Position papers

ACNFP advice on the safety of genetically modified GMB151 soybean for food and feed uses

Reference Number RP1123

Regulated Product Dossier Assessment

Assessment finalised: 5th of April 2024

Summary

Following the submission of application RP1123 to the Food Standards Agency (FSA) under assimilated Regulation (EC) No. 1829/2003 from BASF Agricultural Solutions Seed US LLC, FSA/FSS (Food Standards Scotland) were required to undertake a safety assessment on genetically modified GMB151 soybean. To support the safety assessment by FSA/FSS, the Advisory Committee on Novel Foods and Processes (ACNFP) provided advice to FSA/FSS on the data submitted for the authorisation of genetically modified GMB151 soybean, as outlined in this document.

GMB151 soybean (*Glycine max*) is modified by the addition of the *cry14Ab-1.b* and *hppdPf-4Pa* gene cassettes. The Cry14Ab-1 protein is a member of the Cry (crystal)-type protein family produced by *B. thuringiensis* which are toxic towards insects and nematodes, and confers resistance to soybean cyst nematode (SCN). *Bt* Cry proteins have been used for 50 years as an alternative to synthetic pesticides and are very effective when expressed in genetically modified plants. Planting SCN resistant soybean reduces yield losses caused by SCN infestation. The HPPD-4 protein is a modified form of the 4-hydroxyphenylpyruvate dioxygenase gene from *P. fluorescens*, which confers tolerance to HPPD (4-hydroxyphenylpyruvate dioxygenase) inhibitor herbicides such as isoxaflutole. The *hppdPf-4Pa* gene was engineered by modifying the gene at four positions; E335P, G336W, K339A, and A340N. HPPD is involved in tyrosine catabolism in aerobic organisms, and the formation of isoprenoids in anaerobic organisms.

Soybeans have been domesticated for over three millennia and are now grown as a commercial crop in over 90 countries throughout the world. In 2021, over 129,000 hectares of soybeans were harvested producing over 370,000,000 tonnes of soybeans, and over 58,000,000 tonnes of oil. The major commodities of soybeans are the grain (used to make traditional soy foods such as miso, soy sauce and tofu as well as other products), oil, and meal, despite the known presence of allergens. The scope of the application is for the authorisation for import, processing, and food and feed use of cyst nematode resistant and herbicide tolerant GMB151 soybean. The application does not cover cultivation and therefore no GMB151 soybean will be grown in the UK.

In providing its scientific advice, the ACNFP considered data provided as part of application RP1123. The molecular characterisation of GMB151 soybean determined that there was a single, intact copy of the T-DNA at a single insertion locus, within the 3’ untranslated region of a putative endogenous gene with an unknown function in soybean. Genetic stability of the transgenic locus, and phenotypic stability of transgenic protein expression, were both confirmed over multiple generations. Bioinformatics analyses of the newly expressed proteins. The field trials (including locations and management practices) for the production of test materials for the comparative analysis were considered appropriate, and no differences between GMB151 soybean and the conventional counterpart or the non-GM reference varieties that would raise safety concerns were observed. Studies on both newly expressed proteins found no evidence of potential toxicology. The studies were performed using bacterially-produced proteins, and the committee was satisfied these proteins were equivalent to plant-produced proteins. No safety concerns were raised during the 90-day feeding study. Bioinformatics analysis of the allergenicity potential of Cry14Ab-1 found one low identity match with Asp f 22 enolase from Aspergillus fumigatus, and a partial match with Hordein Barley peptide B03. Detailed analyses of these matches suggested they are not biologically relevant. No matches were found for the HPPD-4 protein. An independent outside contractor assessed the outcomes and methodologies of all bioinformatic analyses and were satisfied that the methods and results were adequate.

The ACNFP concludes that considering the nature of the introduced traits, the lack of differences in the agronomic and compositional analyses, and the proposed levels of exposure, there is no evidence in application RP1123 that the import, processing, and food and feed use of GMB151 soybean would raise any safety concerns. The ACNFP concludes that GMB151 soybean is as safe as its conventional counterpart.

1. Introduction

1.1 Background

On 25th May 2021, the Food Standards Agency (FSA) received application RP1123 (EFSA-GMO-NL-2018-153) for the authorisation of genetically modified cyst nematode resistant and herbicide tolerant GMB151 soybean (unique identifier: BCS-GM151-6), submitted by BASF Agricultural Solutions Seed US LLC (Florham Park, New Jersey) (hereafter referred to as "the applicant") according to Regulation (EC) No. 1829/2003, as assimilated into UK law.

FSA/FSS checked the application for compliance with the relevant requirements of Regulation (EC) No. 1829/2003, and assimilated Regulation (EU) No. 503/2013, and on 8th June 2021, declared the application valid.

FSA and FSS would like to thank the following members of the Advisory Committee on Novel Foods and Processes (ACNFP) who participated in the assessment: Dr Camilla Alexander White, Dr Andy Greenfield, Dr Anton Alldrick, Alison Austin, Prof George Bassel, Dr Mark Berry, Prof Dimitris Charalampopoulos, Dr Cathrina Edwards, Prof Susan Fairweather-Tait, Prof Paul Fraser, Dr Hamid Ghouddusi, Prof Wendy Harwood, Prof Huw Jones, Dr Ray Kemp, Dr Elizabeth Lund, Emeritus Professor Harry McArdle, Rebecca McKenzie, Prof Clare Mills, Dr Lesley Stanley, Prof Hans Verhagen, Dr Maureen Wakefield, and Prof Bruce Whitelaw; Dr Christine Bosch, Dr Antonio Peña-Fernández, and Dr Kimon Andreas Karatzas (associate members); and Prof Pete Lund and Prof Alastair Macrae (co-opted members of ACNFP-PGT Subcommittee).

