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Assessment of genetically modified soybean GMB151 for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2018-153)

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Abstract

Soybean GMB151 was developed to confer tolerance to 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor herbicides and resistance to nematodes. The molecular characterisation data and bioinformatic analyses do not identify issues requiring food/feed safety assessment. None of the identified differences in the agronomic/phenotypic and compositional characteristics tested between soybean GMB151 and its conventional counterpart needs further assessment, except for palmitic acid and heptadecenoic acid in seeds and carbohydrate and crude protein in forage, which does not raise nutritional and safety concerns. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the HPPD-4 and Cry14Ab-1 proteins as expressed in soybean GMB151, and finds no evidence that the genetic modification would change the overall allergenicity of soybean GMB151. In the context of this application, the consumption of food and feed from soybean GMB151 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that soybean GMB151 is as safe as the conventional counterpart and non-GM soybean reference varieties tested, and no post-market monitoring of food/feed is considered necessary. In the case of accidental release of viable soybean GMB151 seeds into the environment, this would not raise environmental safety concerns. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of soybean GMB151. The GMO Panel concludes that soybean GMB151 is as safe as its conventional counterpart and the tested non-GM soybean reference varieties with respect to potential effects on human and animal health and the environment.

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Requestor: Competent authority of the Netherlands

Question number: EFSA-Q-2018-00781

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Summary

The scope of application EFSA-GMO-NL-2018-153 is for food and feed uses, import and processing of the genetically modified (GM) herbicide tolerant and nematode resistant soybean GMB151 in the European Union (EU).

In this scientific opinion, the scientific Panel on Genetically Modified Organisms of the European Food Safety Authority (hereafter referred to as the 'GMO Panel') reports on the outcome of its risk assessment of soybean GMB151 according to the scope of the application EFSA-GMO-NL-2018-153. The GMO Panel conducted the assessment of soybean GMB151 in line with the principles described in Regulation (EU) No 503/2013 and its applicable guidelines for the risk assessment of genetically modified (GM) plants. The molecular characterisation data establish that soybean GMB151 contains a single insert consisting of one copy of the hppdPf-4Pa and the cry14Ab-1.b expression cassettes. The quality of the sequencing methodology and data sets was assessed by the EFSA GMO Panel and is in compliance to the requirements listed in the EFSA Technical Note.[1] Updated bioinformatics analyses of the sequences encoding the newly expressed proteins and open reading frames (ORFs) present within the insert or spanning the junctions between the insert and genomic DNA, do not raise any safety concerns. The stability of the inserted DNA and of the introduced trait is confirmed over several generations. The methodology used to quantify the levels of the Cry14Ab-1 and HPPD-4 proteins is considered adequate. The protein characterisation data comparing the biochemical, structural and functional properties of plant- and microbe-produced Cry14Ab-1 and HPPD-4 proteins indicate that these proteins are equivalent and the microbe-derived proteins can be used in the safety studies.

None of the identified differences in the agronomic/phenotypic and compositional characteristics tested between soybean GMB151 and its conventional counterpart needs further assessment, except for palmitic acid and heptadecenoic acid in seeds and carbohydrate and crude protein in forage, which does not raise nutritional and safety concerns. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the HPPD-4 and Cry14Ab-1 proteins as expressed in soybean GMB151, and finds no evidence that the genetic modification would change the overall allergenicity of soybean GMB151. In the context of this application, the consumption of food and feed from soybean GMB151 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that soybean GMB151 is as safe as the conventional counterpart and non-GM soybean reference varieties tested, and no post-market monitoring of food/feed is considered necessary.

Considering the introduced traits, the outcome of the agronomic and phenotypic analysis and the routes and levels of exposure, soybean GMB151 would not raise safety concerns in the case of accidental release of viable GM soybean seeds into the environment. The post-market environmental monitoring (PMEM) plan and reporting intervals are in line with the intended uses of soybean GMB151.

The GMO Panel considered the overall quality of the performed literature searches acceptable. The literature searches did not identify any relevant publications on soybean GMB151.

The GMO Panel concludes that soybean GMB151 is as safe as its conventional counterpart and the tested non-GM soybean reference varieties with respect to potential effects on human and animal health and the environment.

[1] https://doi.org/10.2903/j.efsa.2018.5345

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1. Introduction

The scope of the application EFSA-GMO-NL-2018-153 is for food and feed uses, import and processing of soybean GMB151 and does not include cultivation in the European Union (EU). Soybean GMB151 was developed to confer tolerance to 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor herbicides such as isoxaflutole and resistance to nematodes.

1.1. Background and Terms of Reference as provided by the requestor

On 9 October 2018, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands application EFSA-GMO-NL-2018-153 for authorisation of soybean GMB151 (Unique Identifier BCS-GM151-6), submitted by BASF Agricultural Solutions Seed US LLC (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003[2]. Following receipt of application EFSA-GMO-NL-2018-153, EFSA informed EU Member States and the European Commission, and made the application available to them. Simultaneously, EFSA published summary of the application.[3]

EFSA checked the application for compliance with the relevant requirements of EFSA guidance documents, and, when needed, asked the applicant to supplement the initial application. On 4 March 2019, EFSA declared the application valid.

From validity date, EFSA and its scientific Panel on Genetically Modified Organisms (hereafter referred to as 'the GMO Panel') endeavoured to respect a time limit of six months to issue a scientific opinion on application EFSA-GMO-NL-2018-153. Such time limit was extended whenever EFSA and/or GMO Panel requested supplementary information to the applicant. According to Regulation (EC) No 1829/2003, any supplementary information provided by the applicant during the risk assessment was made available to the EU Member States and European Commission (for further details, see the section 'Documentation', below). In accordance with Regulation (EC) No 1829/2003, EFSA consulted the nominated risk assessment bodies of EU Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC[4]. The EU Member States had three months to make their opinion known on application EFSA-GMO-NL-2018-153 as of date of validity.

1.2. Terms of Reference as provided by the requestor

According to Articles 6 and 18 of Regulation (EC) No 1829/2003, EFSA and its GMO Panel were requested to carry out a scientific risk assessment of soybean GMB151 in the context of its scope as defined in application EFSA-GMO-NL-2018-153.

According to Regulation (EC) No 1829/2003, this scientific opinion is to be seen as the report requested under Articles 6(6) and 18(6) of that Regulation and thus will be part of the EFSA overall opinion in accordance with Articles 6(5) and 18(5). The relevant information is made available in the EFSA Register of Questions including the information required under Annex II to the Cartagena Protocol; a labelling proposal; a Post-Market Environmental Monitoring (PMEM) plan as provided by the applicant; the method(s), validated by the Community reference laboratory, for detection, including sampling, identification of the transformation event in the food-feed and/or foods-feeds produced from it and the appropriate reference materials.[5]

2. Data and methodologies

2.1. Data

The GMO Panel based its scientific risk assessment of soybean GMB151 on the valid application EFSA-GMO-NL-2018-153, additional information provided by the applicant during the risk assessment, relevant scientific comments submitted by the Member States and relevant peer-reviewed scientific publications.

[2] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23.

[3] Available online: https://open.efsa.europa.eu/questions/EFSA-Q-2018-00781

[4] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 12.3.2001, p. 1–38.

[5] https://open.efsa.europa.eu/questions/EFSA-Q-2018-00781

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2.2. Methodologies

The GMO Panel conducted its assessment in line with the principles described in Regulation (EU) No 503/2013, its applicable guidelines (i.e. EFSA GMO Panel, 2010a,b, 2011a,b, 2015a,b), explanatory notes and statements (i.e. EFSA, 2017a,b, 2019a) for the risk assessment of GM plants. For the assessment of 90-day animal feeding studies, the GMO Panel took into account the criteria included in the EFSA Scientific Committee guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed (EFSA Scientific Committee, 2011) and the explanatory statement for its applicability (EFSA, 2014). The GMO Panel also assessed the applicant's literature searches, which include a scoping review, in accordance with the recommendations on literature searching outlined in EFSA (2010, 2017a). In the frame of the contracts OC/EFSA/GMO/2013/01 and OC/EFSA/GMO/2014/01, contractors performed preparatory work and delivered reports on the methods applied by the applicant in performing bioinformatic and statistical analyses, respectively.

3. Assessment

3.1. Systematic literature review as requested by Commission Regulation (EU) No 503/2013

The GMO Panel assessed the applicant's literature searches on soybean GMB151, which include a scoping review, according to the guidelines given in EFSA (2010, 2017a).

A systematic review as referred to in Regulation (EU) No 503/2013 has not been provided in support to the risk assessment of application EFSA-GMO-NL-2018-153. Based on the outcome of the scoping review, the GMO Panel agrees that there is limited value of undertaking a systematic review for soybean GMB151 at present.

The GMO Panel considered the overall quality of the performed literature searches acceptable. The literature searches did not identify any relevant publications on soybean GMB151.

3.2. Molecular characterisation[6]

3.2.1. Transformation process and vector constructs

Soybean GMB151 was developed by *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*)-mediated transformation. Explants of soybean variety Thorne were co-cultured with a disarmed *A. tumefaciens* strain LBA4404 containing the vector pSZ8832. The plasmid pSZ8832 used for the transformation contains two expression cassettes between the right and left border of the T-DNA, containing the following genetic elements:

- The hppdPf-4Pa expression cassette consists of the P2 × 35S promoter from Cauliflower Mosaic Virus, the TPotpY-1Pf transit peptide containing sequences of the RuBisCO small subunit genes of *Zea mays* and *Helianthus annuus*, the hppdPf-4Pa coding sequence of the 4-hydroxyphenylpyruvate dioxygenase gene of *Pseudomonas fluorescens* and the T35S sequence including the 3′ untranslated region of the 35S transcript of the Cauliflower Mosaic Virus.
- The cry14Ab-1.b expression cassette consists of the Pubi10At sequence including the promoter region of ubiquitin-10 gene of *Arabidopsis thaliana*, the cry14Ab-1.b coding sequence of the delta-endotoxin gene of *Bacillus thuringiensis* and the T35S sequence including the 3′ untranslated region of the 35S transcript of the Cauliflower Mosaic Virus.

The vector backbone contained elements necessary for the maintenance and selection of the plasmid in bacteria.

3.2.2. Transgene constructs in the GM plant

Molecular characterisation of soybean GMB151 was performed by next generation sequencing (NGS), junction sequence analysis (JSA), polymerase chain reaction (PCR) and DNA sequence analysis, in order to determine insert copy number, size and organisation of the inserted sequences and to confirm the absence of plasmid backbone sequences. The approach used is acceptable in terms of coverage and sensitivity. Overall, the quality of the sequencing methodology and data sets was

[6] Dossier: Part II – Section 1.2; additional information: 3/6/2019, 8/10/2019, 30/9/2020 and 4/12/2020.

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assessed by the EFSA GMO Panel and is in compliance to the requirements listed in the EFSA Technical Note (EFSA GMO Panel, 2018).

NGS/JSA of the whole genome demonstrated that soybean GMB151 contains a single insert, consisting of a single copy of the T-DNA. NGS/JSA also confirmed the absence of plasmid backbone sequences in the soybean genome.

Sanger sequencing of PCR-amplified fragments determined the nucleotide sequence of the entire soybean GMB151 event consisting of 7,459 bp of the insert and 1,000 bp of both 5′ and 3′ flanking regions. The Sanger analysis revealed that the insert in soybean GMB151 is characterised by a 482-bp deletion in the promoter P2 × 35S as compared to the T-DNA sequence in the plasmid pSZ8832 used for the transformation.

Both NGS/JSA and Sanger sequencing showed that the 3′-end of the event in soybean GMB151 is characterised by a 39-bp filler DNA. Further analysis revealed that one portion of the filler DNA (21 bp) showed identity to ORIpVS1 sequence from plasmid pSZ8832 while another portion (17 bp) showed identity to a sequence of the soybean genomic 3′-flanking region.

