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**Supporting document 1**

Risk assessment – Application A1196

Food derived from nematode-protected and herbicide-tolerant soybean line GMB151

# Executive summary

### Background

A genetically modified (GM) soybean line with OECD Unique Identifier BCS-GM151-6, hereafter referred to as GMB151, has been developed to be protected from parasitic nematodes and tolerant to p-hydroxyphenyl pyruvate dioxygenase (HPPD) inhibitor herbicides.

Of the range of parasitic nematodes that target soybean, GMB151 is specifically protected against the soybean cyst nematode (*Heterodera glycines*) and root lesion nematodes in the genus *Pratylenchus*. This protection is achieved through expression of the *Bacillus thuringiensis* (Bt) gene *cry14Ab-1.b*, which encodes a novel Bt crystal (Cry) protein, designated Cry14Ab1. Tolerance to HPPD inhibitor herbicides, such as isoxaflutole, is conferred through expression of a modified HPPD enzyme, HPDD-4. The gene for this enzyme is based on the sequence from *Pseudomonas fluorescens*.

In conducting a safety assessment of food derived from GMB151, a number of criteria have been addressed including: characterisation of the transferred genes including their origin, function and stability in the soybean genome; the nature of the introduced proteins and their potential to be either allergenic or toxic in humans; compositional analyses and any resultant changes in the whole food. This approach evaluates the intended and any unintended changes in the plant.

This safety assessment addresses only food safety and nutritional issues of the GM food. It therefore does not address:

• risks related to the environmental release of GM plants used in food production

• risks to animals that may consume feed derived from GM plants

• the safety of food derived from the non-GM (conventional) plant.

Food derived from the non-GM (conventional) plant with an accepted history of safe use is used as the benchmark for the comparative analysis.

### History of use

The host organism is cultivated soybean (*Glycine max* L.). Soybean has a long history of use as food and is cultivated in over 90 countries. Processed products derived from soybean grain, such as refined oil, lecithin, flour and protein concentrates are commonly used in cooking oils, margarines, infant formula, breakfast cereals and confectioneries.

### Molecular characterisation

Comprehensive analyses of soybean line GMB151 indicated there is a single copy of the insert containing both genes, with a small deletion and rearrangement of DNA at the left border. The small deletion removes some of the promoter sequence for HPPD-4 gene but the enzyme is active, indicating this deletion does not impact expression.

The introduced genetic elements and the expression of new proteins in GMB151 were shown by DNA sequence analyses and phenotypic analyses to be stably inherited from one generation to the next across multiple generations. The pattern of inheritance supports the conclusion that the nematode-protective and herbicide-tolerance traits occur within a single locus in the GMB151 genome and are inherited in accordance with Mendelian principles.

### Characterisation and safety assessment of new substances

A range of characterisation studies confirmed the identity of Cry14Ab1 and HPPD-4 expressed in soybean line GMB151. The plant Cry14Ab1 and HPPD-4 proteins had the expected molecular weights (131.5 kDa and 40 kDa respectively), amino acid sequence, immunoreactivity, lack of glycosylation, and enzyme activity. Equivalent forms of Cry14Ab1 and HPPD-4 expressed in bacteria were heat labile and readily digested in *in vitro* studies.

Bioinformatic analyses of Cry14Ab1 and HPPD-4 confirmed the absence of similarity to known allergens. HPPD-4 also has no similarity to known toxins and Cry14Ab1 only showed similarity to other Cry protein toxins as expected. Animal toxicity studies performed on both proteins also confirmed the absence of toxicity in mice.

Cry14Ab1 was detected in whole seed and hull analysed from GMB151. Expression of the protein was highest in the whole seed. The mean level in the whole seed ranged from 72-82 µg/g dry weight, which corresponds to ~0.020% of total protein. HPPD-4 was also detected in the whole seed, hull and protein isolate, with highest expression in the whole seed. The mean level in the whole seed ranged from 3.34-4.91 µg/g dry weight, which corresponds to ~0.001% of total protein.

### Compositional analyses

Detailed compositional analyses were done on grain from GMB151, the control Thorpe cultivar and several commercial non-GM soybean lines. The plants were grown under normal agricultural conditions over eight field-trial sites, located in traditional soybean growing regions of the USA. The analyses included proximates (protein, fat, ash, moisture), fibre components, fatty acids, amino acids, minerals, vitamins and anti-nutrients. The levels of 81 of these key analytes in GMB151 were compared to those of the control and non-GM lines.

Statistically significant differences were found between seeds from GMB151 and the control for 22 of the analytes measured, however all differences were small in magnitude and were within the range established for existing commercial soybean varieties. Overall, the compositional data support the conclusion that there are no biologically significant differences in the levels of key constituents in grain from GMB151 compared to conventional soybean varieties available on the market.

### Conclusion

No potential public health and safety concerns have been identified in the assessment of GMB151. On the basis of the data provided in the present application, and other available information, food derived from GMB151 is considered to be as safe for human consumption as food derived from conventional soybean varieties.

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### List of Abbreviations

|  |  |
| --- | --- |
| APVMA | Australian Pesticides And Veterinary Medicines Authority |
| BC / bc | backcrossed |
| BLOSUM | BLOcks SUbstitution Matrix |
| bp | base pairs |
| Bt | *Bacillus thuringiensis* |
| bw | body weight |
| COMPARE | COMprehensive Protein Allergen REsource |
| Cry | crystalline protein |
| Da | dalton |
| DNA | deoxyribonucleic acid |
| DW | dry weight |
| ED | effective dose |
| ELISA | enzyme-linked immunosorbent assay |
| EPA | Environmental Protection Agency |
| ILSI | International Life Sciences Institute |
| FASTA | Fast alignment search tool – all |
| FAO | Food and Agriculture Organization of the United Nations |
| FSANZ | Food Standards Australia New Zealand |
| g | gram |
| GM | genetically modified |
| HPPD | hydroxyphenyl pyruvate dioxygenase |
| kDa | kilodalton |
| kg | kilogram |
| LOD | limit of detection |
| MALDI-TOF MS | matrix assisted laser desorption ionization-time of flight mass spectrometry |
| mg | milligram |
| min | minute |
| MT | million tonnes |
| NCBI | National Centre for Biotechnology Information |
| ng | nanogram |
| OECD | Organisation for Economic Co-operation and Development |
| OGTR | Office of the Gene Technology Regulator |
| ORF | open reading frame |
| PAGE | polyacrylamide gel electrophoresis |
| PCR | polymerase chain reaction |
| SAS | Statistical Analysis Software |
| SDS | sodium dodecyl sulfate |
| SGF | simulated gastric fluid |
| SIF | simulated intestinal fluid |
| T | tonne |
| T-DNA | transfer DNA |
| U | units |
| μg | microgram |
| US | United States |
| USDA | United States Department of Agriculture |
| WHO | World Health Organisation |

# 1 Introduction

FSANZ has received an application from BASF Agricultural Solutions Seed US LLC to vary Schedule 26 in the *Australia New Zealand Food Standards Code* (the Code). The variation is to add food derived from the genetically modified (GM) nematode-protected and herbicide-tolerant soybean line GMB151, with the OECD Unique Identifier BCS-GM151-6. This line is protected against parasitic nematodes, targeting plant roots and is tolerant to p-hydroxyphenyl pyruvate dioxygenase (HPPD) inhibitor herbicides, such as isoxaflutole.

Protection from parasitic nematodes is conferred by expression of the Cry14Ab1 crystalline (Cry) protein, encoded by the gene *cry14Ab-1.b* derived from the soil bacterium *Bacillus thuringiensis*. FSANZ has assessed and approved several applications where *B. thuringiensis* Cry proteins have been introduced into crops for pest-protection but this is the first application based on the Cry14Ab1 protein.

Tolerance to HPPD inhibitor herbicides is achieved by expression of a modified HPPD enzyme, HPPD-4. The modified protein contains four amino acid changes, encoded by the *hppdPf-4Pa* gene, which is based on the gene from the soil bacterium *Pseudomonas fluorescens*. FSANZ has previously approved two GM lines containing a modified *hppdPf* gene (FSANZ 2011; 2018) however this is the first application for the HPPD-4 enzyme.

Soy lines containing the GMB151 transformation event will not be cultivated in Australia or New Zealand, therefore food from GMB151 may enter the Australian and New Zealand food supply via imported products.

# 2 History of use

## 2.1 Host organism

The information provided here has been summarised from more detailed reports published by the Organisation for Economic Cooperation and Development (OECD 2012), the Grains Research & Development Corporation (GRDC 2016), Australian Oilseed Foundation (AOF 2008) and the US Soybean Export Council (USSEC 2015). Numerical and statistical data have been sourced from the [FAOSTAT website](http://www.fao.org/faostat/en/#data)[[1]](#footnote-1) from the Food and Agriculture Organization of the United Nations, the United States Department of Agriculture [Foreign Agricultural Service website](https://www.fas.usda.gov/commodities/soybeans)[[2]](#footnote-2) and the International Service for the Acquisition of Agri-Biotech Applications (ISAAA 2017).

The host organism is conventional soybean (*Glycine max* (L.) Merrill), belonging to the family *Leguminosae*. The soybean cultivar Thorne was used as the parental variety for the genetic modification described in this application, and thus is regarded as the near-isogenic line for the purposes of comparative assessment with GMB151.