1.2 Terms of Reference

According to Articles 6 and 18 of assimilated Regulation (EC) No. 1829/2003, FSA/FSS were requested to carry out a scientific safety assessment of genetically modified GMB151 soybean for authorisation in the scope of the application, namely the import, processing, and food and feed use of GMB151 soybean.

FSA/FSS sought safety advice from the ACNFP on genetically modified GMB151 soybean, which will inform the FSA/FSS safety assessment. The FSA/FSS safety assessment is to be seen as the opinion requested under Articles 6(6) and 18(6) of that Regulation.

In addition to the present advice on the safety of genetically modified GMB151 soybean, the ACNFP were also asked to advise on the particulars listed under

Articles 6(5) and 18(5) of assimilated Regulation (EC) No. 1829/2003. These articles concern details that must be included in positive opinions/outcomes of assessment of GMO foods and feeds, including labelling details, any relevant conditions or restrictions, and monitoring plans.

2. Applicant details

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3. Data and methodologies

3.1 Data

The data for application RP1123 submitted according to assimilated Regulation (EC) No. 1829/2003 and provided by the applicant at the time of submission are specified below. To inform the FSA/FSS safety assessment of genetically modified GMB151 soybean for food and feed uses in accordance with Articles 11 and 23 of assimilated Regulation (EC) No. 1829/2003, the ACNFP was asked to provide safety advice. It considered the requirements described in applicable guidance for the safety assessment of GM food and feed applications under assimilated Regulation (EC) No. 1829/2003, and based its scientific safety assessment on the

data within application RP1123, additional information provided by the applicant, and any relevant peer reviewed scientific publications.

3.2 Methodologies

The ACNFP conducted its assessment in accordance with the principles described in assimilated Regulation (EU) No. 503/2013, applicable guidance, explanatory notes, and statements (EFSA GMO Panel 2010; EFSA GMO Panel 2011; EFSA GMO Panel, 2015; EFSA GMO Panel, 2017). Independent contractors performed preparatory work and delivered reports on the methods applied by the applicant in performing sequencing and bioinformatics analyses.

4. Assessment

4.1 Molecular characterisation

The molecular characterisation section of the safety assessment considers the methods used to insert the transgenic material, the sequence and structure of the newly expressed protein(s), and the sequences at the insertion locus. Analyses performed by the applicant to determine insertion locus, copy number, and any deletions that occurred during the insertion of transgenic material are assessed. Bioinformatics analyses performed on the transgenic sequences are also assessed to ensure the newly expressed proteins do not raise any safety concerns. Additionally, the expression of the newly expressed proteins is assessed. Finally, bioinformatics analyses performed on the flanking regions either side of the inserted material (and the junctions between them) are assessed to ensure no sequences occur that could raise safety concerns.

4.1.1 Transformation process and vector constructs

Explants from soybean variety Thorne were transformed with transformation vector pSZ8832 (derived from pGSC1700) using *Agrobacterium tumefaciens* strain LBA4404. The helper Ti-plasmid pAL4404 was also used. After exposure, explants were transferred to media containing tembotrione to select for transformed cells, and ticarcillin to remove any remaining *A. tumefaciens*.

The T-DNA region of pSZ8832 contains the *cry14Ab-1.b* and *hppdPf-4Pa* gene cassettes which encode the Cry14Ab-1 and HPPD-4 proteins respectively. Cry14Ab-1 is a member of the Cry (crystal)-type protein family produced by *Bacillus thuringiensis* which are toxic towards insects and nematodes. *Bt* Cry

proteins are pore-forming toxins that when ingested by target organisms are activated by specific proteases, leading to cell death. HPPD-4 is a modified form of the 4-hydroxyphenylpyruvate dioxygenase gene from *Pseudomonas fluorescens*, which confers tolerance to HPPD (4-hydroxyphenylpyruvate) inhibitor herbicides, such as isoxaflutole. The *hppdPf-4Pa* gene was engineered by modifying the gene at four positions; E335P, G336W, K339A, and A340N, which reduces HPPD-inhibitor binding efficiency.

4.1.2 Molecular studies performed on GMB151 soybean

Junction sequence analysis (JSA) and gDNA sequencing of the T2 generation of GMB151 soybean identified two T-DNA/gDNA junctions, demonstrating the presence of a single copy of the complete T-DNA. The absence of backbone vector sequences was also confirmed with the exception of 21bp designated as “filler DNA” (see below).

Analysis of the junction sequences revealed 39 bp of “filler” DNA between the 3’ end of the T-DNA and the 3’ flanking genomic region corresponding to 21 bp of the ORIpVS1 of the transforming plasmid and 17 bp of the 3’ flanking genomic region. The T-DNA insert lacks the 5’ part of the P2x35S sequence which includes the double enhanced promoter region of the Cauliflower Mosaic Virus. One bp difference between the transgenic and insertion loci was found, and a 63 bp target site deletion was observed. Alignment of the GMB151 soybean transgenic locus with the T-DNA region of pSZ8832 demonstrated 100% identity.

The inserted sequence, and both flanking regions, were sequenced by Sanger sequencing. Bioinformatics analysis located the GMB151 insertion locus to *G. max* chromosome 7, within the 3’ untranslated region (non-protein-coding region) of a putative endogenous gene, BON1-associated protein 1-like protein. The function of BON1-associated protein 1-like protein in soybean remains uncharacterised.

Bioinformatics analyses of all putative ORFs at the insertion site found a low identity match with Asp f 22 enolase from *Aspergilus fumigatus* wholly within the *cry14Ab-1.b* gene. This is further discussed in Section 4.3.6. Another putative ORF within the *cry14Ab-1.b* gene matched a contiguous 8 amino acid sequence of the Cas s 5 allergen, but this sequence is out of frame with the *cry14Ab-1.b* coding sequence and there is no start codon upstream. No match with the Cas s 5 protein was found in the overall search, or in the 80-mer sliding window search. No identities were found in any putative ORFs at the *hppdPf-4Pa* insertion locus. Bioinformatics analyses of the insertion site found no sequences likely to

contribute to horizontal gene transfer with bacterial species.