A comparison with the sequenced pre-insertion locus indicated that 63 bp were deleted from the soybean genomic DNA. The possible interruption of known endogenous soybean genes by the insertion in soybean GMB151 was evaluated by bioinformatics analyses of the pre-insertion locus and of the genomic sequences flanking the insert. The results of these analyses indicate that the insertion may have occurred in the 3′ UTR of the predicted gene for BON1-associated protein 1-like protein (NCBI accession number XM_006583276). The coding region of the gene is not affected and since the soybean genome is a partially diploidised tetraploid, paralogues (multiple copies of the gene) are expected. The function of the predicted gene for BON1-associated protein 1-like protein has not been characterised in soybean. In *Arabidopsis*, interruption of BAP1 gene (encoding BON1-associated protein 1) leads to constitutively active defence response and results in a dwarf phenotype (Yang et al., 2007) that has not been observed in soybean GMB151. Overall, these analyses indicate that the insertion of the T-DNA in the 3′UTR of the predicted gene for BON1-associated protein 1-like protein does not lead to unintended effects in soybean GMB151; this is also confirmed by compositional, agronomic and phenotypic characteristics (see Section 3.3).

The results of segregation (see Section 3.2.5) and bioinformatics analyses establish that the insert is located in the nuclear genome.

In addition, updated bioinformatics analyses of the amino acid sequence of the newly expressed HPPD-4 protein reveal no significant similarities to toxins and allergens. Initial bioinformatic analyses of the amino acid sequences of the Cry14Ab-1 revealed no significant similarities to known toxins but similarity of this protein with an allergen was detected. This hit was further assessed by the applicant and it is discussed in Section 3.4.4.1. Subsequently, updated bioinformatic analysis did not reveal any relevant hits. The updated bioinformatic analyses of the newly created open reading frames (ORFs) within the insert and spanning the junctions between the insert and genomic DNA indicated the presence of an eight amino acid exact match between an ORF and a putative chitinase (Cas s 5) from chestnut (*Castanea sativa*). This ORF is found within the transcriptional unit of the Cry14Ab-1.b coding sequence but in a different reading frame and without any translational start codons (ATG). In conclusion, this analysis indicates that the expression of an ORF showing significant similarities to toxins or allergens is highly unlikely.

In order to assess the possibility for horizontal gene transfer (HGT) by homologous recombination (HR), the applicant performed a sequence identity analysis for soybean GMB151 to microbial DNA. The likelihood and potential consequences of plant-to-bacteria gene transfer are described in Section 3.5.1.2.

3.2.3. Protein characterisation and equivalence

Soybean GMB151 expresses two new proteins: Cry14Ab-1 and HPPD-4. HPPD-4 is a modified 4-hydroxyphenylpyruvate dioxygenase from *Pseudomonas fluorescens*, conferring tolerance to HPPD inhibiting herbicides. The GMO Panel has previously evaluated other HPPD proteins (EFSA GMO Panel, 2015a,b, 2020). Protein Cry14Ab-1 belongs to the Cry (crystal)-type protein family, in particular to the 'nematicidal branch' of Cry proteins from *B. thuringiensis* (Sanahuja et al., 2011). Given the technical restraints in producing large enough quantities for safety testing from plants, these proteins were recombinantly produced in *Escherichia coli*. A set of biochemical methods was employed to demonstrate the equivalence between the GMB151 and E. *coli*-produced Cry14Ab-1 and HPPD-4. Purified proteins from these two sources were characterised and compared in terms of their biochemical, structural and functional properties.

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Cry14Ab-1 protein characterisation and equivalence

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and western blot analysis showed that both plant- and microbe-produced Cry14Ab-1 proteins had the expected molecular weight of ~ 131.1 kDa and were comparably immunoreactive to Cry14Ab-1 protein specific antibodies. Glycosylation detection analysis demonstrated that none of the Cry14Ab-1 proteins were glycosylated. Amino acid sequence analysis of the plant-derived Cry14Ab-1 protein by mass spectrometry (MS) methods showed that the protein matched the deduced sequence as defined by the *cry14Ab-1 gene*. These sequence analysis data were consistent with the previously analysed microbe-produced Cry14Ab-1. In addition, the MS data showed that the N-terminal methionine of the plant-produced Cry14Ab-1 protein was truncated and aspartic acid-two was acetylated. Such modifications are common in eukaryotic proteins (e.g. Polevoda and Sherman, 2000). Functional equivalence was demonstrated by an insect feeding bioassay which showed that plant- and microbe-derived Cry14Ab-1 proteins had comparable insecticidal activity.

HPPD-4 protein characterisation and equivalence

SDS–PAGE and western blot analysis showed that both plant- and microbe-produced HPPD-4 proteins had the expected molecular weight of ~ 40.3 kDa and were comparably immunoreactive to HPPD-4 protein specific antibodies. Glycosylation detection analysis demonstrated that none of the HPPD-4 proteins were glycosylated. Amino acid sequence and intact mass analysis of the plant-derived HPPD-4 protein by MS methods showed that the protein matched the deduced sequence and molecular weight as defined by the *hppd-4* gene. These sequence analysis data were consistent with the previously analysed microbe-produced HPPD-4. In addition, the MS data showed that the N-terminal methionine was truncated from both HPPD-4 proteins and the four C-terminal amino acids of the plant-produced HPPD-4 protein were also truncated. These data also indicated the presence of another two variants of the plant-produced HPPD-4; one with an additional N-terminal cysteinic sulfinic acid and the third variant with an N-terminal cysteinic sulfinic acid and the four C-terminal amino acids truncated. Such modifications are common in eukaryotic proteins (e.g. Polevoda and Sherman, 2000). N-terminal sequence analysis by Edman degradation of the plant- and microbe-derived HPPD-4 proteins produced data consistent with those by MS. Functional equivalence was demonstrated by an *in vitro* assay which showed that plant- and microbe-derived HPPD-4 proteins had comparable enzymatic activity. Microbially produced HPPD-4 protein was also screened for its ability to utilise certain endogenous plant substrates. A number of compounds that could be substrates of this enzyme and potentially present in plants in addition to the intended substrate were tested. Although some catalysis was observed at a slow rate and with high protein amount for 3,4-dHPP, none of the compounds is likely to be a genuine in vivo substrate.

The data demonstrated that it is unlikely that HPPD-4 has a metabolic impact within soybean GMB151 different from that of the native (endogenous) enzyme.

The protein characterisation data comparing the biochemical, structural and functional properties of plant- and microbe-produced Cry14Ab-1 and HPPD-4 proteins, indicate that these proteins are equivalent, and the microbial derived proteins can be used in the safety studies.

3.2.4. Information on the expression of the insert

Protein levels of Cry14Ab-1 and HPPD-4 were analysed by an enzyme-linked immunosorbent assay (ELISA) in material harvested in a field trial across three locations in the USA during the 2016 growing season. Samples analysed included leaves (BBCH 13-14, BBCH 16-18, BBCH 60-66, BBCH 76-79), root (BBCH 13-14, BBCH 16-18, BBCH 60-66, BBCH 76-79), flower (BBCH 60-66), forage (BBCH 76-79), whole plant (BBCH 89-99) and seed (BBCH 89-99) from plants treated and not treated with the intended herbicide. The mean values and standard deviations of protein expression levels in seeds (n = 12), forage (n = 12) and flowers (n = 12) of the Cry14Ab-1 and HPPD-4 proteins used to estimate human and animal dietary exposure (see Section 3.4.5) are reported in Table 1.

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Table 1: Mean values (n = 12) and standard deviation of newly expressed protein in seeds [μg/g dry weight (dw) and μg/g fresh weight (fw)] flowers and forage (μg/g dw) from soybean GMB151

Tissue	Isoxaflutole treatment			
	Not treated		Treated	
	μg/g dry weight (dw)	μg/g fresh weight (fw)	μg/g dry weight (dw)	μg/g fresh weight (fw)
Seed (BBCH 89-99)				
Cry14Ab-1	101.82[a] ± 45.77[b] (45.78–176.46)[c]	90.23 ± 41.04 (40.82–157.00)	88.25 ± 40.01 (16.10–153.73)	78.49 ± 35.94 (14.33–137.81)
HPPD-4	4.50 ± 2.93 (1.25–9.92)	3.99 ± 2.62 (1.12–8.83)	4.50 ± 3.60 (1.33–12.84)	4.01 ± 3.23 (1.19–11.51)
Flowers[(d)] (BBCH 60-66)				
Cry14Ab-1	53.37 ± 11.47 (37.06–71.59)		51.81 ± 18.42 (33.13–77.77)	
HPPD-4	57.08 ± 14.43 (43.06–79.62)		74.52 ± 27.82 (41.63–127.86)	
Forage (BBCH 76-79)				
Cry14Ab-1	55.56 ± 10.01 (39.95–69.31)		52.73 ± 10.15 (41.40–72.40)	
HPPD-4	120.18 ± 42.47 (78.96–203.83)		129.03 ± 45.32 (87.61–196.27)	

(a): Mean value.
(b): Standard deviation.
(c): Range.
(d): Whole flowers were collected and analysed as surrogate tissue for the determination of newly expressed proteins in pollen.

3.2.5. Inheritance and stability of inserted DNA

Genetic stability of soybean GMB151 insert was assessed by NGS/JSA from five generations (T2, T4, T5, T6, and BC2F3) and PCR-based segregation analysis from five generations (two F2, two BC2F2, and one BC1F2). The results indicate that all the plants tested retained the single copy of the insert and flanking regions, which were stably inherited in subsequent generations.

Phenotypic stability was assessed by confirming the absence or presence of Cry14Ab-1 and HPPD-4 proteins in leaves collected from five generations. The expression of the Cry14Ab-1 and HPPD-4 proteins was confirmed in the tested tissue.

The results support the presence of a single insertion, segregating in a Mendelian fashion.

3.2.6. Conclusion on molecular characterisation

The molecular characterisation data establish that soybean GMB151 contains a single insert consisting of one copy of the hppdPf-4Pa and the cry14Ab-1.b expression cassettes. Bioinformatics analyses of the sequences encoding the newly expressed proteins and other ORFs within the insert or spanning the junctions between the insert and genomic DNA do not raise any safety concerns. The stability of the inserted DNA and of the introduced trait is confirmed over several generations. The methodology used to quantify the levels of the Cry14Ab-1 and HPPD-4 proteins is considered adequate. The protein characterisation data comparing the biochemical, structural and functional properties of plant- and microbe-produced Cry14Ab-1 and HPPD-4 proteins, indicate that these proteins are equivalent and the microbial derived proteins can be used in the safety studies.

3.3. Comparative analysis[7]

3.3.1. Overview of studies conducted for the comparative analysis

Application EFSA–GMO–NL–2018–153 presents data on agronomic and phenotypic characteristics as well as on forage and seed composition of soybean GMB151 (Table 2).

[7] Dossier: Part II – Section 1.3; additional information: 12/4/2019, 20/8/2019, 18/12/2019 and 31/3/2020.

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Table 2: Overview of comparative analysis studies to characterise soybean GMB151 in application EFSA–GMO–NL–2018–153

Study focus	Study details	Comparator	Commercial non-GM reference varieties
Agronomic and phenotypic characteristics	Field trials, 2017, US, eleven sites[(a)]	Thorne	9[(b)]
Compositional analysis	Field trials, 2017, US, eight sites[(a)]		

(a): Eight field trials conducted in 2017 were used for both the compositional and the agronomic/phenotypic analysis: at Keokuk, IA; York, NE; Shelby, IA; Shelby, IL; Lehigh, PA; Butler, MO; Pawnee, KS and Rush, IN. Three field trials conducted in 2017 were used only for the agronomic/phenotypic analysis: at Johnson, KS; Clinton, IL and Marshall, IA. A field trial established at Carlyle, IL was excluded from the statistical analysis because of flood damage at the start of the growing season.

(b): The following nine soybean varieties were used for both the compositional and the agronomic/phenotypic characterisation with their corresponding maturity group indicated in brackets were E2282 (2.2), E2692 (2.6), E2993 (2.9), E3066 (2.8), E3192 (3.1), E3494 (3.4), NGN 3121STS (3.1), NGN 3292C (2.9) and NGN 3347C (3.4).

