Executive Summary

Bayer CropScience Pty Ltd seeks to vary FSANZ Standard 1.5.2 to allow the use of genetically modified cotton (*Gossypium hirsutum*) derived from transformation event GHB811 *G. hirsutum* in the Australian and New Zealand food industries. Four food products are derived from cotton: oil, meal, hulls and linters. Refined oil is the primary food product consumed by humans in Australia, with the other cotton food products, as well as whole cottonseeds, used as components of animal feed.

Bayer CropScience has developed a dual-herbicide tolerant line of GM cotton (*G. hirsutum*) that will be commercialized in the USA and Brazil and possibly other cotton cultivation countries in the future. Planting double-herbicide tolerant cotton GHB811 varieties provides growers with new options for weed control using isoxaflutole (IFT) and/or glyphosate herbicide. Glyphosate is widely used in cotton and other agricultural production systems. IFT herbicide offers and alternative weed control option for the cotton grower to help manage problem weed species and as an alternative mode of action tool to help slow the spread of herbicide resistant weeds. With IFT, a new mode of action is introduced in cotton that is efficacious against many weeds currently found in cotton fields.

GHB811 cotton was developed through *Agrobacterium*-mediated transformation using the vector pTSIH09 containing *hppdPfW336-1Pa* and *2mepsps* expression cassettes. The OECD identifier is BCS-GH811-4.

(i) The double mutant 5-enol pyruvylshikimate-3-phosphate synthase (*2mepsps*) gene that encodes for the 2mEPSPS protein. The *2mepsps* coding sequence was developed by introducing two point mutations to the wild-type *epsps* gene cloned from maize (*Zea mays*). Expression of the 2mEPSPS protein confers tolerance to glyphosate herbicides. FSANZ has previously assessed the 2mEPSPS protein, as expressed by the *2mepsps* gene, in the Bayer CropScience applications for GlyTol cotton (A614) and FG72 soy bean (A1051).

(ii) The *hppdPf W336* gene encodes for the HPPD W336 protein. The *hppdPf W336* coding sequence was developed by introducing a single point mutation to the wild type *hppd* gene derived from *Pseudomonas fluorescens*. Expression of the HPPD W336 protein confers tolerance to isoxaflutole herbicides. The *hppdPf W336* gene has been used to confer HPPD inhibitor tolerant properties to soy bean in the past. FSANZ has previously assessed the HPPD W336 protein, as expressed by the *hppdPf W336* gene, in the Bayer CropScience application for FG72 soy bean (A1051).

Cotton is primarily used worldwide for its lint. Lint is produced on the seed coat, and is spun into fine strong threads. Only the United States and a few other countries have developed major commercial uses for the seed. Raw unprocessed cottonseed may be fed to ruminants in the form of cottonseed meal and hulls or the seed can be processed for oil, the primary

component consumed by humans. Linters, the short fibers that remain on the hulls after the removal of the lint have both edible and non-edible use.

The incorporation of the GHB811 transgenic locus in the *G. hirsutum* genome and the safety of proteins expressed by introduced genes, *hppdPf W336* and *2mepsps* have been characterized according to international standards for the safety assessment of biotechnology products. This information is included with this application to support the food safety of the 2mEPSPS and HPPD W336 proteins. Open pollinated *G. hirsutum* varieties containing the GHB811 event will be grown commercially in the cotton producing areas of the USA and Brazil.

Molecular characterization determined that a single copy of the complete T-DNA of the pTSIH09 plasmid was inserted at a single locus of the cotton GHB811 genome. These data also demonstrated the absence of vector backbone sequences in cotton GHB811 gDNA. The DNA sequence of the cotton GHB811 transgenic locus and the corresponding insertion locus was determined. Molecular characterization analysis also demonstrated inheritance and stability of the insert across multiple generations.

Bioinformatics analysis of the full DNA sequence revealed no evidence supporting cryptic gene expression or unintended effects resulting from the genetic modification.

Food safety evaluation of the 2mEPSPS and HPPD W336 proteins was undertaken utilising guidance provided by Codex (2009). No health-related adverse effects have been associated with the proteins.