4.1.3 Transgenic protein expression

Expression levels of Cry14Ab-1 and HPPD-4 were determined by ELISA on leaf, root, flowers, forage, whole plant, and grain samples from tissues harvested from GMB151 soybean plants grown in the USA (Pennsylvania, Missouri, and Kansas) in 2016 (Table 1). Forage and grain were analysed as they represent the main point of entry into the food and feed chain.

Table 1. Protein expression of Cry14Ab-1 and HPPD-4 in GMB151 soybean grain and forage (µg/g FW and (µg/g DW))

	Herbicide treatment	**Herbicide treatment**
Grain	**Conventional herbicide treatment[a]**	**Intended herbicide treatment[b]**
Cry14Ab-1	73.93 ± 33.86 (83.14 ± 37.69)[c]	84.99 ± 38.66 (95.91 ± 43.11)
HPPD-4	3.97 ± 3.20 (4.45 ± 3.57)	3.95 ± 2.59 (4.46 ± 2.90)
Forage		
Cry14Ab-1	11.37 ± 3.59 (48.72 ± 9.38)	12.19 ± 3.02 (51.34 ± 9.25)
HPPD-4	30.48 ± 14.07 (129.03 ± 45.32)	29.09 ± 15.55 (120.18 ± 42.47)

[a] Treated with conventional herbicide only

[b] Treated with isoxaflutole pre-emergence (BBCH stage 00) at a target rate of 70.1 (69.2 – 71.1) g ai/ha

[c] Results report as mean ± standard deviation (n=12)

4.1.4Genetic stability

DNA sequencing and JSA confirmed the genetic stability of the insert over five generations (T2, T4, T5, T6, and BC2F3). For each generation, two junctions which mapped partially to the T-DNA sequence and partially to the soybean genome, were observed. Multiple sequence alignments of the obtained junction sequences showed that both novel junctions were conserved across all generations.

Phenotypic stability of Cry14Ab-1 and HPPD-4 expression was determined for five generations using lateral flow strip analysis. Cry14Ab-1 and HPPD-4 expression was consistent across all generations tested. Event-specific PCR was used to test for the presence or absence of the *cry14Ab-1.b* and *hppdPf-4Pa* genes in five segregating generations (2 × F2, 2 × BC2F2, and one BC1F2) to calculate the segregation ratios. Chi-square analysis of the segregation data confirmed that the GMB151 insert is inherited in a predictable manner as expected for a single insert, consistent with Mendelian principles.

4.1.5 Conclusion on the molecular characterisation

The molecular characterisation data presented confirm that GMB151 soybean contains a single transgenic insert. Bioinformatics analyses of this insert, and the flanking sequences, raised no safety concerns. The genetic stability of the insert was confirmed over five generations. The expression levels of the transgenic proteins in GMB151 soybean grain and forage were determined using suitable methodologies, and do not cause a safety concern.

4.2 Comparative analysis

The role of the comparative analysis is to compare the GM plant with its conventional counterpart, a non-GM plant with a similar genetic background, and several non-GM reference varieties with similar properties to the GM plant and conventional counterpart. This comparison takes two forms; firstly, a comparison of the agronomic characteristics of the plant as it grows in the field which looks at the yields derived from the plants, as well as their observable characteristics such as height and colour, and a comparison of the composition of the plant after harvest which considers the nutritional value and safety of the genetically modified plant.

4.2.1 Experimental field trail design

GMB151 soybean, along with Thorne (the non-GM conventional counterpart) and nine non-GM reference varieties (E2282, E2692, E2993, E3066, E3192, E3494, NGN 3121STS, NGN 3292C, and NGN 3347C) were grown at 12 sites in the USA in 2017, however one site was removed from consideration after flood damage. The field trials consisted of entries replicated four times in a randomised complete block design. The entries were;

- Non-GM conventional counterpart (Thorne) with conventional herbicide management
- GMB151 soybean with conventional herbicide management
- GMB151 soybean with trait-specific herbicide treatment (one application of isoxaflutole at BBCH 00-03)
- At least three of the nine reference varieties with conventional herbicide management

The agronomic/phenotypic data and compositional data from these field trials were analysed as specified previously in guidance provided by EFSA (EFSA GMO Panel 2010; EFSA GMO Panel 2011; EFSA GMO Panel, 2015). This includes the application of a test of difference between GMB151 soybean and the conventional counterpart, and a test of equivalence between GMB151 soybean and the non-GM reference varieties.

4.2.2 Suitability of field trails and test materials

The field trial sites were representative of commercial soybean production in the USA, and represented a variety of agro-climatological conditions, soil types, planting conditions, and harvest dates.

Average monthly maximum and minimum temperatures and total monthly precipitation was recorded for each site. Weather conditions were generally normal however some sites recorded cooler than average temperatures, and some sites experienced dryer or wetter conditions than usual. One site was removed from consideration due to flooding at the start of the growing season.

GMB151 soybean and the conventional counterpart (Thorne) were produced following good agricultural practices and with quality assurance mechanisms to ensure genetic identity, purity, and health. GMB151 soybean seeds was tested for the presence or absence of the transgenic insert and PCR analysis of 3000 seeds found no impurities. Good seed germination (in warm germination tests), and good seed health was observed. The non-GM reference varieties were produced in accordance with standards of commercial certified seed production.

Forage and grain were selected for compositional analysis as they represent the main point of entry into the food and feed production and processing chain. Forage and grain samples from 8 of the 11 sites were selected for compositional analysis, selected to represent a broad geographical distribution capturing a range of agro-climatological conditions as well as different crop management systems.