3.3.2. Experimental field trial design and statistical analysis

At each field trial site, the following materials were grown in a randomised complete block design with four replicates: soybean GMB151 not exposed to the intended herbicide, soybean GMB151 exposed to the intended herbicide, the comparator Thorne and three non-GM reference varieties.

The agronomic/phenotypic and compositional data were analysed as specified by EFSA GMO Panel (2010b, 2011a). This includes, for each of the two treatments of soybean GMB151, the application of a difference test (between the GM soybean and the comparator) and an equivalence test (between the GM soybean and the set of non-GM reference varieties).[8] The results of the equivalence test are categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence).[9]

3.3.3. Suitability of selected test materials

3.3.3.1. Selection of the GM soybean line and comparator

Soybean GMB151 was obtained using the non-GM soybean variety Thorne as recipient line. The comparator used in the field trials is the non-GM soybean variety Thorne, which has a genetic background near-isogenic to that of soybean GMB151 as documented by the pedigree. The EFSA GMO Panel considers the selected variety (Thorne) the conventional counterpart for the comparative analysis.

The GM soybean GMB151 and its conventional counterpart belong to maturity group 3 that is appropriate for a wide range of growing environments across North America.

3.3.3.2. Selection of commercial non-GM soybean reference varieties

Commercial non-GM soybean reference varieties with maturity groups ranging from 2.2 to 3.4 were included in the field trials. Based on the information on the maturity group, the GMO Panel considers that the selected non-GM soybean reference varieties are appropriate for the comparative analysis.

3.3.3.3. Seed production and quality

The seeds of soybean GMB151 and the conventional counterpart used in the field trials (see Table 2) were produced, harvested and stored under similar conditions, before being sown in the field trials. The seed lots were verified for their purity via event specific quantitative PCR analysis. The germination of soybean GMB151 and its conventional counterpart was tested under warm and cold temperature conditions. The GMO Panel considers that the starting seed used as test material in the agronomic, phenotypic and compositional studies was of suitable quality.

3.3.3.4. Conclusion on suitability

The GMO Panel is of the opinion that the soybean GMB151, its comparator and the non-GM soybean reference varieties were properly selected and are of adequate quality. Therefore, the test materials are considered suitable for the comparative analysis.

[8] The purpose of the test of equivalence is to evaluate the estimated mean values for the GM crop taking into account natural variability as defined by a set of non-GM references varieties with a history of safe use for consumption as food or feed.

[9] In detail, the four outcomes are: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence).

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3.3.4. Representativeness of the receiving environments

3.3.4.1. Selection of field trial sites

The selected field trial sites were located in commercial soybean-growing regions of the US. Climate and soil characteristics of the selected fields were diverse,[10] corresponding to optimal, near-optimal and sub-optimal conditions for soybean cultivation (Sys et al., 1993). The GMO Panel considers that the selected sites, including the subset chosen for the compositional analysis, reflect commercial soybean-growing regions in which the test materials are likely to be grown.

3.3.4.2. Meteorological conditions

Maximum and minimum mean temperatures and sum of precipitations were provided on a weekly basis. Exceptional weather conditions were reported at four of the selected sites. However, due to the lack of major impacts on plant growth at these sites,[11] the GMO Panel considers that the exceptional weather conditions did not invalidate the selection of the field trial sites for the comparative analyses.

3.3.4.3. Management practices

The field trials included plots containing soybean GMB151, plots with the conventional counterpart and plots with non-GM reference varieties, all managed according to local agricultural practices. In addition, the field trials included plots containing the soybean GMB151 managed following the same agricultural practices, plus exposed to the isoxaflutole containing herbicide that was applied at the BBCH 00-03 growth stage. The GMO Panel considers that the management practices including sowing, harvesting and application of plant protection products were appropriate.

3.3.4.4. Conclusion on representativeness

The GMO Panel concludes that the geographical locations, soil and climate characteristics, meteorological conditions and management practices of the field trials are typical for receiving environments where the test materials could be grown.

3.3.5. Agronomic and phenotypic endpoints

3.3.5.1. Agronomic and phenotypic endpoints tested under field conditions

Thirteen agronomic and phenotypic endpoints plus information on abiotic stressors, disease incidence and arthropod damage were evaluated in the field trials (see Table 2).[12] The endpoints lodging and seed loss were not analysed with formal statistical methods because more than half of the measurements were 0. The remaining 11 endpoints were analysed with the tests of difference and equivalence, with the following results:

- For soybean GMB151 (not treated), statistically significant differences with the conventional counterpart were identified for fruit count and seed moisture; both endpoints fell under equivalence category I.
- For soybean GMB151 (treated), statistically significant differences with the conventional counterpart were identified for crop development, flowering duration, plant height, seed moisture and final stand count; all these endpoints fell under equivalence category I.

3.3.6. Compositional analysis

Soybean GMB151 seeds and forage harvested from 8 sites (Table 2) were analysed for 112 constituents (9 in forage and 103 in seeds), including those recommended by OECD (2012). The

[10] Soil types of the field trials were clay loam, silty clay loam, loam, silt loam and sandy loam; soil organic matter ranged from 1.3% to 4.2%; pH ranged from 5.1 to 7.0; mean temperatures and sum of precipitations for the typical crop growing season ranged respectively from 18.3°C to 24,3°C and from 226 to 849 mm.

[11] Drought at site 06, excessive moisture and flooding at site 09 and excessive rain at sites 05 and 10.

[12] Early stand count, crop development, days to flowering, flowering duration, plant height, lodging, final stand count, days to maturity, seed loss, fruit count, seed weight, seed moisture and yield.

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statistical analysis was not applied to 23 seed constituents because more than one third of the analyses were below the limit of quantification.[13]

The statistical analysis was applied to a total of 89 constituents (80 in seeds[14] and nine in forage[15]); a summary of the outcome of the test of difference and the test of equivalence is presented in Table 3:

- For soybean GMB151 treated with the intended herbicide, all the 34 seed endpoints for which significant differences were found between the GM soybean and the conventional counterpart fell under equivalence category I or II. Among the endpoints for which no significant difference was observed, trypsin inhibitor fell under equivalence category III. For forage, no statistically significant differences were found for any of the nine forage constituents except for neutral detergent fibre (NDF) content that fell under equivalence category I. Among the endpoints for which no significant difference was observed, moisture fell under equivalence category III.
- For soybean GMB151 treated with conventional herbicides, all the 31 seed endpoints for which significant differences were found between the GM soybean and the conventional counterpart fell under equivalence category I or II, except for palmitic acid (C16:0) and heptadecenoic acid (C17:1) that fell under equivalence category III. Among the endpoints for which no significant difference was observed, moisture and trypsin inhibitor fell under equivalence category III. For forage, statistically significant differences were found for ash, carbohydrates, and crude protein. The equivalence test could not be done for carbohydrates and crude protein because of the lack of variation among the non-GM reference varieties; the endpoint ash fell under equivalence category I.[16]

Table 3: Outcome of the comparative compositional analysis in seeds and forage for soybean GMB151. The table shows the number of endpoints in each category

		Test of difference[(a)]			
		Treated[(c)]		**Not-treated[(c)]**	
		Not different	**Significantly different**	**Not different**	**Significantly different**
Test of equivalence[(b)]	Category I/II	50	34[(d)]	53	29[(d)]
	Category III/IV	2[(e)]	–	2[(e)]	2[(f)]
	Not categorised	3[(g)]	–	1[(g)]	2[(h)]
	Total endpoints	89		89	

[13] Caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristoleic acid (C14:1), pentadecanoic acid (C15:0), pentadecenoic acid (C15:1), stearidonic acid (C18:4), nonadecanoic acid (19:0), eicosadienoic acid (20:2), eicosatrienoic acid (20:3), arachidonic acid (C20:4), eicosapentaenoic acid (C20:5), erucic acid (C22:1), docosapentaenoic acid (22:5n3), docosapentaenoic acid (22:5n6), docosahexaenoic acid (22:6), sodium, a-tocotrienol, b-tocotrienol, d-tocotrienol, g-tocotrienol, total tocotrienols, homogentisic acid.

[14] Seed constituents included proximates and fibre content (ash, carbohydrates, moisture, crude protein, crude fat, acid detergent fibre (ADF), neutral detergent fibre (NDF) and total dietary fibre), minerals (calcium, phosphorus, copper, iron, magnesium, manganese, potassium, zinc), amino acids (alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine), fatty acids (myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (17:0), heptadecenoic acid (C17:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C20:1) behenic acid (22:0) and lignoceric acid (C24:0)), vitamins (vitamin E (α-tocopherol), β-tocopherol, δ-tocopherol, γ-tocopherol, total tocoferols, vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B3 (niacin), vitamin B5 (pantothenic acid), vitamin B6 (pyridoxine), vitamin B9 (folic acid), vitamin A (β-carotene), and vitamin K1 (phylloquinone)), isoflavones (daidzein, genistein, glycitein, total isoflavones), endogenous allergens (gly m 1, gly m 3, gly m 4, gly m 5 (β-conglycinin), gly m 6 (glycinin), gly m 7, gly m 8, gly m Bd 28k, gly m Bd 30k, Kunitz trypsin inhibitor 1, Kunitz trypsin inhibitor 3), other compounds (phytic acid, raffinose, soybean lectin, stachyose, trypsin inhibitor).

[15] Forage constituents included moisture, crude protein, crude fat, ash, carbohydrates, acid detergent fibre (ADF), neutral detergent fibre (NDF), calcium and phosphorus.

[16] Ash levels in forage were found to be significantly higher in one of the sites (mean values between 14 and 18.3% DW) as compared to the other sites for all cultivated plants (GM crop, conventional counterpart, reference varieties). The GMO Panel accepted the confirmation by the applicant on the reliability of these data after considering the representativeness of the site, the sampling protocol, the quality control measures implemented by the laboratory (additional information 18/12/2019 and 31/3/2020), and that similar and higher ash levels in soybean forage are described in international databases (ILSI CCDB, https://foodsystems.org/resources/ccdb/).

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(a): Comparison between soybean GMB151 and its conventional counterpart.
(b): Four different outcomes: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence). Not categorised means that the test of equivalence was not applied because of the lack of variation among the non-GM reference varieties.
(c): Treated/not treated with the intended herbicide.
(d): Endpoints with significant differences between soybean GMB151 and its conventional counterpart falling in equivalence category I-II (treated and not treated).
For seeds, both treated and not treated: moisture, carbohydrates, crude protein, glutamic acid, proline, oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), arachidic acid (C20:0), eicosenoic acid (C20:1), behenic acid (C22:1), lignoceric acid (C24:0), gly m Bd 30k, daidzein, genistein, glycitein, total isoflavones, phytic acid, calcium, vitamin B1 (thiamine), vitamin B3 (niacin), vitamin B9 (folic acid), δ-tocopherol. Only treated: NDF, palmitic acid (C16:0), heptadecenoic acid (C17:1), gly m 8, Kunitz trypsin inhibitor 1, Kunitz trypsin inhibitor 3, copper, magnesium, zinc, vitamin B5 (pantothenic acid). Not treated: aspartic acid, tryptophan, palmitoleic acid (C16:1), gly m 5 (β-conglycinin), vitamin A (β-carotene)
For forage: ash (only not treated), NDF (only treated).
(e): Endpoints in seeds and forage with no significant differences between soybean GMB151 and its conventional counterpart and falling under equivalence category III: trypsin inhibitor (seeds, both treated and not treated) and moisture (forage, both treated and not treated).
(f): Endpoints with significant differences between the soybean GMB151 and its conventional counterpart and falling in equivalence category III-IV. Estimated means are reported for these endpoints in Table 4.
(g): Endpoints not categorised for equivalence and without significant differences between the soybean GMB151 and its conventional counterpart: crude fat, carbohydrates and crude protein (forage, treated), crude fat (forage, not treated).
(h): End points not categorised for equivalence and with significant differences between soybean GMB151 and its conventional counterpart: carbohydrates and crude protein (forage, not treated).