Soybean is grown as a commercial food and feed crop in many countries worldwide, with some 96 countries listed as producers in 2018. The major producers of soybeans, accounting for 90% of world production, are the United States (US) (123.7 MT), Brazil (117.9 MT), Argentina (37.8 MT), China (14.2 MT), India (13.8 MT) and Paraguay (11.1 MT). The proportion of GM soybean cultivation in these countries is significant (US - 94%; Brazil - 97%; Argentina -100%; and Paraguay - 96%).

Australia is a net importer of soybean and soybean products, cultivating 63,000T and importing 892,000T. Soybean cultivation in Australia is typically used as a rotational crop, either for green manure or forage and grain for animal feed. New Zealand has no domestic cultivation of soybean and imports 292,000T.

The types of food products derived from soybean are outlined in [Table 1](#Table1). The products from which GMB151-derived lines will be targeted towards are the fractionated products, made from soybean grain, such as refined oils and protein products. Refined oil from soybean is used in cooking oils, shortening, margarine, frozen desserts and confectionery products. Protein products from soybean is used in meat analogues, bakery ingredients, breakfast cereals, baby foods and infant formula. Unprocessed (raw) soybean grain products are not suitable for food, due to presence of anti-nutrients, such as phytic acid and isoflavones (OECD 2012). The processing used with the soybean grain products inactivate these anti-nutrients, making them suitable for food use.

 Table 1: Food product groups derived from soybean

|  |  |
| --- | --- |
| Whole soybean products | Fractionated soybean products |
| soy (bean) sprouts | refined soybean oil |
| baked soybeans | lecithin |
| roasted soybeans | soy flour |
| soy full-fat flour | protein concentrate |
| soy milk | protein isolates |
| tofu |  |
| soy sauce |  |
| edamame |  |

## 2.2 Donor organisms

### 2.2.1 Bacteria

The DNA sequence encoding Cry14Ab1 was originally isolated from *Bacillus thuringiensis* strain ARP001 (Sampson et al. 2012). *B. thuringiensis* is not considered pathogenic to humans (WHO 1999; Raymond and Federici, 2017) although some strains have been co-isolated from foods associated with causing diarrhoea and an incident has been reported of *B. thuringiensis* being isolated from a corneal ulcer, after use of a *B. thuringiensis* pesticide product (Samples and Buettner, 1983).

The presence of *B. thuringiensis* in foods is not considered unusual considering these bacteria are ubiquitous in the environment. Specific strains of these bacteria have been utilised as commercial microbial insecticides in agriculture and forestry since 1938 in France and 1961 in the US, indicating this organism has a long history of safe use (Nexter et al. 2002; CERA 2011). Since 2010, there have been approximately 180 biopesticide products registered in the US and also in China, 120 registered in the European Union, 44 in Australia and 13 in New Zealand, with high usage in forestry and the organic farming industry (Kabaluk et al. 2010; APVMA 2020). The strains used in agricultural biopesticide preparations are unlikely to be the cause of foodborne diarrhoea, since they have been shown to not produce enterotoxins (Cho et al. 2015). In foods associated with diarrhoea, the *B. thuringiensis* bacteria were co-isolated with *B. cereus* strains, of which both were shown to contain genes for enterotoxins.

The DNA sequence that encodes the HPPD enzyme was initially isolated from *P. fluorescens* strain A32 (Sailland et al. 2001). This organism is considered a non-pathogenic, asporogenous saprophyte found in soil, water and on plant surface environments. This organism is generally considered non-pathogenic to humans because it has limited growth at body temperature but has been associated with opportunistic infections in immunocompromised patients or after infusion of contaminated whole blood or blood products (Scales et al. 2014).

### 2.2.2 Plant

Genetic elements from several plant species have been used in the genetic modification of GMB151 (refer to Table 1). *Arabidopsis thaliana* is a member of the mustard family (*Brassicaceae*), which also includes cabbage, turnip and rapeseed, and is commonly known as mouse-ear or thale cress. Although this plant is not traditionally used as food, it is ubiquitous in the environment and is not known to be pathogenic, toxigenic or allergenic to humans.

*Helianthus annuus* (sunflower) is a member of the flowering plant family (*Asteraceae*). Sunflower seeds as whole seeds, oil or protein-rich flour has been consumed for centuries (OECD 2004) demonstrating a long history of safe food use. The seeds do not contain significant amounts of anti-nutrients or toxins and the genetic elements isolated from sunflower are not related to the known anti-nutrients.

*Zea mays* (corn/maize) is a member of the grass family (*Poaceae*). This plant has a very long history of safe food use, with evidence suggesting domestication about 6000-8000 years ago in the Mexican highlands (OECD 2002; OGTR 2008). Although corn does have known anti-nutrients and allergens, the genetic elements isolated from the corn genome are not related to the known anti-nutrients or allergenic compounds.

### 2.2.3 Other organisms

Genetic elements from several other organisms have been used in the genetic modification of GMB151 (refer to [Table 2](#Table2)). These non-coding sequences are used to drive or enhance expression of the two new genes.

# 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
* the genetic stability of the inserted DNA and any accompanying expressed traits.

## 3.1 Transformation Method

In order to create GMB511, plasmid pSZ8832 was transformed into the soybean variety Thorne. Plasmid pSZ8832 contains two gene cassettes that encode the Cry14Ab1 and HPPD-4 proteins ([Figure 1](#Figure1)).



Figure 1: Plasmid map of pSZ8832.The plasmid contains a T-DNA insert region that contains two gene cassettes, running in an anticlockwise direction from the left border (LB) to right border (RB).

The transformation method involved infection of explants of soybean with a disarmed strain of *Agrobacterium* LBA4404 (Hoekema et al. 1983) containing pSZ8832 and the virulence helper plasmid pAL4404. pAL4404 is used to enhance the transformation process and does not introduce any novel genes into the plant tissue. After infection, the explants were grown on media containing tembotrione, a HPPD inhibitor to select positive transformants, and ticarcillin, to suppress growth of the agrobacterium. Transformed tissue was subsequently cultured in media to encourage shoot formation, followed by growth in rooting medium. Rooted plants (T0) were transferred to soil and allowed to self-pollinate, producing T1 seed.

## 3.2 Detailed description of DNA to be introduced

The plasmid pSZ8832 ([Figure 1](#Figure1)), used to generate GMB511, contains two gene cassettes in the T-DNA region. At the right border (RB) is the *cry14Ab-1.b* gene cassette. Expression of the Cry gene is under the control of the ubiquitin-10 promoter from *A. thaliana* and the 35S terminator sequence from the cauliflower mosaic virus (CaMV).

At the left border (LB) is the *hppdPf-4Pa* gene cassette. Expression of *hppdPf-4Pa* is under the control of the double enhanced 35S promoter from the CaMV and the terminator sequence within the 35S 3’ UTR from CaMV. The *hppdPf-4Pa* gene is preceded by a leader sequence from the tobacco etch virus and a transit peptide of the RuBisCo small unit gene, based on the sequence found in *Z. mays* and *H. annuus* (Lebrun et al. 1996). This transit peptide ensures the enzyme is translocated to the plastids, such as chloroplasts, after protein translation.

There are intervening sequences also present in the T-DNA region as outlined in [Table 2](#Table2). These sequences assist with cloning, mapping and sequence analysis.

Table 2: The genetic elements contained in the T-DNA region of pSZ8832, used to create GMB511.

| **Genetic element** | **Relative position** | **Orientation** | **Source** | **Description, Function & Reference** |
| --- | --- | --- | --- | --- |
| Right border (RB) | 1 - 24 |  | *Agrobacterium tumefaciens* | Right border repeat from the T-DNA (Zambryski 1988) |
| Right border region | 25 - 154 |  |  |  |
| *T35S* | 155 - 424 | counter clockwise | *Cauliflower mosaic virus* | 3´ untranslated region of the 35S gene containing the termination sequence (Sanfaçon et al. 1991) |
| Intervening sequence | 425 - 435 |  |  |  |
| *Cry14Ab-1.b* | 436 - 3993 | counter clockwise | *Bacillus thuringiensis* | coding sequence of the Cry14Ab-1 gene (Sampson et al. 2012) |
| *Pubi10At* | 3994 - 5300 | counter clockwise | *Arabidopsis thaliana* | promoter sequence from the ubiquitin-10 gene (Grefen et al. 2010) |
| Intervening sequence | 5301 - 5405 |  |  |  |
| *T35S* | 5406 - 5600 | counter clockwise | *Cauliflower mosaic virus* | 3´ untranslated region of the 35S gene containing the termination sequence (Sanfaçon et al. 1991) |
| Intervening sequence | 5601 - 5612 |  |  |  |
| *hppdPf-4Pa* | 5613 - 6689 | counter clockwise | *Pseudomonas fluorescens* | coding sequence of a variant of the HPPD gene (Porée et al. 2014) |
| *TPotpY-1Pf* | 6690 - 7061 | counter clockwise | *Helianthus annuus**Zea mays* | coding sequence of an optimized transit peptide derivative from the RuBisCO small subunit gene (Lebrun et al. 1996) |
| Intervening sequence | 7062 - 7082 |  |  |  |
| *Ltev* | 7083 - 7209 | counter clockwise | *Tobacco etch virus* | leader sequence of the virus genomic RNA (Allison et al. 1985) |
| Intervening sequence | 7210 - 7215 |  |  |  |
| *P2x35S* | 7216 - 7965 | counter clockwise | *Cauliflower mosaic virus* | double enhanced promoter sequence from the 35S gene (Kay et al. 1987) |
| Left border region | 7966 - 8092 |  |  |  |
| Left Border (LB) | 8093 - 8117 |  | *Agrobacterium tumefaciens* | Left border repeat from the T-DNA (Zambryski 1988) |

## 3.3 Development of the soybean line from original transformation

A breeding programme was undertaken for the purposes of:

• obtaining generations suitable for analysing the characteristics of GMB151

• ensuring that the GMB151 event is incorporated into elite lines for commercialisation of isoxaflutole-tolerant and nematode-protected soybean.