The ACNFP is satisfied that the field trials, and the materials used in the field trails are appropriate for the comparative assessment. The geographical locations, soil conditions, meteorological conditions, and the management practices used were all considered typical of the receiving environments where GMB151 soybean could be grown.

4.2.3 Compositional analysis (agronomic characteristics)

In the comparative analysis of agronomic characteristics, tests between GMB151 soybean not treated with the intended herbicide and the conventional counterpart found no statistically significant differences for 8 of the 11 parameters tested (Table 2). Statistically significant differences were observed for fruit count, seed moisture, and seed weight. However, equivalence with the reference varieties for each was demonstrated.

Table 2. Outcome of the comparative analysis of the agronomic characteristics of GMB151 soybean.

Intended herbicide treatment[c]

	Test of difference[(a)]	**Test of difference[(a)]**
Test of equivalence[(b)]	Not different	Significantly different
Category I	4[d]	5[e]
Category II	1[f]	-
Category III	1[g]	-

Category IV	-	-
Total endpoints	6	5

Conventional herbicide treatment[c]

	Test of difference[(a)]	**Test of difference[(a)]**
Test of equivalence[(b)]	Not different	Significantly different
Category I	8[d]	2[e]
Category II	-	-
Category III	1[g]	-
Category IV	-	-
Total endpoints	9	2

[a] Comparison between GMB151 soybean and the conventional counterpart (Thorne)

[b] The test of equivalence with the reference varieties is categorised into four different outcomes; category I (equivalence with the reference varieties is demonstrated), category II (equivalence is more likely than not), category III (equivalence is less likely than not), and category IV (non-equivalence is demonstrated)

[c] The intended herbicide treatment was one application of isoxaflutole at BBCH 00-03

[d] Intended herbicide treatment: early stand count, days to maturity, fruit count, and seed weight; Conventional herbicide management: early stand count, crop development, flowering duration, plant height, final stand count, days to maturity,

seed weight, and yield

[e] Intended herbicide treatment: Crop development, flowering duration, plant height, seed moisture, and final stand count; Conventional herbicide treatment: Fruit count and seed moisture

[f] Yield

[g] Days to flowering

In the test between GMB151 soybean treated with the intended herbicide and the conventional counterpart, crop development, flowering duration, plant height, final stand count, and seed moisture were all found to be significantly different (Table 2). Again, equivalence with the reference varieties was demonstrated for all of these parameters.

Although the days to flowering for GMB151 soybean (both treated and not treated with the intended herbicide) were within the range of the reference varieties, there was a trend of the conventional counterpart and GMB151 soybean to begin flowering later. This is likely due to the maturity group of Thorne, the conventional counterpart and genetic background for GMB151 soybean (3.5), versus those of the reference varieties (2.2 to 3.4). Considering the natural variability and the relevance of the endpoints to food and feed safety, the ACNFP considered that the observed differences for days to flowering did not change the safety of GMB151 soybean compared to the comparator.

4.2.4 Compositional analysis of forage

In the comparative analysis of composition, GMB151 soybean forage samples were analysed in accordance with the OECD document on soybean (OECD, 2012). In the comparative analysis of composition of forage, statistically significant differences between GMB151 soybean not treated with the intended herbicide and the conventional counterpart were found in ash, carbohydrates, and crude protein, but equivalence with the reference varieties was demonstrated in all cases. Moisture was given equivalence category III (equivalence with the reference varieties less likely than not), but there was insufficient evidence of a difference with the conventional counterpart. Statistically significant differences between GMB151 soybean treated with the intended herbicide and the conventional counterpart were found in neutral detergent fibre only, but equivalence was demonstrated again.

4.2.5 Compositional analysis of grain

In the compositional analysis of GMB151 soybean grain, statistically significant differences between GMB151 soybean not treated with the intended herbicide and the conventional counterpart were found in 30 of the composition parameters tested (Table 3). Of these parameters, equivalence with the reference varieties was demonstrated, except for C16:0 palmitic acid, C17:1 heptadecenoic acid, and vitamin A. Equivalence was found to be more likely than not for vitamin A, but less likely than not for C16:0 palmitic acid and C17:1 heptadecenoic acid.

Statistically significant differences between GMB151 soybean treated with the intended herbicide and the conventional counterpart were found in 33 of the composition parameters tested (Table 3). Equivalence was demonstrated for all of these parameters, except C16:0 palmitic acid and C17:1 heptadecenoic acid, however equivalence was more likely than not for both fatty acids.

No significant differences between GMB151 soybean (either treated or not treated with the intended herbicide) and the conventional counterpart were observed for trypsin inhibitor, however equivalence with the non-GM reference varieties was less likely than not (Table 3).

Table 3. Outcome of the comparative compositional analysis of GMB151 soybean.

Intended herbicide treatment[c]

	Test of difference[(a)]	**Test of difference[(a)]**
Test of equivalence[(b)]	Not different	Significantly different
Category I	46[d]	31
Category II	-	2[e]
Category III	1[h]	-
Category IV	-	-

Not categorised	8	-
Total endpoints	55 Appendix 1	33 Appendix 1

Conventional herbicide treatment[c]

	Test of difference[(a)]	**Test of difference[(a)]**
Test of equivalence[(b)]	Not different	Significantly different
Category I	45	27
Category II	4[f]	1[g]
Category III	1[h]	2[e]
Category IV	-	-
Not categorised	8	-
Total endpoints	58 Appendix 1	30 Appendix 1

[a] Comparison between GMB151 soybean and the conventional counterpart (Thorne)

[b] The test of equivalence with the reference varieties is categorised into four different outcomes; category I (equivalence with the reference varieties is demonstrated), category II (equivalence is more likely than not), category III (equivalence is less likely than not), and category IV (non-equivalence is demonstrated)

[c] The intended herbicide treatment was one application of isoxaflutole at BBCH 00-03