The GMO Panel assessed all significant differences between soybean GMB151 and its conventional counterpart, taking into account the potential impact on plant metabolism and the natural variability observed for the set of non-GM reference varieties. Mean estimates for the endpoints showing significant differences between soybean GMB151 and its conventional counterpart and falling under category III/IV are given in Table 4, together with endpoints with significant differences with the conventional counterpart where the equivalence test was not applied because of the lack of variation among the non-GM reference varieties.

Metabolites closely related to the tyrosine catabolic pathway (Kramer et al., 2014) were included among the constituents analysed in soybean GMB151, i.e. β-tocopherol, δ-tocopherol, γ-tocopherol, α-tocopherol (and their corresponding tocotrienols), β-carotene, vitamin K1, and homogentisic acid. As described above, the outcome of the comparative assessment indicated that no further assessment regarding food and feed safety is required for these compounds.

Table 4: Quantitative results (estimated means and equivalence limits) for compositional endpoints in seeds and forage that are further assessed based on the results of the statistical analysis

	Endpoint	Soybean GMB151[(a)]		Conventional counterpart	Non-GM reference varieties	
		Not treated	Treated		Mean	Equivalence limits
Seeds	Palmitic acid (C16:0) (% FA)	10.6*	10.7*	11.0	11.5	10.6–12.5
	Heptadecenoic acid (C17:1) (% FA)	0.0651*	0.0650*	0.0617	0.0576	0.051–0.065
Forage	Carbohydrate (% dw)	66.3*	67.1	68.7	66.9	–
	Crude protein (% dw)	20.9*	20.5	19.6	19.7	–

(a): For the soybean GMB151, significantly different values are marked with an asterisk, while the outcomes of the test of equivalence are differentiated by greyscale backgrounds. A white background is used for equivalence category I or II and for carbohydrate and crude protein in forage (both treated and not treated), and light grey background corresponds to equivalence category III. dw = dry weight; FA = fatty acids. Treated: treated with the intended herbicide; not treated: treated only with conventional herbicides (see Section 3.3.4.3).

3.3.7. Conclusion on the comparative assessment

Considering the selection of test materials and field trial sites, the crop management practices and the outcome of the agronomic-phenotypic characterisation, the GMO Panel concludes that the field trials are appropriate to support the comparative analysis.

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Taking into account the natural variability observed for the set of non-GM reference varieties, the GMO Panel also concludes that:

1) None of the differences identified in agronomic and phenotypic characteristics between soybean GMB151 and the conventional counterpart needs further assessment.
2) None of the differences identified in forage and seed composition between soybean GMB151 and its conventional counterpart needs further assessment regarding food and feed safety, except for the levels of palmitic acid (C16:0) and heptadecenoic acid (C17:1) in seeds (not treated), and carbohydrate and crude protein in forage (not treated), which are further assessed in Sections 3.4.3 and 3.4.6.

3.4. Food/feed safety assessment

3.4.1. Effects of processing

Soybean GMB151 will undergo existing production processes used for conventional soybean. No novel production process is envisaged. Based on the outcome of the comparative assessment, processing of the GM soybean into food and feed products is not expected to result in products being different from those of conventional non-GM soybean varieties.

3.4.2. Stability of newly expressed proteins

Protein stability is one of several relevant parameters to consider in the weight-of-evidence approach in protein safety assessment (EFSA GMO Panel, 2010c, 2011a, 2017, 2021). The term protein stability encompasses several properties such as thermal stability, pH-dependent stability, proteolytic stability and physical stability (e.g. tendency to aggregate), among others (Li et al., 2019). It has been shown, for example, that when characteristics of known food allergens are examined, one of the most prominent traits attributed to food allergens is protein stability (Helm, 2001; Breiteneder and Mills, 2005; Costa et al., 2021).

Effect of temperature and pH on newly expressed proteins

The applicant provided information on the effects of temperature on Cry14Ab-1 protein. Samples of Cry14Ab-1 from a microbial recombinant system were incubated for 30 min at 4°C, 25°C, 37°C, 55°C, 75°C and 95°C followed by SDS-PAGE and western blotting, by ELISA quantification or by a bioassay measuring its activity. The studies showed that Cry14Ab-1 is unstable, undetectable and has no activity after incubation at temperatures ≥ 75°C. In relation to the effect of pH on the Cry14Ab-1 protein, the molecular mass (~ 131 kDa) and immunoreactivity of the protein was unchanged at pH 1.2 and 7.5.

Similar studies were performed with the HPPD-4 protein showing instability upon temperature treatments of ≥ 55°C. Furthermore, the optimum conditions for this enzyme activity were at pH 8.0 and 22.5°C. The activity of the enzyme was just above the limit of quantification at pH 9.5 while at 65°C no detectable activity was observed.

In vitro *protein degradation by proteolytic enzymes*

The applicant provided information on *in vitro* protein degradation. First, a resistance to degradation by pepsin of the Cry14Ab-1 and HPPD-4 proteins from microbial recombinant systems were investigated in solutions at pH ~ 1.2. The integrity of the test proteins in samples of the incubation mixture taken at various time points were analysed by SDS–PAGE gel electrophoresis followed by protein staining or by western blotting. The Cry14Ab-1 and HPPD-4 proteins were degraded by pepsin within 0.5 min of incubation.

Second, the resistance to degradation by pancreatin of the Cry14Ab-1 and HPPD-4 proteins was also analysed in solutions at pH ~ 7.5. The Cry 14Ab-1 protein was partially degraded within 60 min while the HPPD-4 protein was completely degraded within 10 min of incubation.

3.4.3. Toxicology

3.4.3.1. Testing of newly expressed proteins[17]

Soybean GMB151 expresses two new proteins, Cry14Ab-1 and HPPD-4. The GMO Panel assessed the safety of these proteins considering molecular characterisation and bioinformatic analyses

[17] Dossier: Part II – Section 1.4.1; additional information: 7/4/2020.

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(Section 3.2) and taking into account *in vitro* (Section 3.4.2) and *in vivo* studies. Based on scientific knowledge (Section 3.2), no synergistic or antagonistic interactions between these two proteins which could raise safety concerns for food and feed from soybean GMB151 are expected.

Proteins used for toxicological studies

Cry14Ab-1 and HPPD4 proteins obtained from microbial recombinant systems and equivalent to the respective plant proteins (Section 3.2) were used for safety studies. Post-translational modifications were observed in the plant-produced Cry14Ab-1 and HPPD-4 proteins (Section 3.2.4). These were assessed by the GMO Panel and found not to raise concerns for the safety of humans and animals, since these modifications are commonly present in eukaryotic systems and have negligible impact on the structure and function of the proteins.

Bioinformatics

Bioinformatic analyses of the amino acid sequences of the Cry14Ab-1 and HPPD-4 revealed no significant similarities to known toxins (Section 3.2.2).

28-day repeated dose toxicity studies

Cry14Ab-1

The 28-day oral repeated dose toxicity study on Cry14Ab-1 provided was conducted in accordance with OECD TG 407 (2008) and with the principles of Good Laboratory Practice (GLP). Groups of C57BL/6J mice, (10/sex per group), 7-week-old at the start of dosing, were administered by oral gavage: the Cry14Ab-1 protein[18] at a targeted nominal dose of 1,000 mg/kg body weight (bw) per day (Cry14Ab-1 protein group); or the vehicle[17] alone (vehicle control group). The test substance contained 91% of Cry14Ab-1 protein produced by a microbial recombinant system (*Bacillus thuringiensis*). This protein was considered by the GMO Panel to be equivalent to the protein expressed in soybean GMB151. In-life procedures and observations and terminal procedures were conducted in accordance to OECD (2008), with some exceptions. Blood clotting parameters were not determined due to the relatively small blood volume obtained from mice; related parameters (protein levels, platelet counts and spleen histopathology were unaffected by treatment and no clinical signs of bleeding/haemorrhage were seen in the study; other Cry proteins are not reported to induce adverse effects on blood clotting in mammals). A functional observation battery was not performed. No neurotoxicity was expected from an exposure to the Cry14Ab-1 protein; Cry proteins that have been tested exhibit no neurotoxicity to mammals. In addition, a *B. thuringiensis* Cry14Ab-1 protein did not induce any clinical signs at a high dose level (2,000 mg/kg bw) when administered acutely by gavage in mice, and was rapidly degraded *in vitro*. In addition, the 28-day study design included several parameters that could give indications of possible neurotoxic effects, if any (e.g. clinical observation, physical examination). The GMO Panel concludes that the deviations from the test guideline do not compromise the safety assessment of Cry14Ab-1 protein.

In the statistical analysis, conducted for the two sexes separately, the test and control groups were compared with a *t*-test.

Based on the results of concentration analysis, the administered dose was 1,010 mg/kg bw per day. The results of the substance analysis tests indicated that the dosing preparations were homogeneous and exhibited acceptable stability.

There were no deaths or abnormal clinical signs. The GMO Panel assessed the statistically significant findings observed in the Cry14Ab-1 protein group and concluded that these are not adverse effects of the treatment with Cry14Ab-1 (see Appendix A, Table A.1). No gross pathological findings related to the treatment with Cry14Ab-1 protein were seen at necropsy. Microscopic examinations of a wide range of organs and tissues identified no biologically relevant test substance-related differences in the incidences and severity of the histopathological findings between the groups. A notable increase in the incidence of mononuclear cell infiltration in the liver (6 vs 1) was not statistically significant ($p = 0.057$) and is a common finding in mice of this strain and age.

The GMO Panel concludes that no adverse effects were observed in this 28-day mouse toxicity study on Cry14Ab-1 protein at gavage doses up to 1,000 mg/kg bw per day.

HPPD-4

The 28-day oral repeated dose toxicity study on HPPD-4 provided was conducted in accordance with OECD (2008) and with the principles of GLP. Groups of C57BL/6J mice, (10/sex per group),

[18] Formulated in a vehicle (50 mmol/L Na_2CO_3 buffer containing 0.46 mmol/L 2-mercaptoethanol).

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7-week old at the start of dosing were administered by oral gavage: the HPPD-4 protein[19] at a targeted nominal dose of 1,000 mg/kg bw per day (HPPD-4 protein group); or the vehicle[19] alone (vehicle control group). The test substance contained 98% of HPPD-4 protein produced by a microbial recombinant system (*E. coli*).

In-life procedures and observations and terminal procedures were conducted in accordance to OECD (2008), with some exceptions. Sodium and potassium levels were not determined due to the relatively small volume of blood obtained from mice; it is noted that the vehicle contained sodium and potassium salts, which might have compromised interpretation of any changes. Blood clotting parameters were not determined due to the relatively small blood volume obtained from mice; related parameters (protein levels, platelet counts, and spleen histopathology) were unaffected by treatment and no clinical signs of bleeding/haemorrhage were seen in the study. A functional observation battery was not performed. No neurotoxicity was expected from an exposure to the HPPD protein. In addition, an *E. coli* HPPD-4 protein did not induce any clinical signs at a high dose level (2,000 mg/kg bw) when administered acutely by gavage in mice and was rapidly degraded *in vitro*. In addition, the study design included several parameters that could give indications of possible neurotoxic effects, if any (e.g. clinical observation, physical examination). The GMO Panel concludes that the deviations from the test guideline do not compromise the safety assessment of HPPD-4 protein.

In the statistical analysis, conducted for the two sexes separately, the test and control groups were compared with a *t*-test.

Based on the results of concentration analysis, the administered dose was 1,020 mg/kg bw per day. The results of the substance analysis tests indicated that the dosing preparations were homogeneous and exhibited acceptable stability.

One female from the HPPD-4 group was killed on day 26 after exhibiting evidence of a gavage error, unrelated to the test substance. The GMO Panel assessed the statistically significant findings observed in the HPPD-4 protein group and concluded that these are not adverse effects of the treatment with this protein (see Appendix A, Table A.2). Microscopic examinations of a wide range of organs and tissues identified no biologically relevant test substance-related differences in the incidences and severity of the histopathological findings between the groups.