The breeding pedigree for the various generations is given in [Figure 2](#Figure2).

Following development of the T0 plants (see [Section 3.1](#Section3_1)), a series of self-pollination (selfed) and seed bulking steps proceeded up to generation T8. T3 plants were also crossed with two commercial cultivars (CC) to produce two F1 generations. These F1 generation were selfed, or underwent backcrosses with their respective CCs to produce further F generations.



*Figure 2: Breeding path used in the characterisation of the GMB151 line.*

## 3.4 Characterisation of the inserted DNA and site(s) of insertion

A range of analyses were undertaken to characterise the genetic modification in GMB151. These analyses focused on the nature and stability of the insertion and whether any unintended rearrangements or products may have occurred as a consequence of the transformation process. When characterising GMB151, different generations of plants were analysed and these are outlined in [Table 3](#Table3).

Table 3: Events used in the molecular characterisation studies performed in GMB151

|  |  |  |
| --- | --- | --- |
| **Analysis** | **Generations analysed** | **Control(s) used** |
| Identifying the number of integration sites (Section 3.4.1) | T2, T4, T5, T6, BC2F3 | Thorne, CC1 & Thorne spiked with pSZ8832 |
| Detection of backbone sequence (Section 3.4.2) | T2, T4, T5, T6, BC2F3 | Thorne, CC1 & Thorne spiked with pSZ8832 |
| Insert integrity and site of integration (Section 3.4.3) | T2, T4, T5, T6, BC2F3 | Thorne, CC1 & Thorne spiked with pSZ8832 |
| Inheritance & genetic stability of the inserted DNA (Section 3.4.4) | F2, BC2F2 | CC1 |
| F2, BC1F2, BC2F2 | CC2 |

### 3.4.1 Identifying the number of insertion sites

Next-generation sequence analysis was performed on seed-derived genomic DNA from GMB151, the parental Thorne cultivar and the commercial cultivar (CC1). A reference sequence was generated using Thorne DNA spiked with an equimolar amount of pSV8832. After preparation of a paired-end library with 500-600 bp lengths of sheared genomic DNA, the samples were sequenced using Illumina HiSeq technology. Sufficient sequence fragments were obtained to cover the entire genomes of GMB151, Thorne and CC1, with a depth of coverage > 80x. Comparison of the sequence between the Thorne, CC1 and GMB151 showed that a single integration event has occurred, with only two junction sites detected.

### 3.4.2 Detection of backbone sequence

Next-generation sequencing of seed-derived DNA from GMB151 and the parental Thorne cultivar resulted in zero reads that mapped to the backbone sequences of pSV8832, shown in [Figure 1](#Figure1).

### 3.4.3 Insert integrity and site of integration

Next-generation sequencing of the seed-derived DNA from GMB151, using the plasmid DNA as a reference sequence, showed that a single copy of the DNA was integrated into the host genome. No insertions or rearrangements of the inserted DNA were detected. There was a deletion at the LB of the T-DNA, resulting in the deletion of the LB sequence and a truncation of 481 bp of the P2x35S promoter sequence. At the junction between the LB region and 3’ flanking genome sequence, there has been an insertion of 39 bp filler DNA. Within the filler DNA, there was sequence similarity to the ORIpVS1 sequence from the vector backbone and sequence similarity to the 3’ genomic flanking sequence.

Next-generation sequencing of both GMB151, Thorne and CC1 genomic DNA was also used to identify the site of integration. Based on the identified host genomic DNA sequence flanking the insert, a PCR was designed to produce two overlapping fragments, with primer pairs that bound to the flanking sequences and within the insert. Sequence analysis of the PCR fragments confirmed the insertion site and sequence of the inserted DNA.

### 3.4.4 Inheritance and genetic stability of the inserted DNA

Since it was demonstrated that the insert is present at a single locus in the GMB151 genome, there is the expectation that the genetic elements within this locus would be inherited according to Mendelian principles.

Chi-square (Χ2) analysis was undertaken over several generations ([Table 3](#Table3)) to confirm the segregation and stability of the T-DNA insert in GMB151. The inheritance pattern was analysed by quantitative real-time PCR using primers targeting the *cry14Ab-1.b* and *hppdPf-4Pa* genes and an endogenous soybean gene.

The expected segregation ratio at the F2 generations was 1:2:1 and the critical value to reject the hypothesis of this ratio at the 5% confidence level was 5.99 (Strickberger 1976). As the X2 values calculated from these experiments were < 5.99, the results showed there were no significant differences between the observed and expected segregation ratios in any of the generations ([Table 4](#Table4) and [Table 5](#Table5)). These data support the conclusion that the T-DNA is present at a single locus in GMB151 and was inherited predictably according to Mendelian principles in subsequent generations.

Table 4: Segregation results for GMB151 x CC1 in F2 and BC2F2

|  |  |  |  |
| --- | --- | --- | --- |
|  | **F2** | **BC2F2** |  |
| **Observed** | **Expected** | **Observed** | **Expected** |  |
| **homozygous** | 56 | 61.5 | 57 | 61.5 |  |
| **hemizygous** | 133 | 123 | 120 | 123 |  |
| **null** | 57 | 61.5 | 69 | 61.5 |  |
| **Total plants** | 246 | 246 |  |
| **X2** | 1.634 | 1.317 |  |

Table 5: Segregation results for GMB151 x CC2 in F2, BC1F2 and BC2F2

|  |  |  |  |
| --- | --- | --- | --- |
|  | **F2** | **BC1F2** | **BC2F2** |
| **Observed** | **Expected** | **Observed** | **Expected** | **Observed** | **Expected** |
| **homozygous** | 54 | 54 | 62 | 55.5 | 56 | 57.25 |
| **hemizygous** | 102 | 108 | 101 | 111 | 104 | 114.5 |
| **null** | 60 | 54 | 59 | 55.5 | 69 | 57.25 |
| **Total plants** | 216 | 222 | 229 |
| **X2** | 1.000 | 1.883 | 3.402 |

### 3.4.5 Open reading frame (ORF) analysis

The Applicant used the search program GetORF to identity all start-to-stop ORFs in both the inserted DNA and junctions between the insert and genomic DNA. All six reading frames were analysed. ORFs of ≥ 3 amino acids were initially captured identifying a total of 601 ORFs. From this pool, 115 potential proteins were identified with a minimum length of 30 amino acids. Proteins of ≥ 30 amino acids meet the minimum requirements of a 35% match over an 80 amino acid sequence (Codex 2009). These 115 potential proteins were then used as query sequences in homology searches for known allergens and toxins.

#### 3.4.5.1 Bioinformatic analysis for potential allergenicity

The Applicant provided the results of *in silico* analyses comparing the 115 amino acid sequences identified as potential proteins to known allergenic proteins listed in the Comprehensive Protein Allergen Resource ([COMPARE](http://comparedatabase.org/database/)[[3]](#footnote-3)) database, from the Health and Environmental Science Institute. At the date of the search, there were 2,038 sequences in the allergen database.

The following analyses were performed for the sequence comparison:

(a) Full length search – a FASTA alignment using a BLOSUM50 scoring matrix and E-value threshold conservatively set at 1. Only matches of ≥ 35% similarity over 80 amino acids were considered.

(b) 8-mer exact match search – Alignment made use of the SeqMatchAll tool from the European Molecular Biology Open Software Suite (EMBOSS). Only matches of 100% similarity over 8 amino acids were considered.

Of the 115 potential ORFs used to query the COMPARE database, no similarities were found using the full length search to any of the known allergenic proteins. A single hit was identified with the 8-mer search for a Cas s 5 allergen however subsequent comparison of the full length putative proteins found no similarity to the Cas allergen. Analysis of the ORFs also did not identify a suitable start codon that would lead to a translation event.

#### 3.4.5.2 Bioinformatic analysis for potential toxicity

The Applicant provided results from *in silico* analyses comparing the 115 potential proteins identified as ORFs to known protein toxins identified in the NCBI non-redundant protein database. A FASTA algorithm was used with a BLOSUM50 scoring matrix and the E-value threshold set to 0.1. No matches were found between the putative proteins and any of the known proteins toxins.

### 3.4.6 Conclusion

The data provided by the Applicant showed that a single integration event has occurred at a specific locus. The T-DNA region from pSZ8832, containing the *cry14Ab-1.b* and *hppdPf-4Pa* gene cassettes, has been inserted without rearrangement. A deletion was identified at the LB region resulting in truncation of the promoter for *cry14Ab-1.b*. No backbone sequences from the transforming plasmid have been incorporated. The introduced DNA was shown to be stably inherited across generations.

