation of proteins is known to promote proper protein folding and confer enhanced protein stability, particularly for proteins secreted from the cell or associated with membranes. Glycosylation is also associated with many allergenic proteins. Consideration of potential glycosylation sites using an *in silico* approach may therefore be useful for considering the potential allergenicity of novel proteins for which the patterns of N-glycosylation may differ from their wildtype counterpart.

The bioinformatics study of the 2mEPSPS protein for assessment of potential toxicity and allergenicity also considered the potential N-glycosylation sites in the protein by searching for a described consensus sequence as found in known allergenic proteins. Using this approach, two potential glycosylation sites (at amino acid positions 118 and 394) were identified in the 2mEPSPS sequence. Both of these potential sites are downstream from the two amino acid changes introduced into the maize EPSPS protein at amino acid positions 102 and 106. As neither of these substitutions is within the potential glycosylation sites, the N-glycosylation profile of the 2mEPSPS protein can reasonably be expected to be identical to that of the naturally occurring wildtype EPSPS enzyme.

Furthermore, the 2mEPSPS protein is specifically targeted to the chloroplast where the shikimate biochemical pathway operates in plant cells. It is widely accepted that nuclear-encoded proteins destined for intracellular compartments are not glycosylated in plants. This information provides further weight to the *in silico* analyses indicating that 2mEPSPS in GHB614 cotton is not glycosylated.

4.5.4 Digestibility

Studies submitted:
1. 2mEPSPS Protein – *In vitro* Digestibility Study In Simulated Gastric Fluid, D. Rouquie; completed August 2006. Report of Study SA06101.
2. 2mEPSPS Protein – *In vitro* Digestibility Study In Simulated Intestinal Fluid, D. Rouquie; completed July 2006. Report of Study SA06102.

One of the criteria for assessing potential allergenicity is to determine the stability of novel proteins in conditions that simulate human digestion. Proteins that are rapidly degraded in such conditions are considered less likely to be involved in eliciting an allergic response. It should be noted that ordinarily an ingested protein would first be exposed to pepsin-mediated hydrolysis in the acidic environment of the stomach before being subject to further digestion in the small intestine.

The 2mEPSPS protein was subjected to digestibility studies using simulated human gastric fluid (SGF) containing pepsin and simulated intestinal fluid (SIF) containing porcine pancreatin, which is a mixture of enzymes including amylase, trypsin, lipase, ribonuclease and protease. A pepsin digestibility assay protocol has been standardised in a multi-laboratory evaluation published by Thomas *et al.* (2004), and these studies followed the protocols described in that reference.

Because it was not possible to purify sufficient quantities of 2mEPSPS from GHB614 cotton for use in these studies, *E. coli*-produced 2mEPSPS was used as the test substance in both the SGF and SIF studies. The equivalence of the *E. coli*- and cotton-produced 2mEPSPS proteins was established using a range of biochemical methods including Western blot analysis and enzyme activity assay (see Section 4.2).

Digestibility in SGF was measured by incubating 2mEPSPS protein at 37ºC in reaction mixtures (pH 1.2) with and without pepsin, taking samples at selected time points (0, 0.5, 2, 5, 10, 20 30 and 60 minutes) and subjecting these to SDS-PAGE. The two control proteins, horseradish peroxidase (unstable reference protein) and ovalbumin (stable reference protein), were treated with pepsin under identical incubation conditions to the test substance. Proteins were visualized by staining the gel with Coomassie blue prior to scanning.

In the absence of pepsin, the 2mEPSPS protein band was equally visible in the zero and 60 minute incubation samples. In SGF (with pepsin), there was no full length or partially degraded 2mEPSPS protein observed at 30 seconds and at subsequent time points. In the same experiment, the intensity of the ovalbumin band was undiminished at incubation times up to and including 5 minutes, but at subsequent time points showed a gradual reduction in staining intensity. A band corresponding to intact ovalbumin remained faintly visible after 60 minutes, indicating that digestion of this protein was not complete within 1 hour. The horseradish peroxidase band was not visible after 30 seconds incubation with pepsin, demonstrating that the experimental conditions were appropriate in this study for measuring *in vitro* digestion patterns.

Digestibility in SIF was measured by incubating 2mEPSPS protein at 37ºC in reaction mixtures (pH 7.5), with and without pancreatin, taking samples at selected time points (0, 0.5, 2, 5, 10, 20 30 and 60 minutes) and subjecting these to SDS-PAGE. Degradation of a standard protein (azoalbumin) under identical digestion conditions was used as the control. Proteins were visualized by staining the gel or by transferring the protein to a nitrocellulose membrane for Western blot analysis. The 2mEPSPS protein band was visible in the zero and 60 minute incubation samples without pancreatin, with no decrease in stain intensity over the 60 minutes. In SIF, the 2mEPSPS protein band was only faintly visible after scanning the gel even at time zero. At all subsequent incubation times, there was no full length or partially degraded 2mEPSPS protein observed. Further, the authors reported that digestibility was dramatically increased by pre-heating (data not supplied).