The GMO Panel concludes that no adverse effects were observed in this 28-day mouse toxicity study on HPPD-4 protein at gavage doses up to 1,000 mg/kg bw per day.

Conclusions

Based on the molecular characterisation information, bioinformatic analyses, *in vitro* and *in vivo* studies, the GMO Panel considers that there are no toxicological concerns for the Cry14Ab-1 and HPPD-4 proteins newly expressed in soybean GMB151.

3.4.3.2. Testing of new constituents other than newly expressed proteins[20]

No new constituents other than the newly expressed proteins have been identified in seed and forage from soybean GMB151. Therefore, no further food/feed safety assessment of components other than the newly expressed proteins is required.

3.4.3.3. Information on altered levels of food and feed constituent[21]

Palmitic acid (C16:0) and heptadecenoic acid (C17:1) in seeds (not treated), and carbohydrate and crude protein in forage (not treated) were significantly different in soybean GMB151 when compared with its conventional counterpart, and showed a lack of equivalence with the non-GM reference varieties or could not be categorised (Section 3.3.7). No toxicological concern is identified regarding these compounds. Further information on safety is provided in Section 3.4.5.

3.4.3.4. Testing of the whole genetically modified food and feed[22]

Based on the outcome of the studies considered in the molecular characterisation and comparative analysis, no compositional modifications or indication of possible unintended effects relevant to food/feed safety of soybean GMB151 have been identified. Therefore, animal feeding studies with food/feed derived from soybean GMB151 are not considered necessary by the GMO Panel (EFSA GMO Panel,

[19] Formulated in a vehicle (50 mmol/L Tris-HCl buffer containing 136 mmol/L NaCl, 2.7 mmol/L KCl, 0.07 mmol/L $FeCl_2$).
[20] Dossier: Part II – Section 1.4.2.
[21] Dossier: Part II – Section 1.4.3.
[22] Dossier: Part II – Section 1.4.4; additional information: 31/3/2020.

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2011a). In accordance with Regulation (EU) No 503/2013, the applicant provided a 90-day study in rats receiving diets containing soybean GMB151.

In this study, the applicant tested a group of rats given a diet containing GMB151 soybean and compared this with a control group. In addition, a group of rats given a diet containing a non-GM commercial variety was introduced as reference group. This study is adapted from OECD Test Guideline 408 (OECD, 1998) and complies with the principles of GLP, except for the lack of analytical determination of concentration, homogeneity and stability of the test item in the formulated diets. It is recognised that it may not always be technically possible to generate information on homogeneity and concentration for a test item administrated or formulated, and the lack of such data and its impact on the validity of a study should be justified (OECD, 2018). The GMO Panel acknowledges that there are no practical methods available to analytically determine these for complex test items such as soybean meal in formulated diets and considers adequate the application of proper diet preparation procedures and regular evaluations of the mixing methods. Based on the information received from the applicant, the GMO Panel considers that the diet preparation procedures in place in the facilities where the diets for this study were prepared guaranteed their homogeneity and the proper concentration of the respective test or control items. As regards the stability of the test, control and reference items (defatted toasted soybean meals) in the diet, the applicant considers that, in accordance to product expiration declared by the diet manufacturer, the constituents of the diets used in these studies are stable for the duration of the treatment. The GMO Panel considers this justification acceptable. In addition, the GMO Panel notes that the diets were prepared and analysed in an ISO-9001 certified facility.

A total of 96 Crl:CD(SD) rats (48/sex) were randomly allocated to three treatment groups (one control group, one GMB151 test group and one commercial variety group, n = 16/sex per group) according to a randomised complete block design. The diets contained 30% (w/w) defatted toasted meal from an appropriate conventional counterpart (Thorne), from GMB151 or from a commercial reference soybean variety,[23] respectively. The GMB151 source material was sprayed with the intended herbicide.[24] The seeds used to produce the test and control materials (i.e. defatted toasted meal) were sent to the processing facility in about one month from harvest, then maintained at room temperature for about one month and finally processed into defatted toasted meal. The identity of GMB151 test material was confirmed by PCR. Balanced diets were prepared according to the specifications for PMI Certified Rodent LabDiet® 5002 within 4 months from processing; the diets were analysed for the presence of the GMB151 event. The test, control and reference materials, as well as test, control and reference diets were analysed for proximates, amino acids, minerals, mycotoxins, pesticides[25] and antinutrients. In-life procedures and observations and terminal procedures were conducted in accordance to OECD Test Guideline 408 (OECD, 1998).

In the statistical analysis, rats consuming the test diet were compared with those consuming the control diet. The cage was considered as the experimental unit. The data for continuous parameters, were analysed with analysis of variance (ANOVA) for the two sexes combined;[26] in case a significant sex-by-diet interaction was identified (and for sex-specific endpoints) the results of a sex-specific analysis[27] were considered for the assessment. The data for the reference group were included in the analysis to provide additional information on the range of variability of the parameters.

There were no test diet-related incidents of mortality or clinical signs. No test diet-related adverse findings were identified in any of the investigated parameters. A small number of statistically significant findings were noted but these were not considered adverse effects of treatment for one or more of the following reasons:

- were within the normal variation for the parameter in rats of this age;
- were of small magnitude;
- were identified at only a small number of time intervals with no impact on the overall value;
- exhibited no consistent pattern with related parameters or end-points.

[23] E3494 commercial soybean variety.
[24] Isoxaflutole (Additional information 31/3/2020).
[25] Including residues of the trait-specific herbicide isoxaflutole and its metabolite diketonitrile in the meal.
[26] A mixed effect model was used, with diet, sex and sex-by-diet interaction as fixed effects as block-within-sex as random effect. For locomotor activity data, time interval (with the relevant interaction terms) was included as an additional fixed effect.
[27] Mixed effect model with diet as fixed effect and block as random effect. For locomotor activity data, time interval (with the relevant interaction terms) was included as an additional fixed effect.

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Detailed description of statistically significant findings identified in rats given a diet containing GMB151 soybean is reported in Appendix A, Table A.3.

No gross pathology findings related to the administration of the test diet were observed at necropsy, and the microscopic examinations of a wide range of organs and tissues did not identify relevant differences in the incidence and severity of the histopathological findings related to the administration of the test diet compared to the control group.

The GMO Panel concludes that this study is in line with the requirements of Regulation (EU) No 503/2013 and that no treatment related adverse effects were observed in rats after feeding diets including 30% defatted toasted meal from soybean GMB151 for 90 days.

3.4.4. Allergenicity

The strategies to assess the potential risk of allergenicity focus: i) on the source of the recombinant protein; ii) on the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in already sensitised persons; and iii) on whether the transformation may have altered the allergenic properties of the modified plant. Furthermore, the assessment also takes into account potential adjuvant properties of the newly expressed proteins, which is defined as the ability to enhance an allergic reaction.

3.4.4.1. Assessment of allergenicity of the newly expressed proteins[28]

A weight-of-evidence approach was followed, taking into account all of the information obtained on the newly expressed protein, as no single piece of information or experimental method yielded sufficient evidence to predict allergenicity (Codex Alimentarius, 2009; EFSA GMO Panel, 2011a, 2017; Regulation (EU) No 503/2013).

The *cry14Ab-1.b* and *hppdPf-4Pa* genes originate from *B. thuringiensis* and *P. fluorescens*, respectively, none of which are considered allergenic sources.

Bioinformatic analyses of the amino acid sequences of the Cry14Ab-1 and HPPD-4 proteins, using the criterion of 35% identity in a sliding window of 80 amino acids, initially revealed no significant similarities to known allergens with the exception of a potential similarity of the Cry14Ab-1 protein with an allergen included in the Compare database[29]. Following a request from the GMO Panel, the applicant provided an assessment of such hit which included a structural analysis based on linear amino acid and 3D structure alignments between the Cry14Ab-1 protein and the corresponding allergen. Subsequently, updated bioinformatic analysis using an updated FASTA program did not show any relevant hit because the E-value[30] identified for the previous hit was higher than 100 in the most recent analysis. Considering all the available information, the GMO Panel assessed the relevance of the bioinformatic analyses in relation to its implications on safety. The bioinformatic analysis identified a 35.4% identity with an E-value of ≥ 99 of the Cry14Ab-1 protein vs the Asp f 22 enolase from *Aspergillus fumigatus*. The GMO Panel assessed this alignment in the context of a potential relationship between these two proteins relevant for allergenicity following a case-by-case, weight-of-evidence approach (Reg 503/2013; Codex Alimentarius, 2003-2009; EFSA GMO Panel, 2010c, 2011a), as described below.

Briefly, a low level of identity (35.4%) and a very high E-value (≥ 99) was observed for such alignment. Although the alignment-based criterion involving more than 35% sequence identity to a known allergen over a window of at least 80 amino acids is the minimal requirement for risk assessment (Reg (EU) No 503/2013), the identity threshold is conservatively set and is considered on a case-by-case-basis (EFSA GMO Panel, 2010c). The E-value provides information on the quality of the alignment, and in this case, represents a high probability of finding that sequence by random chance. This is illustrated by the alignment requiring the introduction of 13 gaps in total to ensure that the corresponding 80-mer region fits above the threshold of 35% sequence identity. Furthermore, the longest contiguous amino acid sequence match between the Cry14Ab-1 and Asp f 22 proteins in the 80-mer region is only three amino acids in length. This is in line with the finding that only 5 (and discontinuous) out of the 15 amino acids involved in the unique linear T-cell epitope identified in Asp f 22 matched after the alignment between both proteins (Oseroff et al., 2012). This suggests that there is no linear epitope in common between Cry14Ab-1 protein and Asp f 22 enolase within this 80-mer region. Furthermore, the applicant carried out a structural alignment between the 3D structure of the

[28] Dossier: Part II – Section 1.5.1; additional information: 8/10/2019 and 18/11/2020.

[29] https://comparedatabase.org/

[30] An alignment derived from a FASTA search of a database is accompanied with an E-value, which represents the number of times the corresponding alignment score is expected at chance.

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Cry14Ab-1 protein and the predicted 3D model of the Asp f 22 enolase, showing that Cry14Ab-1 did not have any significant structural alignment with Asp f 22 enolase. This result was also applicable to the 80-mer region of Cry14Ab-1 in which the sequence identity was 35.4% to the Asp f 22 enolase. Therefore, the likelihood of sharing conformational epitopes between both proteins is very low. Lastly, the applicant performed a full-length protein linear amino acid alignment between both proteins, showing a low sequence identity of 7%. This value is in sharp contrast to the sequence identity values found between Asp f 22 and other fungal enolases which have shown immunoglobulin E (IgE) cross-reactivity, such as those from *Penicillium citrinum* (94% of sequence identity), *Alternaria alternate* (88%) (Lai et al., 2002) or *Cladosporium herbarum* (86%) (Simon-Nobbe et al., 2000). Based on this information, the GMO Panel did not find indications of structural relationship between the newly expressed Cry14Ab-1 protein and the Asp f 22 enolase from *Aspergillus fumigatus*.

Furthermore, the GMO Panel assessed the clinical relevance of the allergen to which potential similarity was observed. The available information identified the Asp f 22 enolase as an allergen of weak clinical relevance – there is only evidence of *in vitro* specific IgE-binding, but there is no available information on its biological activity (Banerjee and Kurup, 2003; Chaudhary et al., 2010). In addition, this is a minor allergen because only about 30% of the tested sera from *Penicillium*-sensitised asthmatic individuals had Asp f 22-specific IgE binding (Lai et al., 2002). Furthermore, Asp f 22 has shown potential capacity to sensitise only via the respiratory route (Lai et al., 2002; Singh et al., 2010). Assessing this allergen is challenging because of the scarcity of available human sera for testing (Lai et al., 2002; Singh et al., 2010). According to Codex Alimentarius (2003–2009), a minimum of 24 relevant sera is required to achieve an acceptable level of certainty in the case of minor allergens. In the case of the Asp f 22 allergen, there appears to be insufficient serum samples for testing purposes. Based on the available information, the evidence suggests that Asp f 22 is an allergen of weak clinical relevance.