# 4 Characterisation and safety assessment of novel substances

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.

Only a small number of dietary proteins have the potential to impair health, because they have anti-nutrient properties or they can cause allergies in some consumers (Delaney et al. 2008). As proteins perform a wide variety of functions, different possible effects have to be considered during the safety assessment including potential toxic, anti-nutrient or allergenic effects.

To effectively identify any potential hazards, knowledge of the characteristics, detailed understanding of the biochemical function and phenotypic effects and concentration levels in the edible part of the plant is required. It is also important to determine if the newly expressed protein is expressed in the plant as expected, including whether any post-translational modifications have occurred.

## 4.1 Description of the Cry14Ab1 protein

Cry14Ab1 is a member of the crystal (Cry) family of pore-forming proteins produced by *B. thuringiensis.* Cry proteins are contact pesticides, requiring ingestion by the target pest and passage into the digestive system in order to function (Jurat-Fuentes and Crickmore, 2017). For nematocidal Cry proteins, passage into the intestine of susceptible nematodes results in degeneration and shrinking of the intestines, leading to developmental delays, interruptions to reproduction and death (Marroquin et al. 2000; Wei et al. 2003). How the nematocidal toxins bind to intestinal cells to mediate these effects has not been fully elucidated but some evidence suggests glycolipid receptors may be important (Marroquin et al. 2000; Griffitts et al. 2005). The actual mechanism of how Cry14Ab1 acts has not yet been determined.

There is a limited history of use of Cry14Ab1 in food but *B. thuringiensis* has a long history of use in agriculture. This use in agriculture mean humans have been exposed to over a hundred different Cry proteins, each with their own primary structure yet high similarities in secondary and tertiary structure, without adverse effects.

The *Cry14Ab-1.b* gene prepared by the Applicant encodes a protein of 1185 amino acids, with an expected mass of 131.5 kDa.

### 4.1.1 Characterisation of Cry14Ab1 expressed in GMB151 and equivalence to bacterially-produced forms

Protein was extracted from approximately 30 g ground leaf material from both GMB151 (T6) and the Thorne parental control. Affinity chromatography was performed to isolate any fraction that bound to a Cry14Ab1 antibody. Aliquots of purified protein were run on SDS-PAGE and visualised with Coomassie stain. A sample gel was presented and the results showed a clear band in the GMB151 lane with a molecular mass between 117-200 kDa. There was no equivalent band in the parental control lane. Western blot analysis confirmed that the protein in GMB151 was immunoreactive to a Cry14Ab1-specific antibody, with a size range between 100-150 kDa. The data from the gel analyses places the mass of the protein in the expected range of 117-150 kDa.

N-terminal sequencing identified 13 amino acids that matched the expected sequence ([Figure 3](#Figure3)). Peptide mapping showed that the expressed protein sequence matched the expected Cry14Ab1 sequence, with 71% sequence coverage achieved ([Figure 3](#Figure3)). The protein was shown by a modified Periodic Acid-Schiff staining procedure to be non-glycosylated.



*Figure 3: Tryptic peptide map of the GMB151-produced Cry14Ab1 protein. The deduced amino acid sequence is 1185 amino acids. Boxed regions correspond to peptide sequence coverage achieved using MALDI-TOF MS. The blue coloured amino acids from position 1-13 were identified by N-terminal sequencing.*

Functional activity of plant-expressed Cry14Ab1 was measured with a *Caenorhabditis elegans* nematode toxicity assay. This bioassay uses an engineered *C. elegans* that expresses a red fluorescent protein (RFP) that can be used to monitor biomass (Chase et al. 2004). Expression of the RFP can be measured using a fluorescent plate reader. The results from this assay confirmed that the Cry14Ab1 acts as an nematocide in a dose-responsive manner and an effective dose required to inhibit growth by 50% (ED50) was determined.

The Cry14Ab1 protein was also expressed in *B. thuringiensis*, transformed with a plasmid to express the protein. The bacterially-derived protein was shown by gel electrophoresis to have an apparent molecular mass equivalent to the plant expressed form of Cry14Ab1 and was immunoreactive to Cry14Ab1 antibodies in a western blot. N-terminal sequencing and peptide mapping showed that the sequence matched the expected Cry14Ab1 sequence ([Figure 3](#Figure3)). The protein was also shown to be non-glycosylated and was functional in the nematocide bioassay. This data demonstrates that the bacterial-produced Cry14Ab1 is a suitable standard for the enzyme-linked immunosorbent assay (ELISA) used to detect Cry14Ab1 in plant tissues (as discussed in [Section 4.1.2](#_4.1.2_Expression_of)) and is a suitable surrogate for use in the safety assessment experiments described in [Section 4.1.3](#_Structural_stability_after).

### 4.1.2 Expression of the Cry14Ab1 in GMB151 grain

Cry14Ab1 expression in processed components of soybean was determined using a commercially available ELISA kit. Samples of soybean grain were collected from the parental cultivar Thorne and both untreated and herbicide-treated GMB151, grown across two field-trial sites[[4]](#footnote-4) during the 2016 growing season in the US. A standard curve was generated using microbially expressed Cry14Ab1(see [Section 4.1.1](#_4.1.1_Characterisation_of)). Background controls included no protein vehicle controls and protein isolated from the equivalent processed fractions from Thorne.

Results from the ELISA show that Cry14Ab1 is detectable in the whole seed and seed hulls of both untreated and herbicide-treated GMB151 ([Table 6](#Table6)). No Cry14Ab1 was detected in the more highly processed products: toasted meal, refined oil and the concentrated protein isolate from the meal.

Table 6: Mean expression levels of Cry14Ab1 in GMB151 grain components

|  |  |  |  |
| --- | --- | --- | --- |
| **Matrix** | **Herbicide treated** | **Cry14Ab1 (µg/g FW)1** | **Cry14Ab1 (µg/g DW)2** |
| **Grain** | - | 71.79 | 81.48 |
| + | 81.89 | 92.84 |
| **Toasted meal** | - | < LOD3 | < LOD |
| + | < LOD | < LOD |
| **Refined oil** | - | < LOD | NA4 |
| + | < LOD | NA |
| **Protein isolate** | - | < LOD | < LOD |
| + | < LOD | < LOD |
| **Hulls** | - | 20.90 | 24.24 |
| + | 23.50 | 26.99 |

1. FW – fresh weight. 2. DW - dry weight. 3. < LOD – below the limit of detection for the assay. 4. NA – not applicable.

### 4.1.3 Safety of Cry14Ab1 in GMB151 grain

### Bioinformatic analyses of Cry14Ab1

The Applicant provided the results of *in silico* analyses comparing the Cry14Ab1 amino acid sequence to known allergenic proteins in the COMPARE dataset, using the same search criteria as outlined in [Section 3.4.5.1](#_3.4.5.1__Bioinformatic). The search did not identify any known allergens with homology to Cry14Ab1.

The Applicant also provided the results of *in silico* analyses comparing the amino acid sequence of Cry14Ab1 to proteins identified as “toxins” from the NCBI protein databases. As expected, the top 1000 proteins matched other documented Cry proteins in *B. thuringiensis* and other *Bacillus* strains. The degree of similarity of the Cry proteins was associated with the known target group of the proteins. For other nematocidal proteins, the sequence similarity ranged from 100% for the Cry14Ab1 protein to 20% for a Cry30-like protein. Similarity to Cry1 and Cry2 proteins, targeting Lepidoptera, was low. Comparison of the Cry14Ab1 sequence to a toxin-specific database did not identify toxins of concern for humans or other mammals.

### Structural stability after exposure to heat and digestive enzymes

Bacterial-produced Cry14Ab1 protein was incubated for 30 min at temperatures ranging from 25-95°C. A control sample was kept at 4°C. An aliquot of the control and heated protein samples were run on SDS-PAGE and stained with Coomassie Blue or analysed by western blotting. In both the Coomassie stained gels and western blots, no visible degradation or decrease in band intensity was observed in the control (4°C), 25, 37 or 55°C treated samples, however there were observable loss of band intensity in the 75 and 95°C treated samples. These data indicate that the Cry14Ab1 protein is heat labile at temperatures from 75°C and above.

Bacterial-produced Cry14Ab1 protein (test protein) was incubated with porcine pepsin (10U enzyme/μg protein) or porcine pancreatin (190 μg enzyme/μg protein) at 37°C for 0-60 min, in a simulated gastric fluid (SGF) system at an acidic pH or simulated intestinal fluid (SIF) system at a neutral pH, respectively (Thomas et al. 2004). Controls included a no enzyme control (test protein only) and a test protein control (no enzyme) incubated for 0 and 60 min. The extent of digestion was visualised by protein gel staining and western blotting. The LOD for Cry14Ab1 that can be detected by western blot is 1 ng. Pepsin-sensitive horse radish peroxidase and pepsin-resistant ovalbumin were used to confirm pepsin activity. Pancreatin activity was confirmed with azoalbumin.

The results from the pepsin digestions showed that by 0.5 min, there was no visible Cry14Ab1 remaining in the reaction mix. There was no loss of band intensity in the no enzyme control incubated for 60 min therefore the loss of protein in the reaction mix indicated that Cry14Ab1 was being fully digested by pepsin. Results from the SIF digestion showed that Cry14Ab1 was partially resistant to pancreatin digestion. The main Cry14Ab1 band intensity decreased over time and there was an associated increase in degradation products but there was still presence of full Cry14Ab1 at 60 min. There was no loss of band intensity in the no enzyme control incubated for 60 min therefore the decreased band intensity seen in the reaction mix samples showed that protein loss was due to pancreatin activity rather than protein instability.