[d] See Appendix 1 for all parameters tested

[e] C16:0 palmitic acid and C17:1 heptadecenoic acid

[f] Copper, iron, zinc, and Kunitz trypsin inhibitor 3

[g] Vitamin A

[h] Trypsin inhibitor

The differences observed in C16:0 palmitic acid, C17:1 heptadecenoic acid, trypsin inhibitor, and vitamin A were further analysed. The mean value for C16:0 palmitic acid (10.7% total fatty acids) is within the reference range and the ILSI crop composition database range of 8.03-15.99% (ILSI, 2018), indicating the C16:0 palmitic acid levels are within the range of natural variability. The mean values of C17:1 heptadecanoic acid are also within the range of the reference varieties, and as a low abundance fatty acid, any lack of equivalence would not be expected to impact the value of the food or feed. The mean values for trypsin inhibitor were not significantly different, but equivalence is less likely than not. Again, the mean values were within the reference range. The mean value for vitamin A is also within the range of the reference varieties.

4.2.6 Conclusion on the comparative analysis

The ACNFP assessed the field trials used to generate material for the comparative analyses and considered the locations selected were representative of commercial soybean production, and that the meteorological conditions and management practices used during the field trails were appropriate.

The ACNFP also assessed the results from the comparative analysis, including all the significant differences between GMB151 soybean and its conventional counterpart, and found the information provided did not raise any safety concerns.

4.3 Food/feed safety assessment

The food/feed safety assessment covers the likelihood that the newly expressed protein(s), or the whole genetically modified food or feed, will cause safety concerns when consumed by humans and/or animals. This includes looking at the concentrations of newly expressed proteins in the final products that will be consumed, as well as the anticipated rates of consumption by humans and animals to understand the anticipated magnitude of exposure to the transgenic

proteins. Any toxicological or allergenic effects that can be observed and any nutritional effects that consumption of the products may cause are also assessed.

4.3.1 Effects of processing

Unprocessed soybeans are unsuitable for food use, and their use for animal feed remains limited due to the presence of anti-nutritional factors. Soybeans may be consumed whole – including in an immature form (edamame beans) – after processing such as boiling or roasting to inactivate these anti-nutritional factors, whilst soybean flour is often used as an ingredient in bakery products. Three processing methods are typically used to remove the oil fraction from soybeans; solvent extraction, hydraulic extraction, and expeller extraction (solvent extraction is the most common). This leaves a protein-rich meal byproduct which may be further processed into many different protein ingredients. The extracted oil must then undergo further processing (refining) in which proteins are subjected to harsh conditions that lead to denaturation and loss of biological activity. GMB151 soybean will be processed in the same manner as conventional soybean. Information on the processing steps was provided.

The concentrations of the newly expressed proteins, Cry14Ab-1 and HPPD-4, were determined in processed fractions of GMB151 soybeans, treated and not treated with the intended herbicide. Due to the heat and chemical treatments during the toasting of meal and the oil extraction and refinement procedures, the newly expressed proteins were not detectable at quantifiable concentrations (

4.3.2 Activity and stability of the newly expressed proteins

The studies on both newly expressed proteins were performed with bacterially-produced recombinant proteins rather than the proteins extracted directly from the plants due to the limitations on protein quantity that can be extracted from plant material. Structural and functional equivalence between bacterially-produced and plant-produced proteins was confirmed for both Cry14Ab-1 and HPPD-4 using mass spectrometry, immune-reactivity experiments, peptide mapping and N-terminal sequencing, and quantitative activity assays.

- The molecular weights of the bacterially-produced and plant-produced Cry14Ab-1 proteins were the same (131.1 kDa), as were the two HPPD-4 proteins (40.3 kDa), as confirmed by SDS-PAGE and western blot
- Western blots also confirmed the immuno-reactivity of the proteins against specific antibodies
- The absence of glycosylation was confirmed for all proteins

- Mass spectrometry demonstrated N-terminal acetylation in the GMB151-purified Cry14Ab-1 protein, but not the recombinant protein
- Mass spectrometry of GMB151-purified HPPD-4 identified three major molecular masses;
    - the mature form without the N-terminal methionine and four C-terminal residues
    - the mature form with an N-terminal cysteinic sulfinic acid (and lacking the four C-terminal residues)
    - a mature form with an N-terminal cysteinic sulfinic acid but with the four C-terminal residues
- Mass spectrometry of the microbially-produced HPPD-4 identified a single mass that corresponded to the protein lacking the N-terminal methionine only
- In the Cry14Ab-1 functional assay, the ED50 (effective dose to inhibit *C. elegans* growth by 50%) for the GMB151-purified protein was 11.39 µg/mL, and the recombinant protein was 2.87 µg/mL
- In the HPPD-4 functional assay, GMB151-purified protein had a specific activity of 1.88E-02 nmol/min/µg enzyme and the recombinant protein was 2.77E-02 nmol/min/µg enzyme.

Activity and stability of Cry14Ab-1

The effect of temperature on the activity and stability of Cry14Ab-1 was assessed by the applicant using heat treatment followed by SDS-PAGE and western blot, activity assay, and ELISA. After incubation at 25 °C, 37 °C, 55 °C, and 75 °C, the majority of Cry14Ab-1 remained soluble. After treatment at 75 °C, some smearing was visible on the SDS-PAGE gel and after incubation at 95 °C, no soluble protein remained. The activity of Cry14Ab-1 reduced after treatment at 55 °C and no activity was detected after treatment at 75 °C or 95 °C. ELISA showed a decrease in Cry14Ab-1 concentration after treatment at 55 °C.

Cry14Ab-1 was digested very rapidly (within 30 seconds) in SGF containing pepsin at pH 1.2, and partially digested after 60 minutes in SIF containing pancreatin at pH 7.5.