Therefore, the GMO Panel considers that there are no indications of safety concerns derived from the bioinformatic analysis of the amino acid sequences of the Cry14Ab-1 and HPPD-4 proteins, using the criterion of 35% identity in a sliding window of 80 amino acids, and from the structural comparisons between the Cry14Ab-1 protein and the Asp f 22 enolase relevant for allergenicity.

Studies on protein stability of the Cry14Ab-1 and HPPD-4 proteins are described in Section 3.4.2. Furthermore, post-translational modifications of the HPPD-4 protein were also assessed and no indications of concerns impacting on the allergenicity were identified (Sections 3.2.4 and 3.4.3).

In relation to adjuvanticity, the GMO Panel did not find an indication that the newly expressed proteins Cry14Ab-1 and HPPD-4 at the levels expressed in soybean GMB151 might be adjuvants.

Finally, the applicant provided information on the safety of the Cry14Ab-1 and HPPD-4 proteins regarding their potential hazard to cause a celiac disease response. For such assessment, the applicant followed the principles described in the GMO Panel guidance document (EFSA GMO Panel, 2017). The assessment of the Cry14Ab-1 and HPPD-4 identified no perfect or relevant partial matches with known celiac disease peptide sequences.

In the context of this application, the GMO Panel considers that there are no indications that the newly expressed Cry14Ab-1 and/or HPPD-4 proteins in soybean GMB151 may be allergenic.

3.4.4.2. Assessment of allergenicity of the whole GM plant or crop[31]

Soybean is considered a common allergenic food[32] (OECD, 2012). Therefore, any potential change in the endogenous allergenicity of the GM plant should be assessed (Regulation (EU) No 503/2013). For such assessment, the applicant included in the comparative analysis specific allergens relevant for soybean (Section 3.3.6) quantified using liquid chromatography with tandem MS, which has been previously considered acceptable (EFSA GMO Panel, 2010c, 2017; Fernandez et al., 2013; Selb et al., 2017). These allergens were selected based on the list of potential soybean allergens described in the pertinent OECD document (OECD, 2012) and a scientific rational supporting their selection was provided by the applicant and considered acceptable by the GMO Panel. No changes in the levels of endogenous allergens raising concern are identified by the GMO Panel.

[31] Dossier: Part II – Section 1.5.2.

[32] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

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In the context of this application, the GMO Panel considers that there is no evidence that the genetic modification might substantially change the overall allergenicity of soybean GMB151 when compared with that of the conventional counterpart and the non-GM reference varieties tested.

3.4.5. Dietary exposure assessment to new constituents

In line with Regulation (EU) No 503/2013 the applicant provided dietary exposure estimates to Cry14Ab-1 and HPPD-4 proteins newly expressed in GMB151 soybean. Dietary exposure was estimated based on protein expression levels reported in this application for GMB151 soybean treated with the intended herbicide, the current available consumption data and feed practices, the foods and feeds currently available in the market and the described processing conditions.

3.4.5.1. Human dietary exposure[33]

Chronic and acute dietary exposure estimates to Cry14Ab-1 and HPPD-4 proteins newly expressed in GMB151 soybean were provided by the applicant.

Human dietary exposure was estimated across different European countries on different population groups: young population (toddlers, 'other children'), adolescents, adult population (adults, elderly and very elderly). Since no specific consumption data were available on commodities containing, consisting of or obtained from GMB151 soybean, a conservative scenario with 100% replacement of conventional soybean by the GM soybean was considered. Consumption figures for the relevant commodities (soya bread, textured soy protein, soya drink, tofu, etc.) were retrieved from the EFSA Comprehensive European Food Consumption Database.[34]

Mean protein expression values on fresh weight basis are considered as the most adequate to estimate human dietary exposure (EFSA, 2019a). In this particular application, concentration values for Cry14Ab-1 and HPPD-4 proteins were not corrected for protein extraction efficiency before being used for dietary exposure estimations. Considering the high extraction efficiency values reported,[35] that no safety concerns are identified for these proteins (Section 3.4.3.1) and that overly conservative scenarios are used, the GMO Panel accepted the approach used by the applicant.

Starting with the concentrations reported in seeds for the newly expressed proteins (73.93 μg/kg for Cry14Ab-1 and 3.97 μg/kg for HPPD-4), the applicant used the total protein content in the different soybean derived commodities to estimate the concentration of Cry14Ab-1 and HPPD-4 proteins in the processed commodities. This is considered an overly conservative approach as no losses of newly expressed proteins are assumed during processing and all the protein content in the processed foods is considered as derived from the GM soybean. The only food processed commodity analysed for the presence of Cry14Ab-1 and HPPD-4 proteins was the refined, bleached, and deodorised (RBD) soybean oil. All samples showed levels below the limit of quantification (LOQ) (0.002 μg/g and 0.01 μg/g, respectively); although no proteins are expected to be present in RBD oil, this processed product was considered when estimating dietary exposure using the reported LOQs for Cry14Ab-1 and HPPD-4.

The highest acute dietary exposure was estimated in the age class 'Adults' with Cry14Ab-1 and HPPD-4 exposure estimates of 363.65 and 19.53 μg/kg bw per day, respectively, with textured soy protein as the main contributor to the exposure.

The highest chronic dietary exposure was estimated in the age class 'Toddlers' with Cry14Ab-1 and HPPD-4 exposure estimates of 139.73 and 7.50 μg/kg bw per day, respectively, with soy drinks as the main contributors to the chronic dietary exposure.

An ad hoc dietary exposure scenario was carried out considering the consumption of protein-based supplements ('Protein and amino acids supplements' and 'Protein and protein components for sports people'), under the assumption that these supplements are prepared from GMB151 soybean. Consumption data on protein-based supplements were available for a total of 16 European countries.[36] The highest average acute dietary exposures (consuming days only) were 805 μg/kg bw per day for Cry14Ab-1 in adults and 43.2 μg/kg bw per day for HPPD-4. For high consumers (95th percentile exposure), the highest acute exposures were 1,280 μg/kg bw per day for Cry14Ab-1 and 68.7 μg/kg

[33] Dossier: Part II – Section 1.6.1; additional information: 2/12/2020.

[34] Summary statistics from the EFSA Comprehensive European Food Consumption Database accessed in April 2018 (http://www.efsa.europa.eu/en/applications/gmo/tools).

[35] The protein extraction efficiencies in the analysis of HPPD-4 and Cry14Ab-1 in seeds of GMB151 soybean were 99% and 94.2%, respectively. Starting concentrations used for human dietary exposure estimations without correction for extraction efficiency were: 73.93 μg/kg for Cry14Ab-1 and 3.97 μg/kg for HPPD-4.

[36] https://www.efsa.europa.eu/en/food-consumption/comprehensive-database. Data accessed December 2020.

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bw per day for HPPD-4, also in adults. Similarly, for chronic dietary exposure (consumers only), the highest average estimates were 512 μg/kg bw per day for Cry14Ab-1 and 27.5 μg/kg bw per day for HPPD-4 in adults. For high consumers (95th percentile exposure), the highest chronic exposures were 832 μg/kg bw per day for Cry14Ab-1 and 44.7 μg/kg bw per day for HPPD-4, also in adults.

Furthermore, an ad hoc dietary exposure scenario was carried out for consumers of pollen supplements under the assumption that these supplements might be made of pollen from GMB151 soybean. The expression levels of Cry14Ab-1 and HPPD-4 reported in flowers were used as surrogates for the presence of these proteins in pollen. From the expression values reported in flowers (see Table 1, section 3.2.4), the concentrations of Cry14Ab-1 and HPPD-4 proteins in pollen supplements were calculated, assuming homogeneous distribution of newly expressed proteins in flowers and around 6% moisture content in pollen (48.7 μg/g for Cry14Ab-1 and 70 μg/g for HPPD-4). Consumption data on pollen supplements are available for few consumers across eight different European countries;[28] the low number of consumers available adds uncertainty to the exposure estimations and prevents from estimating exposure for high consumers of pollen supplements. In average consumers of pollen supplements, the highest acute dietary exposure would range from 36.1 μg/kg bw per day for Cry14Ab-1 to 51.9 μg/kg bw per day for HPPD-4, in the elderly population. Similarly, the highest chronic dietary exposure in average consumers would range from 24.1 μg/kg bw per day for Cry14Ab-1 to 34.6 μg/kg bw per day for HPPD-4, also in the elderly population.

3.4.5.2. Animal dietary exposure[37]

Dietary exposure to Cry14Ab-1 and HPPD-4 proteins in soybean GMB151 was estimated across different animal species, assuming the consumption of soybean products commonly entering the feed supply chain (i.e. soybean seed, toasted meal, hulls, forage, hay and silage.

A conservative scenario with 100% replacement of conventional soybean products by the soybean GMB151 products was considered.

Mean levels of Cry14Ab-1 and HPPD-4 proteins in soybean seeds and the highest protein contents in forage from the soybean GMB151 plant (treated with the intended herbicide) used for animal dietary exposure are listed in Table 1 (see Section 3.2.4).

To estimate the mean Cry14Ab-1 and HPPD-4 levels in toasted meal, hulls, hay and silage, a factor of respectively 1.3, 13, 1.5 and 0.5 folds was applied, based on default processing factors (PFs) retrieved from the 'Default PF Forage Items' and 'Default PF by products' tables of the 'Pesticides MRL Guidelines Animal Model 2017′ excel file accessed on 27 May 2020 from the EU Commission website, assuming that no losses of proteins occur during processing.

The applicant estimated dietary exposure to Cry14Ab-1 and HPPD-4 proteins via the consumption of soybean seed, toasted meal and hulls in broiler, layer and turkey, breeding and finishing swine, beef and dairy cattle, ram/ewe and lamb (hulls were not considered in dairy cow and turkey). The consumption of forage, hay and silage was considered only in laying hens. The overall exposure was based on estimates for animal body weight, daily feed intake and inclusion rates (percentage) of soybean seed, toasted meal, hulls, forage, hay and silage in diets (OECD, 2013).

Estimated dietary exposures based on the consumption of soybean meal, seed and hulls are reported in Appendix B (Tables B.1–B.3).

To further integrate the assessment, the GMO Panel estimated the animal dietary exposure to Cry14Ab-1 and HPPD-4 proteins via the consumption of forage in dairy cattle, and of other soybean products (i.e. protein concentrates) entering the feed supply chain, considering the concerned animals for each feed commodity.

Laying hen and dairy cattle for forage

Consumption of soybean forage in dairy cattle is based on estimates for animal body weight and daily feed intake (OECD, 2013), and inclusion rates of soybean forage in animal diets (OECD, 2012). Estimated dietary exposures based on the consumption of soybean forage, hay and silage are reported in Appendix B (Table B.4).

Piglets for protein concentrates

Consumption of soybean protein concentrates in piglet is based on estimates for animal body weight, daily feed intake (EFSA FEEDAP Panel, 2017; EFSA, 2019b) and inclusion rates of protein concentrates in diets (7%) (Guzmàn et al., 2016). To estimate the mean newly expressed protein

[37] Dossier: Part II – Section 1.6.2.; additional information 3/6/2019, and 26/6/2020.

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levels in soybean protein concentrates a factor of 1.75-fold was applied based on the protein content of soybean protein concentrates (70%), relative to soybean seed (OECD, 2012), assuming that no losses of these proteins occur during processing. Estimated dietary exposures to Cry14Ab-1 and HPPD-4 proteins based on the consumption of protein concentrates is respectively 476 and 24.2 μg/kg bw per day.

3.4.6. Nutritional assessment of endogenous constituents

The intended traits of soybean GMB151 are herbicide tolerance and resistance to nematodes, with no intention to alter nutritional parameters. However, levels of carbohydrates and crude protein in forage and palmitic acid (C16:0) and heptadecenoic acid (C17:1) in seeds were significantly different from its conventional counterpart and showed a lack of equivalence with the set of non-GM reference varieties/could not be categorised (Section 3.3.6). The biological relevance of these compounds, the role of soybean as contributor to their total intake and the magnitude and direction of the observed changes were considered during the nutritional assessment.