### Acute toxicity studies

C57BL/6J mice (6/sex/group, aged 8 weeks) were administered 2000 mg/kg bw Cry14Ab1 or the equivalent volume of vehicle control on day 1. Clinical signs were monitored daily, while body weight and food consumption were monitored weekly. At study termination, blood samples were collected for haematology and a gross post-mortem performed. Samples were then collected for histopathology.

No mortalities occurred during the study and no treatment-related clinical signs were observed. Mean weekly body weights and body weight gains in all treatment groups were similar to those of controls. Food consumption was similar in all groups. No macroscopic changes were reported at necropsy therefore histopathology was not required. The data shows Cry14Ab1 at 2000 mg/kg bw did not produce signs of systemic toxicity and was well tolerated by mice.

### 4.1.4 Conclusion

Biochemical analysis of Cry14Ab1 expressed *in planta* showed that the protein had the expected molecular mass, peptide sequence and function. Analysis of an equivalent Cry14Ab1 protein expressed in bacteria confirmed that the protein was heat labile and susceptible to peptic digestion. *In silico* analyses did not identify any significant similarity with known allergens or toxins. An acute toxicity study in mice demonstrated that Cry14Ab1 did not produce systemic toxicity. Taken together, this indicates that Cry14Ab1 is unlikely to be toxic or allergenic to humans.

## 4.2 Description of the HPPD protein

HPPD is a ubiquitously expressed enzyme involved in the catabolism of tyrosine to homogentisate. The fate of homogentisate differs between photosynthetic and non-photosynthetic organisms (Shaner 2003; Moran 2005). In photosynthetic organisms, homogentisate is converted to plastoquinones and alpha-tocopherol (vitamin E). Plastoquinones have a few roles in plants, from acting as an electron carrier between photosystem II and the cytochrome b6f complex, to being a co-factor for the enzyme phytoene desaturase, involved in carotenoid formation. In animals, homogentisate is converted to fumarate and acetoacetate, which can be directed into the citric acid cycle for cellular energy generation.

Isoxaflutole is an inhibitor of HPPD resulting in a decrease in plastoquinones (Pallett et al. 1998; 2001; Little and Pallett, 2003). Decreasing plastiquinone levels directly impacts photosynthesis and phytoene desaturase activity, limiting formation of carotenoids, reducing their photo-protective effect on chlorophylls (Anderson and Robertson, 1960; Mayfield et al. 1986). This in turn leads to photo-bleaching of the foliage and a reduction in the plants’ ability to undergo further photosynthesis, resulting in plant death.

The HPPD protein expressed in GMB151 contains four amino acid changes compared to the source protein from *P. fluorescens* (Sailland et al. 2001). This mutant form of the enzyme shows reduced binding to HPPD-inhibitor herbicides, thus allowing the plant to survive when exposed to these herbicides.

Analysis performed by FSANZ revealed that the HPPD protein from *P. fluorescens* has low sequence homology to plant-derived forms. However, the similarity in sequence between common food crops is also highly variable. Related plants share high homology, illustrated by a comparison of the predicted potato and tomato HPPD proteins that show > 95% homology. Comparison of the HPPD sequence from potato with cotton, soybean and corn showed sequence variation from 55-75%. Humans have therefore been exposed to many different forms of the HPPD protein via the food supply. FSANZ has also previously assessed the HPPD*Pf* W336 protein in soybean line FG72 and cotton line GHB811 (FSANZ 2011; 2018).

The *hppdPf-4Pa* gene prepared by the Applicant encodes a protein of 482 amino acids, consisting of a 124 amino acid transit peptide and a mature protein of 358 amino acids. After cleavage of the plastid transit peptide, the final mature protein will have an expected molecular mass of 40.4 kDa.

### 4.2.1 Characterisation of HPPD-4 expressed in GMB151 and equivalence to bacterially-produced forms

Protein was extracted from ground leaf material from both GMB151 (T7) and the Thorne parental control. Affinity chromatography was performed to isolate any fraction that bound to an HPPD-4 antibody. Aliquots of purified protein were run on SDS-PAGE and visualised with Coomassie stain. A sample gel was presented and the results showed a clear band in the GMB151 lane with a molecular mass around 37 kDa. There was no equivalent band in the parental control lane. Western blot analysis confirmed that the protein in GMB151 was immunoreactive to a HPPD-4-specific antibody, with a size just above 37 kDa. The data from the gel analyses places the mass of the protein around 37 kDa.

Mass spectrometry identified three polypeptides in the extract from GMB151, with unique molecular masses ([Table 7](#Table7)). N-terminal sequencing showed the presence of two amino-termini versions, differing in the region around the cleavage site between the transit peptide and mature protein (Emanuelsson et al. 1999; Rowland et al. 2015; FSANZ 2018). The sequence for the amino-terminus of Form 1 & 2 was XMADLYENPM whereas Form 3 had the sequence ADLYENPM, without the methionine at position 1. This difference in the N-terminal sequence between the polypeptides was also confirmed by peptide mapping. ([Table 7](#Table7); [Figure 4](#Figure4)). Peptide mapping also showed differences at the carboxyl-terminus, where there was absence of the final four amino acids (-LTAD) in Form 2 & 3. The potential truncation of the protein at the carboxyl-terminus may be explained by degradation of the short fragment resulting from the pre-analysis trypsin digest. Peptide mapping did show that the protein sequence between the termini matched the expected HPPD-4 sequence, with 73% sequence coverage achieved ([Figure 4](#Figure4)).

Table 7: Characterisation of HPPD-4 protein by mass spectrometry

|  |  |  |
| --- | --- | --- |
| **Form** | **Mass (Da)** | **Amino acids** |
| 1 | 40415.1 | 1-358 |
| 2 | 40041.7 | 1-354 |
| 3 | 39748.3 | 2-354 |



Figure 4: Tryptic peptide map of the GMB151-produced HPPD-4 protein. The deduced amino acid sequence is 358 amino acids as shown. Boxed regions correspond to peptide sequence coverage achieved using MALDI-TOF MS, from the plant-produced protein sample.

The protein was shown to be functional *in planta* due to the demonstrated resistance to the HPPD-inhibitor tembotrione during selection of transformants ([Section 3.1](#_3.1_Transformation_Method)) and tolerance to isoxaflutole during cultivation ([Section 5.2](#_5.2_Study_design)). Finally, the protein was shown by a modified Periodic Acid-Schiff staining procedure to be non-glycosylated. This data indicates that no unintentional glycosylation has occurred in the modified plant expressed protein.

The mature HPPD*-*4 protein was also expressed in *E. coli*. The bacterially-derived protein was shown by electrophoresis and western blotting to have a similar mass as the protein extracted from GMB151. Western blotting also showed that the protein was immunoreactive to a HPPD antibody. Mass spectrometry showed the mass was 40181.1 Da. Peptide mapping and N-terminal sequencing confirmed the sequence matched HPPD-4 with no N-terminal methionine. The expressed protein was also shown to be non-glycosylated. These data demonstrates that the bacterially-produced protein is a suitable standard for the ELISA used to detect HPPD-4 in plant tissues (as discussed in [Section 4.2.2](#_4.2.2_Expression_of)) and is a suitable surrogate for use in the safety assessment experiments described in [Section 4.2.3](#_Structural_stability_after_1).

### 4.2.2 Expression of the HPPD-4 in GMB151 grain

HPPD-4 expression was also examined in the same processed components of soybean analysed for Cry14Ab1 ([Section 4.1.2](#_4.1.2_Expression_of)) using a commercially available ELISA. The standard curve was generated using microbially expressed HPPD-4, of which the characterisation is described in [Section 4.2.1](#_4.2.1_Characterisation_of). Background controls included no protein vehicle controls and protein isolated from the equivalent processed fractions from Thorne.

The data from the ELISA shows that HPPD-4 is detectable in the whole seed, seed hulls and protein isolate in both untreated and herbicide-treated GMB151 ([Table 8](#Table8)). No HPPD-4 was detected in the more highly processed products: toasted meal and refined oil.

Table 8: Mean expression levels of HPPD-4 in GMB151 grain components

|  |  |  |  |
| --- | --- | --- | --- |
| **Matrix** | **Herbicide treated** | **HPPD-4 (µg/g FW)1** | **HPPD-4 (µg/g DW)2** |
| **Grain** | - | 3.34 | 3.79 |
| + | 4.91 | 5.57 |
| **Toasted meal** | - | < LOD3 | < LOD |
| + | < LOD | < LOD |
| **Refined oil** | - | < LOD | NA4 |
| + | < LOD | NA |
| **Protein isolate** | - | 0.78 | 0.80 |
| + | 0.47 | 0.48 |
| **Hulls** | - | 0.23 | 0.27 |
| + | 0.36 | 0.42 |

1. FW – fresh weight. 2. DW - dry weight. 3. < LOD – below the limit of detection for the assay. 4. NA – not applicable.

### 4.2.3 Safety of HPPD-4 in GMB151 grain

### Bioinformatic analyses of HPPD-4

The Applicant provided the results of *in silico* analyses comparing the HPPD-4 amino acid sequence to known allergenic proteins in the COMPARE dataset, using the same search criteria as outlined in [Section 3.4.5.1](#_3.4.5.1__Bioinformatic). The search did not identify any known allergens with amino acid sequence similarity to this protein.