4.6 Conclusion

GHB614 cotton expresses one novel protein, 2mEPSPS, which retains enzyme activity in the presence of glyphosate. The protein is expressed at relatively low levels in cottonseed and various processed sub-fractions of the seed. The average concentration for 2mEPSPS in fuzzy seed was approximately 21 μg/g fresh weight.

A large number of studies have confirmed the identity and physicochemical and functional properties of the 2mEPSPS protein as expressed in GHB614 cotton, and examined its potential to be either toxic or allergenic in humans when present in foods. These studies have demonstrated that the protein conforms in size and amino acid sequence to that expected, does not exhibit any post-translational modification including glycosylation, and also demonstrates the expected enzymatic activity.

In terms of its potential toxicity and allergenicity, it is worth noting that the 2mEPSPS protein has been evaluated previously as the novel protein present in glyphosate-tolerant corn line GA21 which was approved in Australia and New Zealand in 2000. This modified enzyme is derived from the native EPSPS enzyme in maize (99.5% amino acid homology), and is closely related to other EPSPS enzymes from plants and microorganisms which are natural constituents of human diets. Bioinformatic studies with the 2mEPSPS protein sequence has confirmed the absence of any significant amino acid sequence similarity to known protein toxins or allergens and digestibility studies have demonstrated that the protein would undergo rapid degradation in the digestive tract, similar to other dietary proteins. Acute oral toxicity studies in mice have confirmed the absence of toxicity. The weight of evidence shows that the 2mEPSPS protein is not toxic and unlikely to be allergenic in humans.

5. COMPOSITIONAL ANALYSES

The main purpose of compositional studies is to determine if any unexpected changes in composition have occurred to the food and to establish its nutritional adequacy. Compositional analysis can also be important for evaluating the intended effect where there has been a deliberate change to the composition of food.

The classic approach to the compositional analysis of GM food is a targeted one; rather than analysing every single constituent, which would be impractical, the aim is to analyse only those constituents most relevant to the safety of the food or that may have an impact on the whole diet. Important analytes therefore include the key nutrients, toxicants and anti-nutrients for the food in question. The key nutrients and anti-nutrients are those components in a particular food that may have a substantial impact in the overall diet. They may be major constituents (fats, proteins, carbohydrates or enzyme inhibitors as anti-nutrients) or minor constituents (minerals, vitamins). Key toxicants are those toxicologically significant compounds known to be inherently present in an organism, such as compounds whose toxic potency and level may be significant to health (e.g. solanine in potatoes).

In the case of cottonseed, the key components that should be considered in the comparison include protein, fat, carbohydrate, amino acids, fatty acids, vitamins, minerals and the anti-nutrients, gossypol and cyclopropenoid fatty acids (OECD 2004). Cottonseed oil (refined, bleached and deodorized) typically contains 27% saturates, 18% monounsaturates and 55% polyunsaturates. Refined cottonseed oil is free of gossypol (Gunstone *et al.* 1994). The total tocopherol (α- and β-tocopherol) content of cottonseed oil is about 60 mg/100 ml (NCPA, 2000). Cottonseed meal, whole cottonseed or delinted cottonseed are typically used as animal feed.

5.1 Study design and conduct

To determine whether unexpected changes have occurred in the composition of GHB614 cotton as a result of the modification, and to assess its nutritional adequacy, compositional analyses were done on fuzzy seed collected from GHB614 cotton and the non-GM counterpart, Coker 312, grown in field trials typical of commercial agricultural production.

Nine field trials were conducted in 2005 at sites representing primary cotton-growing regions of the south-eastern United States. At each test site, six plots of transgenic event GHB614 cotton and three non-transgenic plots of Coker 312 were planted. Three of the six plots containing GHB614 cotton were sprayed three times with glyphosate herbicide. Each application of glyphosate herbicide was at a rate of 0.75 pounds of active ingredient (glyphosate acid equivalent) per acre.

Ginned cottonseed (fuzzy seed) samples were collected from each trial. Replication was provided from the triplicate plots of each planted regimen, rather than from multiple samples from each plot. Each sample was representative (a composite) of cotton bolls harvested from multiple areas within the plot. Ginning was carried out at the field trial locations with small research scale cotton gins. A total of 135 samples were generated for analysis. Following compositional analysis, the results were statistically analysed using ANOVA (analysis of variance) at a significance level of 0.01.