3.4.6.1. Human nutrition

The human nutritional assessment covers the observed changes in the levels of palmitic acid and heptadecenoic acid in seeds (see Section 3.3.6)

Palmitic acid is the most abundant saturated fatty acid in conventional soybean seed oil; a decrease of 3–4% was observed as compared to the conventional counterpart in both the treated and the not treated GM crop. Considering the numerous sources of saturated fatty acids in the diet and that palmitic acid is also endogenously synthesised in humans (Carta et al., 2017), the relatively small decrease observed in soybean GMB151 does not represent a nutritional concern.

As regards heptadecenoic acid, it is a very minor fatty acid in soybean seeds, usually not even analysed when characterising the fatty acid profile of soybean oil. Mean levels reported were well below 1% in both treated and not treated soybean GMB151, and around 5% higher than the levels reported for its conventional counterpart (see Section 3.3.6). Considering that no toxicological concerns related to heptadecenoic acid are known and the very low concentration of this fatty acid in soybean, the relatively small increase observed in soybean GMB151 does not represent a nutritional concern.

3.4.6.2. Animal nutrition

The animal nutritional assessment covered the observed changes in the levels of palmitic acid and heptadecenoic acid in seeds and carbohydrates and crude protein in forage (see Section 3.3.6)

Palmitic acid and heptadecenoic acid are not essential fatty acids in animals and the magnitude of their respective decrease and increase in soybean seeds does not represent a nutritional concern.

- Palmitic acid is a common saturated fatty acid found in oils and fats of vegetable and animal origin used in animal diets (Duran-Montgé et al., 2007; Loften et al., 2014; Tancharoenrat et al., 2014); it is also endogenously synthesised in animal body, i.e. milk palmitic acid arises both from diet and *de novo* synthesis (Palmquist, 2006). Feeding palmitic acid could affect fat fatty acid composition, for instance feeding highly concentrated sources of palmitic acid to dairy cows increases significantly C16:0 in milk fat (De Souza and Lock, 2019; Ghasemi et al., 2020;., Loften et al., 2014). Therefore, also considering the extent of the decrease observed, soybean GMB151 does not represent a concern in animal nutrition.
- The monounsaturated heptadecenoic acid is a minor constituent of ruminant fats (Shorland and Jessop, 1955); along with other odd-chain fatty acids, is assumed to be mainly of rumen microbial origin and, after intestinal absorption, is deposited in tissues or exported to milk fat (Alves et al., 2006; Pfeuffer and Jaudszus, 2016). Considering that no toxicological concerns related to heptadecenoic acid are known, the very low concentration of this fatty acid in soybean GMB151, and the magnitude of the increase, no nutritional concern is identified.

Forage is an important feed source for herbivores that can utilise it because of the capacity for microbial digestion of cell wall constituents. Forage guarantees the proper function of gastrointestinal tract that is essential for the activity of microbes; moreover, forage alone is able to satisfy nutritional requirements of animals up to a certain level, e.g. low producing animals. Therefore, forage is not provided to animals with the only purpose to fulfil nutritional requirements and the magnitude of the respective decrease and increase in carbohydrates and crude protein content in soybean GMB151 forage does not represent a nutritional concern.

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3.4.7. Post-market monitoring of GM food/feed

The GMO Panel concluded that soybean GMB151, as described in this application, does not raise any nutritional concern and is as safe as the non-GM comparator and the non-GM reference varieties tested. Therefore, the GMO Panel considers that post-market monitoring of food and feed from this GM soybean, as described in this application, is not necessary.

3.4.8. Conclusions on the food/feed safety assessment

The proteins Cry141Ab.1 and HPPD-4 newly expressed in soybean GMB151 do not raise safety concerns for human and animal health. No interactions between the newly expressed proteins relevant for food and feed safety were identified. Similarly, the GMO Panel did not identify indications of safety concerns regarding allergenicity or adjuvanticity related to the presence of the newly expressed proteins in soybean GMB151, or regarding the overall allergenicity of this GM soybean. Based on the outcome of the comparative assessment and the nutritional assessment, the GMO Panel concludes that the consumption of soybean GMB151 does not represent any nutritional concern, in the context of the scope of this application. The GMO Panel concludes that soybean GMB151, as described in this application, is as safe as the non-GM comparator and the non-GM reference varieties tested, and no post-market monitoring of food/feed is considered necessary.

3.5. Environmental risk assessment and monitoring plan[38]

3.5.1. Environmental risk assessment

Considering the scope of the application EFSA–GMO–NL–2018–153, which excludes cultivation, the environmental risk assessment (ERA) of soybean GMB151 mainly takes into account: (1) the exposure of microorganisms to recombinant DNA in the gastrointestinal tract of animals fed GM material and of microorganisms present in environments exposed to faecal material of these animals (manure and faeces); and (2) the accidental release into the environment of viable soybean GMB151 seeds during transportation and/or processing (EFSA GMO Panel, 2010a).

3.5.1.1. Persistence and invasiveness of the GM plant

Cultivated soybean (*Glycine max* (L.) Merr.) is a species in the subgenus *Soja* of the genus *Glycine*. The species originated from eastern Asia and is a highly domesticated crop, generally unable to survive in the environment without appropriate management (Lu, 2005).

Occasional feral GM soybean plants may occur outside cultivation areas, but survival is limited mainly by a combination of low competitiveness, absence of a dormancy phase and susceptibility to plant pathogens and cold climatic conditions (OECD, 2000). Soybean can grow as volunteers and the presence of volunteers of G. max was occasionally reported in some areas of Italy where soybean is intensively cultivated (Celesti-Grapow et al., 2010). However, as for the same reasons mentioned above, soybean seeds usually do not survive during the winter (Owen, 2005). Thus, the establishment and survival of feral and volunteer soybean in the EU is currently limited and transient.

It is unlikely that the intended traits of soybean GMB151 will provide a selective advantage to soybean plants, except when they are exposed to the intended herbicides or infested by insect pests that are susceptible to the Cry14Ab-1.b protein. However, this fitness advantage will not allow the GM plant to overcome other biological and abiotic factors (described above) limiting plant's persistence and invasiveness. Therefore, the presence of the intended traits will not affect the persistence and invasiveness of the GM plant.

In conclusion, the GMO Panel considers it unlikely that soybean GMB151 will differ from conventional soybean hybrid varieties in its ability to survive until subsequent seasons, or to establish occasional feral plants under European environmental conditions in case of accidental release into the environment of viable soybean GMB151 seeds.

3.5.1.2. Potential for gene transfer

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through HGT of DNA, or through vertical gene flow via cross-pollination from feral plants originating from spilled seeds.

[38] Dossier: Part II – Sections 5 and 6.

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Plant to microorganism gene transfer

Genomic DNA can be a component of food and feed products derived from soybean. It is well documented that such DNA becomes substantially degraded during processing and digestion in the human or animal gastrointestinal tract. However, bacteria in the digestive tract of humans and animals, and in other environments, may be exposed to fragments of DNA, including the recombinant fraction of such DNA.

Current scientific knowledge of recombination processes in bacteria suggests that horizontal transfer of non-mobile, chromosomally–located DNA fragments between unrelated organisms (such as from plants to bacteria) is not likely to occur at detectable frequencies under natural conditions (for further details, see EFSA, 2009).

Homologous recombination is known to facilitate horizontal transfer of non-mobile, chromosomal DNA fragments to bacterial genomes. This requires the presence of at least two stretches of DNA sequences that are similar in the recombining DNA molecules. In the case of sequence identity with the transgene itself, recombination would result in gene replacement. In the case of identity with two or more regions flanking recombinant DNA, recombination could result in the insertion of additional DNA sequences in bacteria and thus confer the potential for new properties.

In addition to homology-based recombination processes, at a lower transformation rate, the non-homologous end joining and microhomology-mediated end joining are theoretically possible (Hülter and Wackernagel, 2008; EFSA, 2009). Independently of the transfer mechanism, the GMO Panel did not identify a selective advantage that a theoretical HGT would provide to bacterial recipients in the environment.

The bioinformatic analysis for event GMB151 revealed no homology with known DNA sequences from bacteria which would facilitate homologous recombination.

In summary, there is no indication for an increased likelihood of horizontal transfer of DNA from soybean GMB151 to bacteria. Given the nature of the recombinant DNA, the GMO Panel identified no safety concern linked to an unlikely but theoretically possible HGT.

Plant-to-plant gene transfer

The potential for occasional feral soybean GMB151 plants originating from seed import spills to transfer recombinant DNA to sexually compatible plants and the environmental consequences of this transfer were considered.

For plant-to-plant gene transfer to occur, imported GM soybean seeds need to germinate and develop into plants in areas containing sympatric wild relatives and/or cultivated soybean with synchronous flowering and environmental conditions favouring cross-pollination. It must be noted that most soybean GMB151 seeds are processed in the countries of production or in ports of importation.

Vertical gene transfer from soybean (*G. max*) is limited to the species of the subgenus *Soja* to which G. max belongs to, as well as the wild relatives *G. soja* and *G. gracilis*. Although wild relatives exist elsewhere, no wild relatives of the subgenus *Soja* have been reported in Europe (Dorokhov et al., 2004; Lu, 2005). Therefore, vertical gene transfer from GM soybean is restricted to cultivated soybean (*G. max*).

Soybean is an annual, almost completely self-pollinating crop with a percentage of cross-pollination usually below 1% (OECD, 2000; Ray et al., 2003; Lu, 2005; Yoshimura et al., 2006; Abud et al., 2007), although natural cross-pollination rates can fluctuate significantly among different soybean varieties under particular environmental conditions, such as favourable climate for pollination and an abundance of pollinators (Caviness, 1966; Gumisiriza and Rubaihayo, 1978; Kikuchi et al., 1993; Ahrent and Caviness, 1994; Ray et al., 2003; Lu, 2005).

The potential of spilled soybean seeds to establish, grow and produce pollen is extremely low and transient (see Section 3.5.1.1). Therefore, the likelihood/frequency of cross-pollination between occasional feral GM soybean plants resulting from seed spillage, and weedy or cultivated soybean plants is also considered extremely low. Even if cross-pollination would occur, the GMO Panel is of the opinion that the likelihood of environmental effects as a consequence of the spread of genes from occasional feral GM soybean plants in Europe will not differ from that of conventional soybean varieties for the reasons given in Section 3.5.1.1 even if exposed to the intended herbicides.

3.5.1.3. Interactions of the GM plant with target organisms

Taking the scope of application EFSA-GMO-NL-2018-153 into account (no cultivation), potential interactions of occasional feral soybean GMB151 plants arising from seed import spills with the target organism are not considered a relevant issue.

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3.5.1.4. Interactions of the GM plant with non-target organisms

Given that environmental exposure of non-target organisms to spilled GM seeds or occasional feral GM soybean plants arising from spilled soybean GMB151 seeds is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM soybean, potential interactions of soybean GMB151 with non-target organisms are not considered by the GMO Panel to raise any environmental safety concern.

3.5.1.5. Interactions of the GM plant with the abiotic environment and biogeochemical cycles

Given that environmental exposure to spilled seeds or occasional feral soybean GMB151 plants arising from seed import spills is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM soybean, potential interactions with the abiotic environment and biogeochemical cycles are not considered by the GMO Panel to raise any environmental safety concern.

3.5.2. Post-market environmental monitoring

The objectives of a post-market environmental monitoring (PMEM) plan, according to Annex VII of Directive 2001/18/EC, are: (1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the ERA are correct; and (2) to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment that were not anticipated in the ERA.

Monitoring is related to risk management, and thus a final adoption of the PMEM plan falls outside the mandate of EFSA. However, the GMO Panel gives its opinion on the scientific rationale of the PMEM plan provided by the applicant (EFSA GMO Panel, 2011b).