The Applicant also provided the results of *in silico* analyses comparing the amino acid sequence of HPPD-4 to proteins identified as “toxins” from the NCBI protein databases. The search did match to other HPPD proteins from a range of species but, did not match to proteins with known toxicity. Comparison of the HPPD-4 sequence to a toxin-specific database did not identify any similarities to known toxins within the threshold limits.

### Structural stability after exposure to heat and digestive enzymes

Following the same protocols described in [Section 4.1.3](#_Structural_stability_after), HPPD-4 was shown to become insoluble, possibly forming aggregates, at temperatures ≥ 55°C, was fully digested with pepsin by 0.5 min and with pancreatin by 10 min. This data indicates that HPPD-4 is heat denatured and is readily degraded by enzymes found in the digestive system.

### Acute toxicity studies

The applicant provided an acute toxicity study on HPPD-4, using the same test guidelines outlined in [Section 4.1.3](#_Acute_toxicity_studies).

Similar to the results for Cry14Ab1, there were no mortalities or treatment-related clinical signs reported during the study. Mean weekly body weights and body weight gains in all treatment groups were similar to those of controls. Food consumption was similar in all groups. No macroscopic changes were reported at necropsy therefore histopathology was not required. The data shows HPPD-4 at 2000 mg/kg bw did not produce signs of systemic toxicity and was well tolerated by mice.

### 4.2.4 Conclusion

Biochemical analysis of HPPD-4 expressed *in planta* showed that the protein had the expected molecular mass, peptide sequence and function. Analysis of an equivalent HPPD-4 protein expressed in bacteria confirmed that the protein was heat labile and susceptible to peptic and pancreatic digestion. *In silico* analyses did not identify any significant similarity with known allergens or toxins. An acute toxicity study in mice demonstrated that HPPD-4 did not produce systemic toxicity. Taken together, this indicates that HPPD-4 is unlikely to be toxic or allergenic to humans.

## 4.4 Novel herbicide metabolites in GM herbicide-tolerant plants

FSANZ has previously assessed the novel herbicide metabolites for isoxaflutole. In the isoxaflutole-tolerant soybean event FG72 expressing the HPPD*Pf* W336 protein, (A1051; FSANZ 2011), the parental isoxaflutole and three major metabolites were identified. The same metabolites were also identified in non-GM corn varieties exposed to isoxaflutole (Pallett et al. 1998; Sailland et al. 2001), indicating that the metabolites are consistent across plant species. It is expected that no new isoxaflutole metabolites would be generated in soybean event GMB151.

# 5 Compositional analysis

The main purpose of compositional analyses is to determine if, as a result of the genetic modification, an unexpected change has occurred to the food. These changes could take the form of alterations in the composition of the plant and its tissues and thus its nutritional adequacy. Compositional analyses can also be important for evaluating the intended effect where there has been a deliberate change to the composition of the food.

The classic approach to the compositional analyses of GM food is a targeted one. Rather than analysing every possible 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 such 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.

## 5.1 Key Components

The key components to be analysed for the comparison of transgenic and conventional soybean are outlined in the OECD Consensus Document on Compositional Considerations for New Varieties of Soybean (OECD 2012), and include: proximates and fibre, amino acids, fatty acids, tocopherol (vitamin E), vitamin K1 and the anti-nutrients phytic acid, stachyose. raffinose, lectins and isoflavones.

## 5.2 Study design

Eight successful field trials were conducted for GMB151 in the US in the 2017 growing seasons. The sites[[5]](#footnote-5) were selected to match the typical geographical and field management styles of the commercial soybean growing regions. The agronomic practices and pest control measures used were location-specific and were typical for all aspects of soybean cultivation including soil preparation, fertiliser application, irrigation and pesticide-based control methods. The materials tested in the field trials included GMB151, the parental varietal Thorne and a range of reference varieties. Three reference varieties were grown at each site and were selected from E2282, E2692, E2993, E3066, E3192, E3494, NGN 3121STS, NGN 3292C and NGN 3347C

The field trials were established in a randomised complete block design, with four replicates of each plot. Plots were separated by a combination of fallow alleyways and planted buffers of conventional non-GM soybean. Replicate plots of GMB151 were cultivated with and without herbicide treatment. Isoxaflutole was applied at the pre-emergence stage at 70.1 g active ingredient (ai) per hectare (ha). The spray volume was 139 L/ha.

Soybean grains were harvested from all plots at maturity. After harvest, samples were despatched to an analytical laboratory under full identity labelling. The analyses were performed at EPL Bio Analytical Services. The compositional analyses were based on internationally recognised procedures including official methods specified by the Association of Official Analytical Chemists (AOAC), the USDA and published articles or technical notes from industrial-based sources.

A total of 81 different analytes were measured. Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC). For each analyte, ‘descriptive statistics’ (mean and standard error) were generated. A linear mixed model analysis of variance was then applied for combined data, and locations, covering the eight replicated field trial sites. The mixed model analysis was also applied to the data from each site separately. In assessing the significance of any difference between the mean analyte value for GMB151 and the parental control, a P-value of 0.05 was used. Where statistically significant differences were observed in the combined data from all sites (presented in Tables 9-13), analysis of the data from each site was used to determine if the differences were common to the majority of sites.

In order to complete the statistical analysis for any component in this study, it was deemed that more than 2/3rd of the values must be greater than the assay LOQ. If analytes had more than 2/3rd of observations below the LOQ for that assay, they were excluded from the overall summary analysis. Values for all components were expressed on a dry weight basis with the exception of moisture, expressed as percent fresh weight, and fatty acids, expressed as percent of total fatty acids.

Compositional data from the non-GM reference varieties grown concurrently in the same trial as GMB151 and the parental control, were combined across all sites and used to calculate a 99% tolerance interval for each component to define the natural variability in soybean varieties that have a history of safe consumption. Any statistically significant differences between GMB151 and the control Thorne were also compared to this tolerance interval to assess whether the differences were likely to be biologically meaningful.

## 5.3 Analyses of key components in soybean grain

### 5.3.1 Proximates and fibre

Analysis of the proximate and fibre levels in both untreated and treated GMB151 compared to the parental control ([Table 9](#Table9)), showed statistically significant differences in moisture, carbohydrate and crude protein. There was also a statistically significant difference in the neutral detergent fibre content between the treated GMB151 and parental control. While there was a statistically significant change, the magnitude of these differences was actually small (~1.7-7.4%) and the mean levels fall within the reference ranges and tolerance interval, indicating that the differences are not biologically significant.

Table 9: Comparison of Proximates and Fibre (% DW)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **Non-GM counterpart** | **GMB151 untreated** | **GMB151 treated** | **Non-GM reference varieties** | **Tolerance Interval** |
| **Mean ± SD** | **Mean ± SD** | **Mean ± SD** | **Min-max** | **lower-upper** |
| Moisture1 | 12.2 ± 2.70 | 11.5 ± 2.332 | 11.3 ± 2.30 | 8.9 - 19.4 | 4.0 - 19.7 |
| Ash | 4.93 ± 0.23 | 4.91 ± 0.19 | 4.94 ± 0.24 | 4.09 - 5.63 | 4.27 - 5.60 |
| Carbohydrate | 35.2 ± 0.85 | 34.4 ± 0.97 | 34.5 ± 1.14 | 32.3 - 39.1 | 32.1 - 40.0 |
| Crude protein | 39.7 ± 1.07 | 40.4 ± 1.37 | 40.4 ± 1.37 | 33.6 - 44.1 | 32.6 - 45.5 |
| Crude fat | 20.2 ± 0.77 | 20.3 ± 0.99 | 20.1 ± 0.92 | 17.5 - 23.8 | 15.8 - 24.2 |
| Acid Detergent Fibre | 16.2 ± 2.10 | 15.5 ± 1.63 | 15.7 ± 1.41 | 10.6 - 21.2 | 9.8 - 20.9 |
| Neutral Detergent Fibre | 16.3 ± 1.52 | 16.1 ± 0.97 | 15.7 ± 1.00 | 13.1 - 19.1 | 11.7 - 20.1 |
| Total Dietary Fibre | 17.5 ± 1.64 | 17.4 ± 1.68 | 17.4 ± 2.07 | 12.1 - 21.9 | 12.2 - 23.6 |

1. Moisture content is expressed as % fresh weight (% FW) whereas the other values are expressed as % dry weight (% DW). 2. Cells highlighted in blue show statistically significant data.

### 5.3.2 Amino acids

There were no statistically significant changes in the levels of amino acids in the untreated and treated GMB151 compared to control ([Table 10](#Table10)).