5-enolpyruvylshiklmate-3-phosphate synthase (EPSPS) catalyzes the penultimate step of the shikimate pathway, which is responsible for the synthesis of aromatic amino acids and other aromatic compounds in plants, fungi and microorganisms including apicomplexan parasites (Herrmann, K. M.; 1995; M-269843-01; published; Node B.1 (a)). As such, it has been shown that EPSPS enzymes are ubiquitous in nature and are present in food and feed derived from plant and microbial sources. No health-related adverse effects have been associated with these proteins.

The *2mepsps* gene was generated by introducing mutations into the *epsps* gene from maize (*Z mays* L.) that result in two amino acid substitutions. The modified EPSPS (2mEPSPS) enzyme has a decreased binding affinity for glyphosate, allowing it to maintain sufficient enzymatic activity in the presence of glyphosate herbicides (Lebrun, M. *et al*., 1997; M-216526-01; Node A.1 (a)). Since the 2mEPSPS protein is derived from maize and has only two amino acid modifications, the safety profile of the novel protein is expected to remain unchanged relative to its wild-type counterpart. EPSPS proteins are present in food and feed from plant and microbial sources with good safety records. Therefore, EPSPS proteins have a history of safe use. The 2mEPSPS protein has been assessed previously by FSANZ in association with approval of the herbicide tolerant cotton event GHB614 (FSANZ A614), and

the herbicide tolerant soy bean event FG72 (FSANZ A1051). As food safety of this protein has been established previously, the information provided for the protein within this application will be limited to studies confirming its amino acid sequence and up to date data to confirm lack of amino acid sequence homology with known toxins and allergens.

The coding sequence of the 4-hydroxyphenylpyruvate dioxygenase (HPPD) protein was isolated from the *Pseudomonas fluorescens* strain A32. *P. fluorescens* is a Gram-negative, rod-shaped, motile, asporogenous, aerobic bacterium. *P. fluorescens*, is ubiquitous in the environment, including soil, water and food (OECD; 1997; M-357528-01; Node A.1 (a), (i)). It has many beneficial uses in agriculture, human health and bioremediation. It is not described as allergenic, toxic or pathogenic to healthy humans and animals and has an overall history of safe use. The HPPD W336 protein has no amino acid sequence homology to known allergens and is rapidly degraded in simulated gastric fluid and simulated intestinal fluid assays. The HPPD W336 protein has no amino acid sequence similarity to known toxins and exhibited no effects in acute oral mouse toxicity tests. The protein is known to have a good history of safe use. The HPPD W336 protein too has been assessed for food safety by FSANZ within the approved herbicide tolerant soy bean event FG72 (FSANZ A1051). As food safety for this protein has been established previously, information for this protein within this application will be limited to studies confirming the amino acid sequence of the protein and up to date data to confirm lack of amino acid sequence homology with known toxins and allergens.

The nature of N-glycosylation sites, heat stability and degradation in simulated digestive environments have been established previously for both of these proteins. In addition to this effects of the proteins have been independently tested within acute oral mouse toxicity testing, the associated data which has been presented to FSANZ in association with the approval of FG72 soybean (A1051). It is therefore concluded that GHB811 cotton has negligible impact on the nutritional value of foods derived from cotton.

LIST OF APPENDED ELECTRONIC DOCUMENTS RELEVANT TO EXECUTIVE SUMMARY

Node B.1 (a) Herrmann, K.M. (1995) The shikimate pathway: Early steps in the biosynthesis of aromatic compounds. The Plant Cell, 7:907-919. Document no. M-269843-01.

Node A.1 (a) Lebrun, M., Sailland, A., Freyssinet, G. (1997) 5-enol pyruvylshikimate-e-phosphate synthase mutee, gene codant pour cette proteine et plantes transformees contenant ce gene. Patent Application: WO9704103-A 1. Document no. M-216526-01.

Node A.2 (a), (i) Organisation for Economic Co-Operation and Development (OECD) (1997) Series on harmonization of regulatory oversight in biotechnology No.6 -

Consensus document on information used in the assessment of environmental applications involving Pseudomonas. Document no. M-357528-01.