Compositional analysis of the cottonseed samples included proximates (protein, fat, ash and moisture), acid detergent fibre (ADF), neutral detergent fibre (NDF), minerals (calcium, iron, magnesium, phosphorus, potassium and zinc), amino acids, fatty acids, vitamin E (alpha tocopherol) and carbohydrates by calculation. In addition, the three known anti-nutrients found in cotton (gossypol, phytic acid and cyclopropenoid fatty acids) were analysed. Methods of analysis were based on internationally recognised procedures (e.g., AOAC International methods) or other published methods. The results of the combined site comparisons are presented in Tables 6 – 11. The results from individual trial sites were also evaluated but are not presented in this report.

Table 6: Combined Mean Proximate Composition of Ginned Cottonseed of Cotton Line GHB614 and Control Line Coker 312 (percentage dry matter)

Analyte	Non-GM Control Coker 312	GHB614 cotton unsprayed	GHB614 cotton sprayed
Crude fat (%)	17.71 ± 1.46	17.15 ± 1.49	17.09 ± 1.37
Crude protein (%)	23.47 ± 2.51	23.16 ± 2.70	23.42 ± 2.55
Ash (%)	4.25 ± 0.31	4.26 ± 0.36	4.24 ± 0.34
ADF (%)	40.81 ± 3.43	41.00 ± 2.77	40.66 ± 2.31
NDF (%)	50.06 ± 3.10	50.20 ± 3.53	49.66 ± 2.68
Carbohydrate (calc.)	54.58 ± 2.66	55.43 ± 3.01	55.25 ± 2.41
*Moisture (%)	9.63 ± 3.42	9.42 ± 2.11	8.92 ± 1.39

Data represent an average of three replicate samples at nine field test sites.
* Moisture is expressed as % fresh weight.

Table 7: Combined Mean Mineral and Vitamin E Composition of Ginned Cottonseed of Cotton Line GHB614 and Control Line Coker 312 (percentage dry matter)

Analyte	Non-GM Control	GHB614 cotton unsprayed	GHB614 cotton sprayed
Calcium (%)	0.14 ± 0.03	0.14 ± 0.03	0.13 ± 0.04
Phosphorus (%)	0.62 ± 0.08	0.63 ± 0.06	0.63 ± 0.06
Iron (%)	0.0058 ± 0.002	0.0058	0.0064
Magnesium (%)	0.38 ± 0.03	0.38 ± 0.02	0.38 ± 0.02
Potassium (%)	1.18 ± 0.08	1.18 ± 0.08	1.18 ± 0.08
Zinc (%)	28.3 ± 5.2	29.2 ± 4.7	29.2 ± 5.4
α-Tocopherol (ppm)	106 ± 18	105 ± 13	103 ± 13
Total Tocopherol (ppm)	153 ± 23	154 ± 22	155 ± 24

Table 8: Combined Mean Amino Acid Composition of Ginned Cottonseed of Cotton Line GHB614 and Control Line Coker 312 (percentage dry matter)

Amino Acid	Non-GM Control	GHB614 cotton unsprayed	GHB614 cotton sprayed
Alanine	0.96 ± 0.09	0.96 ± 0.12	0.96 ± 0.11
Arginine	2.60 ± 0.34	2.68 ± 0.48	2.62 ± 0.46
Aspartic Acid	2.27 ± 0.25	2.31 ± 0.30	2.30 ± 0.29
Cystine*	0.36 ± 0.04	0.35 ± 0.04	0.37 ± 0.06
Glutamic Acid	4.78 ± 0.56	4.86 ± 0.77	4.85 ± 0.68
Glycine	0.96 ± 0.10	0.98 ± 0.13	0.98 ± 0.12
Histidine	0.64 ± 0.07	0.65 ± 0.10	0.64 ± 0.09
Isoleucine	0.69 ± 0.09	0.70 ± 0.12	0.70 ± 0.10
Leucine	1.34 ± 0.14	1.35 ± 0.20	1.35 ± 0.17
Lysine	1.03 ± 0.10	1.04 ± 0.13	1.04 ± 0.11
Methionine*	0.38 ± 0.04	0.37 ± 0.04	0.39 ± 0.05
Phenylalanine	1.24 ± 0.14	1.26 ± 0.21	1.26 ± 0.18
Proline	0.86 ± 0.10	0.88 ± 0.10	0.88 ± 0.07
Serine	1.02 ± 0.10	1.05 ± 0.13	1.04 ± 0.13
Threonine	0.76 ± 0.07	0.78 ± 0.10	0.78 ± 0.09
Tryptophan	0.31 ± 0.04	0.32 ± 0.03	0.32 ± 0.03
Tyrosine	0.59 ± 0.06	0.61 ± 0.09	0.61 ± 0.07
Valine	0.97 ± 0.12	0.99 ± 0.17	1.00 ± 0.15

* Statistically significant differences for site and treatment (see Section 5.2)

Table 9: Combined Mean Fatty Acid Composition of Ginned Cottonseed of Cotton Line GHB614 and Control Line Coker 312 (relative per cent)