Table 10: Comparison of Amino Acids (% DW)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **Non-GM counterpart** | **GMB151 untreated** | **GMB151 treated** | **Non-GM reference varieties** | **Tolerance Interval** |
| **Mean ± SD** | **Mean ± SD** | **Mean ± SD** | **Min-max** | **lower-upper** |
| Alanine | 1.74 ± 0.066 | 1.75 ± 0.071 | 1.76 ± 0.083 | 1.48 - 2.00 | 1.43 - 1.98 |
| Arginine | 2.72 ± 0.182 | 2.77 ± 0.217 | 2.78 ± 0.245 | 2.05 - 3.48 | 1.90 - 3.50 |
| Aspartic Acid | 4.75 ± 0.149 | 4.84 ± 0.283 | 4.82 ± 0.286 | 3.93 - 5.66 | 3.68 - 5.80 |
| Cysteine | 0.514 ± 0.053 | 0.529 ± 0.051 | 0.527 ± 0.055 | 0.314 - 0.645 | 0.285 - 0.692 |
| Glutamic Acid | 7.49 ± 0.25 | 7.68 ± 0.43 | 7.64 ± 0.46 | 6.15 - 8.81 | 5.85 - 9.21 |
| Glycine | 1.76 ± 0.09 | 1.79 ± 0.10 | 1.79 ± 0.11 | 1.41 - 1.96 | 1.40 - 2.06 |
| Histidine | 1.08 ± 0.067 | 1.09 ± 0.076 | 1.08 ± 0.091 | 0.845 - 1.25 | 0.815 - 1.32 |
| Isoleucine | 1.88 ± 0.081 | 1.90 ± 0.088 | 1.90 ± 0.100 | 1.52 - 2.13 | 1.50 - 2.19 |
| Leucine | 3.08 ± 0.12 | 3.12 ± 0.14 | 3.11 ± 0.16 | 2.53 - 3.51 | 2.46 - 3.59 |
| Lysine | 2.89 ± 0.24 | 2.92 ± 0.23 | 2.92 ± 0.19 | 2.33 - 3.41 | 2.11 - 3.56 |
| Methionine | 0.513 ± 0.039 | 0.516 ± 0.039 | 0.528 ± 0.035 | 0.376 - 0.583 | 0.361 - 0.625 |
| Phenylalanine | 2.06 ± 0.15 | 2.09 ± 0.16 | 2.09 ± 0.20 | 1.58 - 2.56 | 1.50 - 2.61 |
| Proline | 2.07 ± 0.088 | 2.11 ± 0.100 | 2.11 ± 0.118 | 1.66 - 2.46 | 1.61 - 2.46 |
| Serine | 2.11 ± 0.11 | 2.13 ± 0.12 | 2.13 ± 0.13 | 1.71 - 2.35 | 1.67 - 2.46 |
| Threonine | 1.63 ± 0.071 | 1.64 ± 0.085 | 1.64 ± 0.095 | 1.33 - 1.79 | 1.30 - 1.88 |
| Tryptophan | 0.542 ± 0.025 | 0.527 ± 0.041 | 0.531 ± 0.022 | 0.370 - 0.603 | 0.398 - 0.624 |
| Tyrosine | 1.24 ± 0.10 | 1.26 ± 0.10 | 1.25 ± 0.12 | 0.92 - 1.46 | 0.88 - 1.56 |
| Valine | 1.90 ± 0.076 | 1.92 ± 0.084 | 1.93 ± 0.104 | 1.55 - 2.21 | 1.52 - 2.23 |

### 5.3.3 Vitamins and minerals

Sodium was excluded from the statistical summary as there was more than 2/3rd of observations below the LOQ.

Statistically significant decreases were observed for vitamin B1 and B9, and the minerals calcium, copper and zinc in the herbicide-treated GMB151 compared to control ([Table 11](#Table11)). There was also an increase in vitamin B5 in the herbicide-treated GMB151 compared to control. The magnitude of the change ranged from 8-23%. However, the mean levels fall within the reference ranges and tolerance interval, indicating that this difference was not biologically significant.

Table 11: Comparison of Vitamins and Minerals (mg/kg DW)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **Non-GM counterpart** | **GMB151 untreated** | **GMB151 treated** | **Non-GM reference varieties** | **Tolerance Interval** |
| **Mean ± SD** | **Mean ± SD** | **Mean ± SD** | **Min-max** | **lower-upper** |
| Vitamin E | 27.6 ± 9.59 | 27.4 ± 9.67 | 28.1 ± 9.36 | 12.3 - 53.2 | 0 - 57.2 |
| Vitamin B1 | 1.80 ± 0.72 | 1.67 ± 0.75 | 1.46 ± 0.741 | 0.41 - 2.54 | 0 - 2.93 |
| Vitamin B2 | 4.44 ± 0.74 | 4.51 ± 0.70 | 4.33 ± 0.88 | 2.26 - 5.92 | 2.32 - 6.03 |
| Vitamin B3 | 26.7 ± 6.57 | 29.2 ± 6.34 | 29.3 ± 6.58 | 13.7 - 46.9 | 5.7 - 47.0 |
| Vitamin B5 | 8.64 ± 1.76 | 9.27 ± 2.23 | 9.50 ± 2.22 | 5.42 - 14.60 | 4.01 - 16.05 |
| Vitamin B6 | 4.59 ± 1.18 | 4.63 ± 1.13 | 4.81 ± 1.20 | 2.12 - 7.69 | 0.85 - 8.43 |
| Vitamin B9 | 5.50 ± 1.64 | 6.43 ± 2.02 | 6.77 ± 2.50 | 2.19 - 13.30 | 0 - 12.57 |
| Vitamin K1 | 0.703 ± 0.330 | 0.700 ± 0.334 | 0.707 ± 0.335 | 0.344 - 1.440 | 0 - 1.592 |
| Calcium | 2547 ± 362 | 2435 ± 345 | 2343 ± 334 | 1480 - 3689 | 1004 - 3987 |
| Copper | 14.8 ± 4.50 | 14.8 ± 3.44 | 13.3 ± 2.61 | 6.8 - 23.2 | 3.2 - 20.7 |
| Iron | 118 ± 29.2 | 118 ± 41.1 | 115 ± 45.3 | 59 - 364 | 0 - 233 |
| Magnesium | 2580 ± 201 | 2497 ± 204 | 2498 ± 268 | 1954 - 3177 | 1758 - 3278 |
| Manganese | 39.2 ± 9.72 | 40.4 ± 9.79 | 38.2 ± 10.46 | 23.8 - 74.7 | 3.7 - 68.1 |
| Phosphorus | 6135 ± 762 | 6170 ± 673 | 6090 ± 730 | 4789 - 7773 | 4088 - 8109 |
| Potassium | 19545 ± 1214 | 19937 ± 1601 | 19862 ± 1425 | 16973 - 22831 | 15691 - 24021 |
| Zinc | 58.4 ± 11.47 | 57.7 ± 13.47 | 54.2 ± 9.90 | 33.0 - 85.6 | 18.0 - 80.6 |

1. Cells highlighted in blue show statistically significant data.

### 5.3.4 Fatty Acids

The following fatty acids were excluded from the statistical summary as they had more than 2/3rd of observations below the LOQ: 8:0 caprylic acid, 10:0 capric acid, 12:0 lauric acid, 14:1 myristoleic acid, 15:0 pentadecanoic acid, 15:1 pentadecenoic acid, 18:4 stearidonic acid, 19:0 nonadecanoic acid, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, 20:4 arachidonic acid, 20:5 eicosapentaenoic acid, 22:1 erucic acid, 22:5 N3 docosapentaenoic acid, 22:5 N6 docosapentaenoic acid and 22:6 docosahexaenoic acid.

Statistically significant differences were observed for palmitic acid (🠇 3-4% change), heptadecenoic acid (🠅 5%), oleic acid (🠅 7-8%), linoleic acid (🠇 2%), eicosenoic acid (🠅 4-5%) and behenic acid (🠅 14-16%) in both the untreated and treated GMB151 lines compared to control ([Table 12](#Table12)). A statistically significant decrease was observed in lignoceric acid (🠇 12% change) in treated GMB151 compared to control. For the changes reported for eicosenoic and behenic acids, the significant differences were observed at 2 of 8 and 4 of 8 sites, respectively. The magnitude of these changes for these fatty acids are small and the mean values fall within the reference ranges and tolerance interval, indicating that these differences are not biologically significant.