Activity and stability of HPPD-4

The effect of temperature on HPPD-4 was assessed by the applicant using the same methods. After treatment at 25 °C and 37 °C, the majority of HPPD-4 was soluble, but after treatment at 55 °C and higher, the protein began to lose stability and appear insoluble on an SDS-PAGE gel. The optimal temperature of

HPPD-4 was room temperature and after treatment at 55 °C, 75°C, and 95 °C, there was no detectable protein activity. Specific activity at 55 °C was

The effect of pH on HPPD-4 activity was assessed by the applicant and determined the optimum pH as 8.0 by testing the specific activity of the HPPD-4 enzyme in pHs ranging from 5.5 to 9.5.

The natural substrate of HPPD-4 is 4-HPP (4-hydroxyphenylpyruvic acid), and potential substrates occurring in plants are phenyl pyruvate, 3,4-dihydroxyphenylpyruvate, α-ketoisocaproate, and α-keto-γ-(methylthio)butyrate. No differences in the specific activities of HPPD-4 against all natural and potential substrates were observed.

HPPD-4 was digested very rapidly (within 30 seconds) in SGF containing pepsin at pH 1.2, and was partially digested after 5 minutes in SIF containing pancreatin at pH 7.5, and completely digested after 10 minutes.

4.3.3 Toxicological testing of the newly expressed proteins

The ACNFP considered the toxicological safety of both newly expressed proteins during its safety assessment using the molecular characterisation data, bioinformatic analyses, and any *in vitro* or *in vivo* studies performed by the applicant.

Bioinformatic analyses were performed for both newly expressed proteins using the FASTA algorithm, with the BLOSUM50 scoring matrix. For Cry14Ab-1 and HPPD-4 no biologically relevant similarities were identified (most matches were from proteins of the same families with no known toxic properties).

To determine any potential toxic effects of the newly expressed proteins, 28-day repeated dose toxicity studies in mice were performed in compliance with OECD TG 407 (OECD, 2008). Neither the Cry14Ab-1 nor the HPPD-4 proteins, when administered for at least 28 days at a measured dose of 1000 mg/kg bw/day, induced any treatment-related changes.

4.3.4 Toxicological testing of new constituents other than the newly expressed proteins

No new constituents other than the newly expressed proteins, Cry14Ab-1 and HPPD-4, were identified in GMB151 soybean, therefore no assessment of any constituents other than the newly expressed proteins is required.

4.3.5 Toxicological testing of the whole genetically modified food or feed

In accordance with assimilated Regulation (EU) No. 503/2013, the applicant provided a 90-day feeding study of Sprague Dawley (Crl:CD(SD)) rats fed a diet consisting of 30% (w/w) toasted GMB151 soybean meal treated with the intended herbicide, the conventional counterpart (Thorne), or a non-GM reference variety (E3494). The study was performed in accordance with OECD TG 408 (OECD, 2018) with the exception of tests on the homogeneity and stability of the formulated diets.

No effects on any of the parameters tested were observed during the study.

4.3.6 Assessment of allergenicity

In accordance with assimilated Regulation (EU) No. 503/2013, the applicant used a weight-of-evidence approach to assess the allergenicity potential of Cry14Ab-1 and HPPD-4 as no single method is sufficient to predict allergenicity (Codex Alimentarius, 2009).

Neither of the source organisms for the newly expressed proteins (*B. thuringiensis* – Cry14Ab-1, *P. fluorescens* – HPPD-4) are considered as sources of allergens.

Two *in silico* searches (an overall identity search and an 80-mer sliding window search) against the COMPARE database found a low identity (35.4%) match between an 80-mer from Cry14Ab-1 and a 77 amino acid stretch of Asp f 22 enolase from *Aspergillus fumigatus*, an inhalant allergen which is cross-reactive with the homologous protein, Pen c 22 from *Penicillium citrinum*. There was no contiguous 8 amino acid identity with Asp f 22, indicating it is unlikely to possess any potential allergenic, IgE cross-reactive, linear epitopes with Asp f 22, and there were no matches with five other fungal enolases, including Pen c 22.

The amino acid sequences of the newly expressed proteins were divided into 9-mer blocks and then searches for a perfect sequence match to peptide sequences known to carry CD toxic motifs, for the transglutaminase 2 (TG2) deamidation motif (Q/E-X1-P-X2, X1 = L, Q, F, S, or E, X2 = Y, F, A, V, or Q), and a sequence identity search allowing up to 3 sequence mismatches (except a Q for E swap at positions P1 and P9) were performed. For the Cry14Ab-1 protein, only a partial match with a CD toxic motif from Hordein Barley peptide B03 (with three mismatches) was found. This is very unlikely to have biological activity due to the lack of any TG2 deamidation sites.

The ACNFP considered the bioinformatics analyses performed by the applicant, particularly the matches between Cry14Ab-1 and the inhalant allergen Asp f 22 enolase from *Aspergillus fumigatus* and the CD toxic motif in Hordein Barley peptide B03, and found no safety concerns for either newly expressed protein.

4.3.7 Anticipated intake/extent of use

In accordance with assimilated Regulation (EU) No. 503/2013, the applicant provided anticipated dietary intake of Cry14Ab-1 and HPPD-4 by using protein expression data in GMB151 soybean treated with the intended herbicide and international and national food consumption data. As no data on GMB151 soybean consumption exists, a worst-case scenario approach was used where 100% of conventional soybean commodities were replaced with GMB151 soybean, and no protein is lost during processing.

The anticipated acute and chronic human intake of the newly expressed proteins is considered minor due to the low expression of the newly expressed proteins. The average chronic dietary exposures for humans to the newly expressed proteins ranged from 0 to 18.59 µg/kg bw/day for Cry14Ab-1, and 0 to 1.00 µg/kg bw/day for HPPD-4, across different European countries.