Fatty Acid	Non-GM Control	GHB614 cotton unsprayed	GHB614 cotton sprayed
Saturated			
Myristic (C14:0)	0.76 ± 0.09	0.75 ± 0.09	0.75 ± 0.10
Palmitic (C16:0)	24.28 ± 0.93	24.21 ± 1.00	24.30 ± 1.00
Stearic (C18:0)	2.35 ± 0.10	2.24 ± 0.12	2.24 ± 0.13
Arachidic (C20:0)	0.30 ± 0.02	0.29 ± 0.02	0.29 ± 0.03
Behenic (C22:0)	0.15 ± 0.01	0.14 ± 0.01	0.14 ± 0.01
Unsaturated			
Palmitoleic (C16:1)	0.62 ± 0.05	0.64 ± 0.05	0.65 ± 0.05
Oleic (C18:1)	15.10 ± 0.85	14.33 ± 0.84	14.38 ± 0.91
Polyunsaturated			
Linoleic (C18:2)	54.94 ± 1.82	56.14 ± 1.87	55.99 ± 2.04
Linolenic (C18:3)	0.61 ± 0.04	0.45 ± 0.05	0.46 ± 0.04
Other components	0.97 ± 0.17	0.87 ± 0.13	0.66 ± 0.11

Table 10: Combined Mean Cyclopropenoid Fatty Acid Composition of Ginned Cottonseed of Cotton Line GHB614 and Control Line Coker 312 (relative)

Analyte	Non-GM Control Coker 312	GHB614 cotton unsprayed	GHB614 cotton sprayed
Sterculic	0.163 ± 0.066	0.119 ± 0.037	0.125 ± 0.037
Malvalic	0.204 ± 0.124	0.145 ± 0.070	0.156 ± 0.074
Dihydrosterculic	0.152 ± 0.022	0.092 ± 0.012	0.090 ± 0.000

Data represent an average of three replicate samples at nine field test sites. Some individual analyses returned a value <0.10, and were changed to 0.09 for inclusion into the calculations.

5.2 Anti-nutrients

Cotton is not considered harmful to humans, however the plant does produce a number of anti-nutrients including gossypol, phytic acid and cyclopropenoid fatty acids (OECD 2004). The levels of these antinutrients in GHB614 cotton were compared with the levels in conventional cotton and the results (Tables 10 and 11) discussed in Section 5.3.

Gossypol is a terpenoid compound naturally occurring throughout the cotton plant, including seeds. It is an important source of plant resistance to damage by herbivores and insects. The levels of gossypol in food and feed products derived from cottonseed must be minimised in order to avoid toxic effects. Gossypol is in the free state in whole cottonseed and is bound to lysine or other components during processing into meal. Once bound in this way, the gossypol is not generally available to animals that consume cottonseed, however sensitivity to gossypol is considerably different between animal species. The amount of free gossypol has been considered the guide used by many nutritionists in making recommendations on feeding of cottonseed products to humans, as free gossypol is toxic. As noted previously, refined cottonseed oil is free of gossypol (Gunstone et al., 1994).

Phytic acid is also considered an important anti-nutrient, particularly for non-ruminant animals, since it can significantly reduce the bioavailability of essential divalent minerals calcium, iron and zinc. Phytic acid can be present at levels around 3-4% in cottonseed flour, depending on the type of cotton.

The cyclopropenoid fatty acids, sterculic (C:19) and malvalic (C:18) acid, are unique to cotton (0.1 – 1.3% of cottonseed oil). Their presence in foods can result in adverse health effects and therefore levels must be minimized for food and feed safety. These fatty acids are largely deactivated or removed from cottonseed oil by hydrogenation or during deodorisation at 230-235ºC.

Table 11: Combined Mean Antinutrient Composition of Ginned Cottonseed of Cotton Line GHB614 and Control Line Coker 312

Analyte % dry matter	Non-GM Control Coker 312	GHB614 cotton unsprayed	GHB614 cotton sprayed
Gossypol free	0.50 ± 0.07	0.48 ± 0.08	0.50 ± 0.08
Gossypol total	0.66 ± 0.09	0.67 ± 0.08	0.67 ± 0.09
Phytic Acid	1.70 ± 0.08	1.69 ± 0.21	1.67 ± 0.18

5.3 Statistical Analyses

The compositional data for all analytes obtained from all sites were analysed by ANOVA using a significance level of 0.01 ($\alpha = 0.01$). Treatments consisted of non-GM control plants (Treatment A), unsprayed transgenic GHB614 plants (Treatment B) and sprayed GHB614 plants (Treatment C). Independent variables evaluated were the site and treatment. Significant differences were observed for the interaction of site and treatment for crude fat, fibre (neutral detergent), phytic acid and valine. Iron was the only analyte that did not show a significant difference for site or treatment. Significant differences were observed with cystine and methionine for both site and treatment.