Table 12: Comparison of Fatty Acids (% Total Fatty Acids)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **Non-GM counterpart** | **GMB151 untreated** | **GMB151 treated** | **Non-GM reference varieties** | **Tolerance Interval** |
| **Mean ± SD** | **Mean ± SD** | **Mean ± SD** | **Min-max** | **lower-upper** |
| C14:0 Myristic Acid | 0.070 ± 0.007 | 0.069 ± 0.007 | 0.070 ± 0.008 | 0.057 - 0.087 | 0.052 - 0.090 |
| C16:0 Palmitic Acid | 11.0 ± 0.24 | 10.6 ± 0.27 | 10.7 ± 0.27 | 10.7 - 12.4 | 10.2 - 12.8 |
| C16:1 Palmitoleic Acid | 0.083 ± 0.005 | 0.081 ± 0.004 | 0.085 ± 0.005 | 0.065 - 0.101 | 0.059 - 0.108 |
| C17:0 Heptadecanoic Acid | 0.091 ± 0.005 | 0.090 ± 0.007 | 0.090 ± 0.006 | 0.073 - 0.108 | 0.064 - 0.116 |
| C17:1 Heptadecenoic Acid | 0.062 ± 0.004 | 0.065 ± 0.004 | 0.065 ± 0.005 | 0.026 - 0.067 | 0.043 - 0.072 |
| C18:0 Stearic Acid | 4.38 ± 0.32 | 4.34 ± 0.32 | 4.38 ± 0.34 | 3.79 - 5.24 | 3.41 - 5.36 |
| C18:1 Oleic Acid | 21.8 ± 1.37 | 23.6 ± 2.39 | 23.4 ± 2.05 | 17.2 - 28.0 | 15.3 - 28.8 |
| C18:2 Linoleic Acid | 53.7 ± 1.18 | 52.6 ± 1.80 | 52.6 ± 1.51 | 49.4 - 56.9 | 47.2 - 58.3 |
| C18:3 Linolenic Acid | 7.81 ± 0.43 | 7.49 ± 0.52 | 7.52 ± 0.51 | 6.26 - 10.40 | 5.00 - 10.98 |
| C20:0 Arachidic Acid | 0.315 ± 0.018 | 0.320 ± 0.015 | 0.324 ± 0.019 | 0.277 - 0.393 | 0.250 – 0.403 |
| C20:1 Eicosenoic Acid | 0.182 ± 0.019 | 0.189 ± 0.022 | 0.191 ± 0.019 | 0.135 - 0.256 | 0.115 - 0.260 |
| C22:0 Behenic Acid | 0.331 ± 0.010 | 0.378 ± 0.013 | 0.382 ± 0.012 | 0.320 - 0.390 | 0.291 - 0.401 |
| C24:0 Lignoceric Acid | 0.111 ± 0.021 | 0.101 ± 0.024 | 0.098 ± 0.032 | 0.038 - 0.180 | 0 - 0.200 |

1. Cells highlighted in blue show statistically significant data.

### 5.3.5 Anti-nutrients

A statistically significant increase (10%) was observed in phytic acid in the GMB151 treated line compared to Thorne ([Table 13](#Table13)). This increase was only observed in measurements from a single site from the eight field trial sites. The mean value falls within the reference range and tolerance interval, indicating that this differences is not biologically significant. Statistically significant decreases (13-17%) were observed for both individual and total isoflavones in untreated and treated GMB151. The changes fall within the reference range and tolerance intervals, indicating these minor differences are not biologically significant.

Table 13: Comparison of Anti-nutrients

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **Non-GM counterpart** | **GMB151 untreated** | **GMB151 treated** | **Non-GM reference varieties** | **Tolerance Interval** |
| **Mean ± SD** | **Mean ± SD** | **Mean ± SD** | **Min-max** | **lower-upper** |
| Phytic acid | 1.45 ± 0.25  | 1.53 ± 0.23  | 1.60 ± 0.25  | 0.88 – 2.11 | 0.77 – 2.26 |
| Lectins | 2.54 ± 0.71  | 2.44 ± 0.67  | 2.43 ± 0.76  | 0.78 – 4.64 | 0.23 – 4.41 |
| Raffinose | 1.02 ± 0.23 | 1.03 ± 0.26 | 0.99 ± 0.21 | 0.40 - 1.38 | 0.39 - 1.40 |
| Stachyose | 3.18 ± 0.18 | 3.18 ± 0.18 | 3.19 ± 0.25 | 2.59 - 4.50 | 2.40 - 4.53 |
| Trypsin inhibitor | 35.8 ± 5.66 | 35.9 ± 4.74 | 36.3 ± 5.88 | 18.1 - 41.8 | 15.1 - 44.4 |
| Isoflavones (% DW) |
|  Daidzein | 850 ± 217 | 712 ± 199 | 709 ± 203 | 380 - 1696 | 0 - 1788 |
|  Genistein | 808 ± 211 | 690 ± 192 | 703 ± 211 | 428 - 1498 | 236 - 1582 |
|  Glycitein  | 160 ± 29.0 | 136 ± 32.2 | 134 ± 18.2 | 102 - 350 | 24 - 320 |
|  Total isoflavones | 1818 ± 436 | 1538 ± 396 | 1546 ± 421 | 947 - 3455 | 364 - 3506 |

## 5.4 Conclusion

Of the 81 analytes measured in soybean grain, mean values were provided for 64 analytes. A summary of the statistically significant analytes in GMB151 is provided in [Table 14](#Table14). These differences fall well within the reference ranges of the commercial non-GM lines. Like any food crop, nutrient and anti-nutrient composition of soybean grain can be impacted by cultivation site and agricultural practices. The differences reported here are consistent with the normal biological variability that exists in soybean.

Overall, the compositional data are consistent with the conclusion that there are no biologically significant differences in the levels of key constituents in GMB151 when compared with conventional soybean cultivars already available in agricultural markets.

Table 14: Summary of statistically significant compositional differences between GMB151 and the parental control Thorne.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameter** | **Non-GM counterpart** | **GMB151 untreated** | **GMB151 treated** | **Are values within the reference ranges?** |
| **Mean ± SEM** | **Mean ± SEM** | **Mean ± SEM** | **Yes / No** |
| Moisture | 12.2 ± 2.70 | 11.5 ± 2.33 | 11.3 ± 2.30 | Yes |
| Carbohydrate | 35.2 ± 0.85 | 34.4 ± 0.97 | 34.5 ± 1.14 | Yes |
| Crude protein | 39.7 ± 1.07 | 40.4 ± 1.37 | 40.4 ± 1.37 | Yes |
| Neutral Detergent Fibre | 16.3 ± 1.52 | 16.1 ± 0.97 | 15.7 ± 1.00 | Yes |
| Vitamin B1 | 1.80 ± 0.72 | 1.67 ± 0.75 | 1.46 ± 0.74 | Yes |
| Vitamin B5 | 8.64 ± 1.76 | 9.27 ± 2.23 | 9.50 ± 2.22 | Yes |
| Vitamin B9 | 5.50 ± 1.64 | 6.43 ± 2.02 | 6.77 ± 2.50 | Yes |
| Calcium | 2547 ± 362 | 2435 ± 345 | 2343 ± 334 | Yes |
| Copper | 14.8 ± 4.50 | 14.8 ± 3.44 | 13.3 ± 2.61 | Yes |
| Zinc | 58.4 ± 11.47 | 57.7 ± 13.47 | 54.2 ± 9.90 | Yes |
| C16:0 Palmitic Acid | 11.0 ± 0.24 | 10.6 ± 0.27 | 10.7 ± 0.27 | Yes |
| C17:1 Heptadecenoic Acid | 0.062 ± 0.004 | 0.065 ± 0.004 | 0.065 ± 0.005 | Yes |
| C18:1 Oleic Acid | 21.8 ± 1.37 | 23.6 ± 2.39 | 23.4 ± 2.05 | Yes |
| C18:2 Linoleic Acid | 53.7 ± 1.18 | 52.6 ± 1.80 | 52.6 ± 1.51 | Yes |
| C20:1 Eicosenoic Acid | 0.182 ± 0.019 | 0.189 ± 0.022 | 0.191 ± 0.019 | Yes |
| C22:0 Behenic Acid | 0.331 ± 0.010 | 0.378 ± 0.013 | 0.382 ± 0.012 | Yes |
| C24:0 Lignoceric Acid | 0.111 ± 0.021 | 0.101 ± 0.024 | 0.098 ± 0.032 | Yes |
| Phytic acid | 1.45 ± 0.25  | 1.53 ± 0.23  | 1.60 ± 0.25 | Yes |
| Total isoflavones | 1818 ± 436 | 1538 ± 396 | 1546 ± 421 | Yes |
|  Daidzein | 850 ± 217 | 712 ± 199 | 709 ± 203 | Yes |
|  Genistein | 808 ± 211 | 690 ± 192 | 703 ± 211 | Yes |
|  Glycitein  | 160 ± 29.0 | 136 ± 32.2 | 134 ± 18.2 | Yes |

1. Cells highlighted in red show data where GMB151 is significantly lower than the parental Thorne cultivar.

2. Cells highlighted in green show data where GMB151 is significantly higher than the parental Thorne cultivar.

# 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 wellbeing. In most cases, this can be achieved through a detailed understanding of the genetic modification and its consequences, together with an extensive compositional analysis of the food, such as that presented in [Section 5](#_5_Compositional_analysis) of this report.

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 or other animal species will add little to the safety assessment (Bartholomaeus et al. 2013; OECD 2003). If the compositional analysis indicates biologically significant changes, either intended or unintended, to the levels of certain nutrients in the GM food, additional nutritional studies should be undertaken to assess the potential impact of the changes on the whole diet.

GMB151 is the result of a simple genetic modification to confer protection from parasitic nematodes and tolerance to HPPD inhibitor herbicides, with no intention to significantly alter nutritional parameters in the food. The compositional analyses have demonstrated that the genetic modification has not altered the nutritional adequacy of GMB151 as a source of food when compared with that of conventional soybean varieties. The introduction of foods derived from GMB151 into the food supply is therefore expected to have negligible nutritional impact.

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2. <https://www.fas.usda.gov/commodities/soybeans> [↑](#footnote-ref-2)
3. <http://comparedatabase.org/database/> [↑](#footnote-ref-3)
4. Field trial sites for testing Cry14Ab1 expression levels: Kimballton; IA; Fisk, MO, US. [↑](#footnote-ref-4)
5. The location of the eight field trial sites: Richland, IA; York, NE; Elk Horn, IA; Stewardson, IL; Germansville, PA; Fisk, MO; Larned, KS; Carlyle, IL. [↑](#footnote-ref-5)