Ad hoc dietary exposure scenarios were carried out for specific groups thought to be at greater risk of increased exposure (consumers of soy-based protein supplements, consumers of pollen supplements). The highest calculated exposures for these products did not raise any safety concerns.

Anticipated animal dietary intakes were calculated using a worst-case scenario approach, assuming that 100% of raw soybean and soybean food products were derived from GMB151 soybean, the highest protein contents (µg/g dw) for forage/silage and average protein contents (µg/g dw) for seed, meal, and hulls were considered, and the maximum percentage of soybean commodities were included in animal feeds, according to OECD data (OECD, 2013). Using this method, the highest daily intakes were calculated for Australian beef cattle, fed a diet of up to 100% soybean forage. The cattle would be exposed to up to 40 g/kg bw/day soybean forage, corresponding to 2.68 mg/kg bw/day Cry14Ab-1 and 7.85 mg/kg bw/day HPPD-4.

4.3.8 Nutritional assessment

As the intended traits of GMB151 soybean are for agronomic purposes only (resistance to cyst nematodes and tolerance to HPPD inhibitors), no change in the

nutritional value of the product is expected. The only significant changes observed in the comparative analysis were changes to the levels of C16:0 palmitic acid and C17:1 heptadecanoic acid in GMB151 soybean compared to the conventional counterpart, therefore not presenting a safety/nutritional concern.

4.3.9 Conclusion of the food/feed safety assessment

The ACNFP assessed the food/feed safety of the newly expressed proteins in terms of their toxicological potential, allergenic potential, and nutritional quality. It concluded that the newly expressed proteins shared no biologically relevant identity with known toxins and allergens, and the overall allergenicity of GMB151 soybean was not different to conventional soybean. The ACNFP concluded that based on the comparative analysis and the nutritional assessment, GMB151 soybean is not nutritionally disadvantageous, and is as safe as conventional soybean varieties.

4.4 Environmental risk assessment and monitoring plan

4.4.1 Environmental risk assessment

The environmental risk assessment (ERA) of GMB151 soybean was considered by the Advisory Committee on Releases to the Environment (ACRE).

The scope of the application does not include cultivation and only covers the import, processing, and food and feed use of GMB151 soybean. No deliberate release of viable plant material or derived products is expected. Therefore, only accidental release of viable GM seeds or propagating material during import, transportation, storage, handling, and processing will be considered.

ACRE considered the ability of GMB151 soybean to persist under GB environmental conditions, interaction of feral GMB151 soybean with the environment, and the potential for horizontal gene transfer (HGT) to the environment. ACRE concluded that GMB151 soybean would not raise safety concerns in the event of accidental release of viable seeds or propagating material into the environment.

ACRE’s advice is available on the GOV.UK website.

4.4.2 Post-market environmental monitoring (PMEM) plan

The PMEM plan provided by the applicant proposes general surveillance to identify the occurrence of unanticipated adverse effects due to the unintended release of GMB151 soybean. Exposure (via accidental release) can be controlled by clean-up measures, and the application of current practices used for the control of any adventitious soybean plants, such as manual or mechanical removal, and the application of herbicides.

General surveillance will be predominantly based on collaboration with third parties, such as operators involved in the import, handling, and processing of GMB151 soybean. These third parties will report any potential unanticipated adverse effects to the authorisation holder, who will investigate.

The authorisation holder will submit an annual report including results of the general surveillance and any unanticipated adverse effects. If information that confirms an adverse effect becomes available, the authorisation holder will investigate, and based on a scientific evaluation, define, and implement management measures to protect human and animal health, or the environment, as necessary.

ACRE considered the PMEM plan provided by the applicant, in conjunction with the ERA. As the ERA did not identify potential adverse effects to the environment, it was not considered necessary for case-specific monitoring to be implemented. The proposed PMEM plan and monitoring intervals are appropriate for the intended uses of GMB151 soybean.

5. Overall conclusions and recommendations

To support the safety assessment by FSA/FSS, the ACNFP was asked to provide advice on the data submitted for the authorisation for import, processing, and food and feed use of genetically modified GMB151 soybean in accordance with assimilated Regulation (EU) No. 1829/2003. GMB151 soybean is modified by the addition of the Cry14Ab-1 and HPPD-4 proteins from *B. thuringiensis* and *P. fluorescens* respectively. *Bt* Cry proteins have been used for 50 years as an alternative to synthetic pesticides and their use in soybeans can reduce yield losses caused by soybean cyst nematode infestation. HPPD-4 confers tolerance to HPPD inhibitor herbicides such as isoxaflutole.

The molecular characterisation data established that GMB151 soybean contains a single transgenic insert. Bioinformatics analyses of this insert, and the flanking sequences, raised no safety concerns. The stability of the insert was confirmed over five generations. The expression levels of the transgenic proteins in GMB151

soybean grain and forage were determined using suitable methodologies, and do not cause a safety concern.

The field trials used to generate material for the comparative analyses were deemed appropriate, and the locations selected were considered representative of commercial soybean production. The meteorological conditions and management practices used during the field trails were appropriate. The ACNFP also assessed the results from the comparative analysis, including all the significant differences between GMB151 soybean and its conventional counterpart, and found no safety concerns when compared to reference varieties.

The food/feed safety of the newly expressed proteins was assessed, and no safety concerns were raised in terms of their toxicological potential, allergenic potential, and nutritional quality. Based on the comparative analysis and the nutritional assessment, GMB151 soybean does not cause any nutritional concerns.

Overall, the ACNFP concludes that GMB151 soybean is as safe as its conventional counterpart with respect to its potential effects on human and animal health.