For both of these analytes, the p-value for interaction was very close to 0.01, and therefore the observed difference in treatment may have been due to the interaction between site and treatment also. For all other analytes, there were significant differences for site, but not for treatment. T-tests comparing the analyte values for non-GM control samples and GHB614 cotton samples, derived from either sprayed or unsprayed plants, showed no significant difference for any of the analytes tested.

5.4 Composition of processed fractions

Seed samples from cotton line GHB614 and the non-GM parental line Coker312, grown in the field trials outlined above, were used to produce processed cottonseed products including linters, delinted seed, meal, toasted meal, hulls, crude oil and deodorized, bleached refined oil. Compositional data were obtained from the transgenic sprayed and non-transgenic processed cottonseed samples. Proximates, amino acids and mineral analyses of ginned and delinted cottonseed, cottonseed meal and toasted cottonseed meal were determined using published AOAC methods. Detailed fatty acid analyses were carried out on the crude oil and deodorized, refined cottonseed oil and the results are presented in Table 12. The results from other compositional analyses of the crude and refined cottonseed oils are presented in Table 13.

Table 13: Compositional Analyses of Crude Oil and Deodorized, Bleached Refined Oil Samples of Cottonseed from Cotton Line GHB614 and Control Line Coker 312

Analyte	Crude Oil		Deodorised, refined Oil	
	Non-GM Coker 312	GHB614 cotton sprayed	Non-GM Coker 312	GHB614 cotton sprayed
α – tocopherol (mg/100 g)	21.7	21.5	29.1	26.8
β – tocopherol (mg/100 g)	<2.00	<2.00	<2.00	<2.00
δ – tocopherol (mg/100 g)	<2.00	<2.00	<2.00	<2.00
γ – tocopherol (mg/100 g)	<2.00	<2.00	<2.00	<2.00
Total vitamin E (tocopherols) (mg/100 g)	21.7	21.5	29.1	26.8
Free gossypol	NA	NA	NA	NA
- gossypol (%)	0.36	0.38	<0.01	<0.01
+ gossypol (%)	0.47	0.47	<0.01	<0.01
Gossypol – Total (%)	0.83	0.85	<0.02	<0.02
Dihydrosterculic acid (%)	0.185	<0.100	0.162	<0.100
Malvalic acid (%)	0.634	0.423	0.430	0.357
Sterculic acid (%)	0.395	0.281	0.301	0.280

No statistical analysis of the compositional results for the crude and refined cottonseed oils was provided, however the results do not show any differences in oil composition between the parental line and GHB614 cotton.

Table 12: Compositional Analyses of Crude Oil and Deodorized, Bleached Refined Oil Samples of Cottonseed from Cotton Line GHB614 and Control Line Coker 312

Fatty Acid Profile % relative	Crude Oil		Deodorised, refined Oil	
	Non-GM Coker 312	GHB614 cotton sprayed	Non-GM Coker 312	GHB614 cotton sprayed
Octanoic (C8:0)	<0.10	<0.10	<0.10	<0.10
Decanoic (C10:0)	<0.10	<0.10	<0.10	<0.10
Undecanoic (C11:0)	<0.10	<0.10	<0.10	<0.10
Dodecanoic (C12:0)	<0.10	<0.10	<0.10	<0.10
Tridecanoic (C13:0)	<0.10	<0.10	<0.10	<0.10
Myristic (C14:0)	0.63	0.65	0.63	<0.10
Myristoleic (C14:1)	<0.10	<0.10	<0.10	<0.10
Pentadecanoic (C15:0)	<0.10	<0.10	<0.10	<0.10
Pentadecenoic (C15:1)	<0.10	<0.10	<0.10	<0.10
Palmitic (C16:0)	23.63	24.12	23.62	24.00
Palmitoleic (C16:1)	0.58	0.61	0.54	0.58
Hexadecadienoic (C16:2)	<0.10	<0.10	<0.10	<0.10
Hexadecatrienoic (C16:3)	<0.10	<0.10	<0.10	<0.10
Hexadecatetraenoic (C16:4)	0.16	0.14	<0.10	<0.10
Heptadecanoic (C17:0)	<0.10	<0.10	<0.10	<0.10
Heptadecenoic (C17:1)	<0.10	<0.10	<0.10	<0.10
Stearic (C18:0)	2.62	2.52	2.61	2.51
Oleic (C18:1)	15.57	15.08	15.47	14.98
Linoleic (C18:2)	54.74	55.21	55.06	55.39
Linolenic (C18:3)	0.56	0.38	0.47	0.39
Octadecatetraenoic (C18:4)	<0.10	<0.10	<0.10	<0.10
Arachidic (C20:0)	0.29	0.30	0.29	0.29
Eicosenoic (C20:1)	<0.10	<0.10	0.12	0.11
Eicosadienoic (C20:2)	<0.10	<0.10	<0.10	<0.10
Eicosatrienoic (C20:3)	<0.10	<0.10	<0.10	<0.10
Arachidonic (C20:4)	<0.10	<0.10	<0.10	<0.10
Eicosapentaenoic (C20:5)	<0.10	<0.10	<0.10	<0.10
Heneicosapentaenoic (C21:5)	<0.10	<0.10	<0.10	<0.10
Behenic (C22:0)	0.13	0.13	0.12	0.12
Erucic (C22:1)	0.24	0.18	0.17	0.14
Docosadienoic (C22:2)	<0.10	<0.10	<0.10	<0.10
Docosatrienoic (C22:3)	<0.10	<0.10	<0.10	<0.10
Docosatetraenoic (C22:4)	<0.10	<0.10	<0.10	<0.10
Docosapentaenoic (C22:5)	<0.10	<0.10	<0.10	<0.10
Docosahexaenoic (C22:6)	<0.10	<0.10	<0.10	<0.10
Lignoceric (C24:0)	<0.10	<0.10	<0.10	<0.10
Nervonic (C24:1)	<0.10	<0.10	<0.10	<0.10
Other components	0.85	0.68	0.90	0.84

5.5 Conclusion

Compositional analyses were done to establish the nutritional adequacy of GHB614 cotton, and to compare it to conventional cotton varieties. The components analysed were proximates, fatty acids, amino acids, vitamin E, minerals, and the anti-nutrients gossypol, phytic acid and the cyclopropenoid fatty acids.

Overall, no differences of biological significance were observed between GHB614 cotton and its conventional counterpart. Some minor differences in some of the key constituents were noted, however the magnitude of the differences observed between the sprayed GM line and the unsprayed non-GM line was very small. Such differences most likely reflect normal biological variability. According to a range of literature values for the non-GM parental line Coker 312, conventional cotton varies significantly in composition with the site, agricultural conditions and season of production. Given this natural variability, food from GHB614 cotton is therefore considered to be compositionally equivalent to food from conventional cotton varieties.

6. NUTRITIONAL IMPACT

In assessing the safety of a GM food, a key factor is the need to establish that the food is nutritionally adequate and will support typical growth and well being. In most cases, this can be achieved through an understanding of the genetic modification and its consequences, together with an extensive compositional analysis of the food.

Where a GM food has been shown to be compositionally equivalent to conventional varieties, the evidence to date indicates that feeding studies using target livestock species will add little to the safety assessment and generally are not warranted (OECD 2003).

If the compositional analysis indicates biologically significant changes to the levels of certain nutrients in the GM food, additional nutritional assessment should be undertaken to assess the consequences of the changes and determine whether nutrient intakes are likely to be altered by the introduction of such foods into the food supply. This assessment should include consideration of the bioavailability of the modified nutrient.

In this case, GHB614 cotton is the result of a simple genetic modification to confer tolerance to glyphosate herbicide, with no intention to significantly alter nutritional parameters in the food. In addition, extensive compositional analyses have been undertaken to demonstrate the nutritional adequacy of food derived from GHB614 cotton and these indicate the GM cotton is equivalent in composition to its non-GM counterpart. The Applicant has however submitted a feeding study comparing the nutritional performance of GHB614 cottonseed meal with that derived from the conventional variety when added to animal feed. This study is evaluated below as additional supporting information.

6.1 Feeding study in broiler chickens

Study submitted:
1. Broiler Chicken Feeding Study with Glyphosate-tolerant GHB614 Cotton, J.M. Stafford; completed June 2007. Springborn Smithers Study No. 13798.4115

The growing broiler is very sensitive during the first 40 days of life to changes in nutrient quality in its diet, as the birds increase body weight approximately 45-fold during this period.

Broiler chicks are thus often used as a model to assess the wholesomeness of feed components, including GM commodities such as corn, cotton and soybean.

The purpose of this study was to compare the wholesomeness of GHB614 cotton (herbicide treated) to its conventional (non-GM) counterpart, as well as to a commercial cotton variety. The study was conducted using rapidly growing broiler chicks (Ross #708). Effects on health, survival, weight gain, feed consumption, feed conversion and marketable carcass quality (muscle: breast, thigh, leg and wing), and abdominal fat pad weights and yields were evaluated.

Cottonseed meal varieties used in the diets were toasted and analysed prior to shipment to the testing laboratories. Using the analytical information, a poultry nutritionist devised formulations for Starter (birds aged 0-7 days), Grower (birds aged 8-21 days), and Finisher (birds over 21 days old) growth phase diets for each cottonseed variety. The prescriptions were designed to be equivalent in energy/calories and protein, and as similar as possible in terms of limiting amino acids with respect to the cottonseed variety and the growth phase.