6. References

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7.Appendix 1

Results of the statistical analyses performed on all analytes tested in the comparative assessment of GMB151 soybean

Intended herbicide treatment

Category I (equivalence demonstrated)[a]

i. Not significantly different

Ash, crude fat, acid detergent fibre, total dietary fibre, alanine, arginine, aspartic acid, cystine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine, valine, C14:0 myristic acid, C16:1 palmitoleic acid, C17:0 heptadecanoic acid, C18:0 stearic acid, iron, manganese, phosphorus, potassium, α-tocopherol, vitamin B2, vitamin B6, vitamin K1, lectins, raffinose, stachyose, Gly m 1, Gly m 3, Gly m 4, Gly m 5, Gly m 6, Gly m 7, Gly m Bd 28k, β-tocopherol, γ-tocopherol, total tocopherols, and vitamin A (46)

ii. Significantly different

Moisture, carbohydrates, crude protein, neutral detergent fibre, glutamic acid, proline, C18:1 oleic acid, C18:2 linoleic acid, C18:3 linolenic acid, C20:0 arachidic acid, C20:1 eicosenoic acid, C22:0 behenic acid, C24:0 lignoceric acid, calcium, copper, magnesium, zinc, vitamin B1, vitamin B3, vitamin B5, vitamin B9, total daidzein, total genistein, totally glycitein, total isoflavones, phytic acid, Gly m 8, Kunitz trypsin inhibitor 1, Kunitz trypsin inhibitor 3, Gly m Bd 30k, and δ-tocopherol (31)

Category II (equivalence more likely than not)

i. Not significantly different

N/A

ii. Significantly different

C16:0 palmitic acid and C17:1 heptadecenoic acid (2)

Category III (equivalence less likely than not)

i. Not significantly different

Trypsin inhibitor (1)

ii. Significantly different

N/A

Category IV (non-equivalence demonstrated)

i. Not significantly different

N/A

ii. Significantly different

N/A

Statistical analyses not performed

C18:4 stearidonic acid, C19:0 nonadecanoic acid, C20:2 eicosadienoic acid, C20:5 eicosapentaenoic acid, C22:5 N6 docosapentaenoic acid, C22:6 docosexaenoic acid, sodium, and Kunitz trypsin inhibitor 2 (8)

Conventional herbicide management

Category I (equivalence demonstrated)

i. Not significantly different

Ash, crude fat, acid detergent fibre, neutral detergent fibre, total dietary fibre, alanine, arginine, cystine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tyrosine, valine, C14:0 myristic acid, C17:0 heptadecanoic acid, C18:0 stearic acid, magnesium, manganese, phosphorus, potassium, α-tocopherol, vitamin B2, vitamin B5, vitamin B6, vitamin K1, lectins, raffinose, stachyose, Gly m 1, Gly m 3, Gly m 4, Gly m 6, Gly m 7, Gly m 8, Kunitz trypsin inhibitor 1, Gly m Bd 28k, β-tocopherol, γ-tocopherol, and total tocopherols (45)

ii. Significantly different

Moisture, carbohydrates, crude protein, aspartic acid, glutamic acid, proline, tryptophan, C16:1 palmitoleic acid, , C18:1 oleic acid, C18:2 linoleic acid, C18:3 linolenic acid, C20:0 arachidic acid, C20:1 eicosenoic acid, C22:0 behenic acid, C24:0 lignoceric acid, calcium, vitamin B1, vitamin B3, vitamin B9, total daidzein, total genistein, totally glycitein, total isoflavones, phytic acid, Gly m 5, Gly m Bd 30k, and δ-tocopherol (27)

Category II (equivalence more likely than not)

i. Not significantly different

Copper, iron, zinc, and Kunitz trypsin inhibitor 3 (4)

ii. Significantly different

Vitamin A (1)

Category III (equivalence less likely than not)

i. Not significantly different

Trypsin inhibitor (1)

ii. Significantly different

C16:0 palmitic acid and C17:1 heptadecenoic acid (2)

Category IV (non-equivalence demonstrated)

i. Not significantly different

N/A

ii. Significantly different

N/A

Statistical analyses not performed

C18:4 stearidonic acid, C19:0 nonadecanoic acid, C20:2 eicosenoic acid, C20:5 eicosapentaenoic acid, C22:5 N6 docosapentaenoic acid, C22:6 docosexaenoic acid, sodium, and Kunitz trypsin inhibitor 2 (8)

[a] The comparative analysis comprises a test of equivalence with the non-GM reference varieties and a test of difference with the conventional counterpart, in this case the soybean Thorne. The results of the test of equivalence are categorised into four groups; equivalence with the reference varieties is demonstrated, equivalence with the reference varieties is more likely than not, equivalence with the reference varieties is less likely than not, and non-equivalence with the reference varieties is demonstrated.

Abbreviations

Acronym	Definition
ACNFP	Advisory Committee on Novel Foods and Processes
ACRE	Advisory Committee on Releases to the Environment

ADF	Acid Detergent Fiber
BLAST	Basic Local Alignment Search Tool
bp	Base pair
bw	Body weight
CD	Coeliac disease
CHM	Conventional Herbicide Management
COMPARE	COMprehensive Protein Allergen REsource
DNA	Deoxyribonucleic acid
DW	Dry weight
EC	European Commission
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EU	European Union
FSA	Food Standards Agency
FSS	Food Standards Scotland
gDNA	Genomic DNA

GM	Genetically modified
GMO	Genetically modified organism
HPPD	Hydroxyphenylpyruvate dioxygenase
ILSI	International Life Sciences Institute
JSA	Junction sequence analysis
kDa	Kilodalton
LLOQ	Lower limit of quantification
NDF	Neutral Detergent Fibre
OECD	Organisation for Economic Co-operation and Development
ORFs	Open reading frames
PCR	Polymerase chain reaction
PMEM	Post-market environmental monitoring
PMM	Post-Market Monitoring
RBD	Refined, bleached, and deodorised
SCN	Soybean cyst nematode

SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
T-DNA	Transfer-deoxyribonucleic acid
TIH	Treated with Intended Herbicide