For each of the three treatment groups (GHB614 cotton, non-transgenic counterpart variety, non-transgenic commercial variety) there were 140 broilers in 14 pens (7 pens of males and 7 pens of females), for a total of 420 birds housed as 10 broilers/pen. Birds were randomised to treatment groups and received one of the three test diets immediately at cage assignment and throughout the 42 days of the study. The lighting regime was adapted from that currently used in regional, commercial, broiler chicken operations to reduce certain metabolic disturbances associated with rapid growth and extended photoperiod. Temperature and humidity were monitored daily. Water and feed were generally provided *ad libitum* throughout the study, however birds were fasted in darkness for a minimum of 8 hours prior to measurements of body weight on days 21, 35 and 42 (study termination).

All birds were monitored at least once a day for health status, overt signs of toxicity, and mortality. Body weights were recorded initially and at days 7, 21, 35 and 42. Feed consumption was measured for each pen on a weekly basis and used to calculate feed conversion ratios. Carcass and tissue weights were recorded for 126 of the 420 broilers in this study (21 birds/gender/treatment group). Statistical analysis was conducted on performance, carcass yield and meat quality parameters.

Chick mortality (14 birds across the three treatment groups, equivalent to 3% in this study) was considered to be on the low side of normal for the species and study conditions and was not related to treatment. The reduced mortality was attributed to a less extreme daylight schedule applied in this study which reduced commonly observed light-related metabolic abnormalities. Overall, including the 14 deaths, twenty-nine birds showed clinical signs that are typically seen in feeding studies of this type and were not related to the dietary treatments.

All data on the following parameters were statistically analysed: feed consumption, body weight and total weight gain, feed conversion, chilled carcass weight, abdominal fat pad weight, leg weight, thigh weight, wing weight and breast weight. The statistical analyses indicated significant differences among the treatment groups for several test (dependent) variables however most of the differences were between the two non-transgenic control groups.

As expected, there were significant differences in feed consumption between genders during all weeks as males consumed more than females, however there were no significant treatment/gender interactions over the study period. At the end of the study, the two factor ANOVA indicated a significant mean weight difference between the treatment groups; mean male body weight in the non-transgenic control group tended to be lower than that in males in the non-transgenic commercial variety. None of the body weight differences identified among the treatment groups could be related to the dietary treatment itself.

There were no significant differences in mean leg weight, thigh weight or wing weight across the treatment groups for the broilers fed diets containing GHB614 cottonseed, the control or commercial cottonseed. There were statistically significant differences in mean abdominal fat pad weight and breast weight between treatments and genders, with the non transgenic control group recording a mean fat pad weight significantly lower than either the GHB614 cotton group or the commercial variety. Similarly, mean female breast weight was lower in the control group than either the GHB614 cotton or the commercial variety. As expected, mean breast weight was significantly higher in male birds than in female birds across treatments.

The results showed no differences among the three diets in the percentage of moisture, protein, and fat in the thigh and breast meat of broilers.

In conclusion, no biologically relevant differences were observed in the parameters measured between broilers fed the GHB614 cotton diet and the control diet. For the individual treatment comparisons, broilers in general had similar performance values and carcass yield and meat composition, regardless of whether the diets contained cottonseed meal from GHB614 cotton, the conventional counterpart or commercial cotton hybrids.

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Attachment 3

SUMMARY OF PUBLIC SUBMISSIONS

1. New South Wales Food Authority

- Costs associated with monitoring and compliance testing for GM foods should be factored into the benefit cost analysis.

2. Madeleine Love

- Objects to food from GM sources on several grounds [not stated].
- FSANZ should operate under the Precautionary Principle as the science is exploratory.
- Women in general (and as many men) have been deceived because the labelling of GM food products does not allow them to recognise all products from a GM source. Producers have therefore gained unfairly because the public is not informed to their desired level.
- FSANZ should give more emphasis to the protection of human health and safety and less emphasis to consistency with food standards of international populations, which have a greater incidence of chronic health problems.

3. New Zealand Food Safety Authority

- Will provide comment on this Application at the Draft Assessment stage.

4. Queensland Health

- Will review the Application following completion of the safety assessment.
- Details on the Applicant's petition to the United States Department of Agriculture and the United States Food and Drug Administration will need to be comprehensively presented in the Draft Assessment Report.
- Costs associated with monitoring and compliance testing will need to be included in the benefit cost analysis. Because of limited resources for these activities, a national enforcement strategy for GM food, which includes education, needs to be progressed without further delay.

5. Australian Food and Grocery Council

- Supports approval of the Application, subject to completion of a satisfactory safety assessment by FSANZ.
- Current labelling requirements for GM foods provide appropriate information to enable consumers informed choice. The assessment process undertaken by FSANZ provides consumers with independent information.
- Following from a review of GM food labelling in 2004, it was concluded that labelling requirements in Australia and New Zealand are amongst the most comprehensive in the world.
- Current labelling requirements are therefore totally adequate and no additional labelling of GM foods is necessary.

6. Ivan Jeray

- Strongly opposed to approval of this Application on safety, economic, environmental and ethical grounds.
- CSIRO abandoned a GM pea because it caused illness in mice (Organic Federation of Australia Newsletter, December 2005). The Precautionary Principle cannot be discounted.

- FSANZ cannot guarantee that the herbicide glufosinate ammonium is safe, particularly when used in higher quantities.
- FSANZ cannot guarantee that GM rice will not contaminate the food supply and the environment, potentially destroying non-GM markets.
- There is no independent evidence to show that the general public will eat GM food.
- FSANZ cannot guarantee and enforce the labelling of this product. Every Australian and New Zealand consumer has the right to know the source of food.
- FSANZ's website and notification circular did not disclose the presence of a GM food in the Application title.

7. Rosemary McKean

- Expresses concern that the current labelling requirements for GM foods do not provide consumers with adequate information to allow informed choice.
- GM foods should carry a clear **'GM'** or **'GE'** in bold colour on the front of the package.
- Does not want to consume or support GM foods and was horrified when she accidentally discovered a cake icing product she had used labelled as GM in the ingredient list.
- A public awareness program should be implemented informing consumers that GM foods are being sold in Australia.
- Method of production labelling is needed for canola, soy and cottonseed oils so that consumers can avoid eating these if they so choose. Manufacturers cannot provide this information.
- This Application should be rejected until method of production and supply chain traceability is introduced.

8. Country Women's Association

- Recommends that proof of rigorous, scientific testing be provided before FSANZ considers this Application.
- All foods and other products derived from GA crops should be clearly labelled as many people have strong ethical or health concerns regarding GM foods.

9. Ceres Natural Foods Pty Ltd (Pureharvest)

- Opposed to the approval of this Application on the grounds that there is not enough independent peer-reviewed studies on safety and that consumers, and the public, are not protected by labelling legislation.
- There is insufficient research published on GM crops and the majority of the research undertaken is biased because it has mostly been industry-driven and funded.
- Foods derived from GM cotton do not have to be labelled and this does not provide consumers with the opportunity to make an informed decision on whether to avoid the product.
- There are no identifying markers on cottonseed oil used by the food preparation industry (restaurants, cafes, take-aways etc) to inform consumers whether it is GM.

10. Ann Lazzaro

- Opposed to the approval of this Application [comments identical to submission from Ceres Natural Foods P/L]

11. Food Technology Association of Australia

- Supports approval of food derived from glyphosate-tolerant cotton line GHB614.

12. Paul Elwell-Sutton

- Opposed to this Application and all foods derived from GM organisms of any kind because:

  - transgenic material is inherently unstable;
  - insertion of genes affects other genes and the proteins in a cell, and can promote cancer;
  - possible transfer of antibiotic resistance to gut bacteria;
  - independent studies find health hazards in animals, which biotechnology companies ignore; and
  - foods from GM sources cannot be guaranteed not to contain transgenes, promoters or markers which could affect the food in ways never seen before.

- Senior staff at the United States Food and Drug Administration have a conflict of interest and fail to take into account the hazards associated with GM foods.
- FSANZ staff need to demonstrate that GM foods can be assessed independently of the data supplied by the Applicant. Without this, current approvals are invalid.
- Herbicide residues are higher in GM crops. For example, permitted glyphosate residue levels in food have been increased in New Zealand by a factor of 200.
- Since the widespread introduction of GM foods in the United States, food allergies and food-borne diseases have increased ten fold. Research into a possible link is needed.
- A robust labelling regime for GM foods is lacking in New Zealand, making analysis of the long-term effects very difficult.
- Consumption of GM foods by laboratory animals can lead to premature death, weight loss, intestinal lesions, neurological damage, cancers and kidney malfunction, and similar effects have been observed for stock fed GM foods.
- GM foods are excluded from the Monsanto staff cafeteria because of safety concerns.
- Most consumers do not want GM foods.
- It is hypocritical to allow GM foods while not permitting commercial production of GM foods in New Zealand.

13. Pancake Parlour Restaurant Group Pty Ltd

- Urges FSANZ to consider the following observations in relation to effects of GM foods on humans and flora and fauna from which food is derived:

  - The FDA (USA) 1992 policy states that there is no information showing GM foods differ from other foods "in any meaningful or uniform way", yet a former staff member says the process is inadequate;
  - Long term feeding studies are not done, nor are human clinical trials, yet millions of people eat GM soy, corn, cotton or canola;
  - Ermakova's research is particularly significant – the first of its kind;
  - Rates of asthma, eczema and hay fever increased between 1991 and 2003. This period is significant because it has been stated that GM foods might lead to hard-to-detect allergens, toxins, new diseases or nutritional problems;
  - Bees are vanishing from the landscape;
  - Extensive research done by Francis Chaboussou (INRA) shows that sprays affect insects and soil fertility and leave the food lacking nutrients, which causes bodies to crave more highly nutritious foods which could lead to health and obesity issues.