As the ERA did not identify potential adverse environmental effects from soybean GMB151, no case specific monitoring is required.

The PMEM plan proposed by the applicant for soybean GMB151 includes: (1) the description of a monitoring approach involving operators (federations involved in import and processing), reporting to the applicant, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment; (2) a coordinating system established by EuropaBio for the collection of information recorded by the various operators; and (3) the review of relevant scientific publications retrieved from literature searches (Lecoq et al., 2007; Windels et al., 2008). The applicant proposes to submit a PMEM report on an annual basis and a final report at the end of the authorisation period.

The GMO Panel considers that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of soybean GMB151. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

3.5.3. Conclusion of the environmental risk assessment and monitoring plan

The GMO Panel concludes that it is unlikely that soybean GMB151 would differ from conventional soybean varieties in its ability to persist under European environmental conditions. Considering the scope of application EFSA-GMO-NL-2018-153, interactions of occasional feral soybean GMB151 plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from soybean GMB151 to bacteria does not indicate a safety concern. Therefore, considering the introduced traits, the outcome of the agronomic and phenotypic analysis, and the routes and levels of exposure, the GMO Panel concludes that soybean GMB151 would not raise safety concerns in the event of accidental release of viable GM soybean seeds into the environment.

The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of soybean GMB151.

4. Overall conclusions

The GMO Panel was asked to carry out a scientific assessment of soybean GMB151 for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003.

The molecular characterisation data establish that soybean GMB151 contains a single insert consisting of one copy of the hppdPf-4Pa and the cry14Ab-1.b expression cassettes. Bioinformatics analyses of the sequences encoding the newly expressed proteins and other ORFs within the insert or spanning the junctions between the insert and genomic DNA do not raise any safety concerns. The

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stability of the inserted DNA and of the introduced trait is confirmed over several generations. The methodology used to quantify the levels of the Cry14Ab-1 and HPPD-4 proteins is considered adequate. The protein characterisation data comparing the biochemical, structural and functional properties of plant- and microbe-produced Cry14Ab1 and HPPD-4 proteins, indicate that these proteins are equivalent and the microbe-derived proteins can be used in the safety studies.

None of the identified differences in the agronomic/phenotypic and compositional characteristics tested between soybean GMB151 and its conventional counterpart needs further assessment, except for palmitic acid and heptadecenoic acid in seeds and carbohydrate and crude protein in forage, which does not raise nutritional and safety concerns. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the HPPD-4 and Cry14Ab-1.b proteins as expressed in soybean GMB151, and finds no evidence that the genetic modification would change the overall allergenicity of soybean GMB151. In the context of this application, the consumption of food and feed from soybean GMB151 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that soybean GMB151 is as safe as the conventional counterpart and non-GM soybean reference varieties tested, and no post-market monitoring of food/feed is considered necessary.

In the case of accidental release of viable soybean GMB151 seeds into the environment, this would not raise environmental safety concerns. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of soybean GMB151. The literature searches did not identify any relevant publications on soybean GMB151.

The GMO Panel concludes that soybean GMB151 is as safe as its conventional counterpart and the tested non-GM soybean reference varieties with respect to potential effects on human and animal health and the environment.

5. Documentation as provided to EFSA

1) Letter from the Competent Authority of The Netherlands received on 09 October 2018 concerning a request for authorization of the placing on the market of soybean GMB151 submitted in accordance with Regulation (EC) No 1829/2003 by BASF Agricultural Solutions Belgium.
2) Application EFSA-GMO-NL-2018-153 validated by EFSA, 04 March 2019.
3) Request for supplementary information to the applicant, 05 March 2019.
4) Receipt of supplementary information from the applicant, 12 April 2019.
5) Request for supplementary information to the applicant, 16 April 2019.
6) Receipt of supplementary information from the applicant, 03 June 2019.
7) Request for supplementary information to the applicant, 18 June 2019.
8) Request for supplementary information to the applicant, 02 August 2019.
9) Receipt of supplementary information from the applicant, 19 August 2019.
10) Receipt of supplementary information from the applicant, 08 October 2019.
11) Request for supplementary information to the applicant, 14 October 2019.
12) Receipt of supplementary information from the applicant, 18 December 2019.
13) Request for supplementary information to the applicant, 29 January 2020.
14) Receipt of supplementary information from the applicant, 31 March 2020.
15) Request for supplementary information to the applicant, 07 April 2020.
16) Receipt of supplementary information from the applicant, 26 June 2020.
17) Request for supplementary information to the applicant, 03 July 2020.
18) Receipt of supplementary information from the applicant, 30 September 2020.
19) Request for supplementary information to the applicant, 26 October 2020.
20) Request for supplementary information to the applicant, 10 November 2020.
21) Receipt of supplementary information from the applicant, 18 November 2020.
22) Receipt of supplementary information from the applicant, 02 December 2020.
23) Receipt of supplementary information from the applicant, 04 December 2020.

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Abbreviations

ADF	acid detergent fibre
ATG	translational start codons
bp	base pair
bw	body weight
dw	dry weight
ELISA	enzyme-linked immunosorbent assay
ERA	environmental risk assessment
FA	fatty acid
fw	fresh weight
GLP	good laboratory practice
GM	genetically modified
GMO	genetically modified organism
GMO Panel	EFSA Panel on Genetically Modified Organisms
HGT	horizontal gene transfer
HR	homologous recombination
HPPD	4-Hydroxyphenylpyruvate dioxygenase
IgE	immunoglobulin E
JSA	Junction Sequence Analysis
LOQ	limit of quantification
MS	mass spectrometry
NCBI	National Center for Biotechnology Information
NGS	next generation sequencing
NDF	neutral detergent fibre
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PCR	polymerase chain reaction
PMEM	post-market environmental monitoring
RBD	refined, bleached, and deodorised
SDS–PAGE	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis
T-DNA	transfer-deoxyribonucleic acid
UTR	untranslated region

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Appendix A – Statistically significant findings in AP153 toxicological studies compared to controls

Table A.1: 28-day toxicity study on Cry14Ab-1 protein in mice: statistically significant findings[(a)]

Statistically significant parameter/endpoint	Finding	GMO Panel interpretation
Mean body weight (g)	Decrease between Day 8 and 15 (males)	Transient change, minimal (0.2g). No impact on overall body weights at study termination. Not an adverse effect of treatment.
Mean WBC, absolute (10^9/L) Mean neutrophils, absolute (10^9/L) and relative (%) Mean lymphocytes, absolute 10^9/L)	Increase, females (+48%, +66% and +18%, +45% respectively)	Not associated with evidence of inflammation or infection. The GMO Panel notes that the values in Cry14Ab-1 treated animals are driven by 3 animals with particularly high WBC counts and counts of individual white cell classes, and that the total WBC counts are well within the normal range defined by the historical controls. No associated findings in the gastrointestinal tract, lymphopoietic organs and tissues or at histological examination of the bone marrow. No similar findings in males. Not an adverse effect of treatment.
Mean blood urea (mmol/L)	Increase, males (+26%)	No similar increase in females and no associated histopathological findings in the kidney. Not an adverse effect of treatment.
Mean adrenal weight, absolute (g) and relative to body weight (%) Mean kidney weight, absolute (g) and relative to body weight (%)	Increase, males (around +20% and +11%, respectively)	No adverse effects were seen on histopathology of the adrenals or kidneys nor on adrenal or kidney weights in females and the values are consistent with the reported historical control data. Not an adverse effect of treatment.
Mean heart weight, absolute (g)	Increase, males (6%)	Low magnitude, not associated with histopathological findings. Not seen in females. Not an adverse effect of treatment.
Mean brain weight, absolute (g) and relative to body weight (%)	Decrease in females (around 5%)	Low magnitude, not associated with histopathological findings. Not seen in males. Not an adverse effect of treatment.

(a): Statistically significant differences in animals given the test item as compared to concurrent controls.

Table A.2: 28-day toxicity study on HPPD-4 protein in mice: statistically significant findings[(a)]

Statistically significant parameter/endpoint	Finding	GMO Panel interpretation
Mean body weight gain (g)	Decrease in Week 4 (males)	Low magnitude (0.7g) and with no impact on overall body weights at study termination. Not an adverse effect of treatment.
Mean food consumption (g/day)	Increase in Week 2 (females)	Low magnitude (11%) and with no impact on overall food consumption or body weights at study termination. Not an adverse effect of treatment.
Mean thymus weight, absolute (g) Relative to body weight (%)	Decrease (females)	Around 34% decrease due to two mice with abnormally low values; no adverse effects were seen on histopathology of the thymus nor on thymus weights in males. Not an adverse effect of treatment.

(a): Statistically significant differences in animals given the test item as compared to concurrent controls.

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Table A.3: 90-day toxicity study on soybean GMB151 in rats: statistically significant findings[(a)]

Statistically significant parameter/endpoint	Finding	GMO Panel interpretation
Body weight gain (g) Cumulative body weight gain (g)	Increases and decreases at different time periods	Sporadic changes at individual time points. No impact on body weight gain over the entire study period. Not an adverse effect of treatment.
Mean Basophils, absolute (thou/μL)	Decrease (combined sexes)	Low magnitude, within normal variation and consistent with reference diet animals. Not an adverse effect of treatment.
Mean eosinophils, % Mean eosinophils, absolute (thou/μL)	Increase (males, absolute; females and combined sexes, % and absolute)	Low magnitude (35%), within normal variation and consistent with reference diet animals. Not an adverse effect of treatment.
Mean lymphocytes, % Mean lymphocytes, absolute (thou/μL) Mean WBC, absolute (thou/μL)	Decrease (males)	Low magnitude (< 20%) and within normal variation Not an adverse effect of treatment.
Mean red cell distribution width (RCDW), %	Increase (females)	Low magnitude (< 10%) and within normal variation. Not an adverse effect of treatment.
Mean albumin (g/dL)	Decrease (combined sexes)	Low magnitude (< 10%), within normal variation and consistent with reference diet animals. Not an adverse effect of treatment.
Mean AST (U/L)	Increase (females)	Low magnitude (ca 20%), within normal variation; no consistent pattern in other liver marker enzymes and no liver weight or pathology findings. Not an adverse effect of treatment.
Mean potassium (mEq/L)	Increase (females)	Low magnitude (< 10%), within normal variation and consistent with reference diet animals. Not an adverse effect of treatment.
Mean sodium (mEq/L)	Decrease (males, combined sexes)	Low magnitude (< 1%), within normal variation and consistent with reference diet animals. Not an adverse effect of treatment.
Mean total protein (mEq/L)	Decrease (males, combined sexes)	Low magnitude (< 10%) and within normal variation Not an adverse effect of treatment.
Mean urine pH	Increase (females)	Low magnitude (< 10%), within normal variation and consistent with reference diet animals. Not an adverse effect of treatment.

(a): Statistically significant differences in animals given the test item as compared to concurrent controls.

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Appendix B – Animal dietary exposure estimation to Cry14Ab-1 and HPPD-4 proteins

Table B.1: Dietary exposure to Cry14Ab-1 and HPPD-4 proteins (µg/kg bw per day) in livestock, based on the consumption of soybean meal

	Dietary exposure (µg/kg bw per day)	
	Cry14Ab-1	**HPPD-4**
Beef	551	28.1
Dairy	1,100	56.3
Ram/ewe	956	48.8
Lamb	1,220	62.2
Breeding	794	40.5
Finishing	1,030	52.7
Broiler	3,240	165
Layer	1,960	100
Turkey	3,690	188

bw: body weight.

Table B.2: Dietary exposure to Cry14Ab-1 and HPPD-4 proteins (µg/kg bw per day) in livestock, based on the consumption of soybean seed

	Dietary exposure (µg/kg bw per day)	
	Cry14Ab-1	**HPPD-4**
Beef	212	10.8
Dairy	339	17.3
Ram/ewe	294	15
Lamb	750	38,3
Breeding	204	10.4
Finishing	530	27
Broiler	1,250	63.5
Layer	906	46.2
Turkey	946	48.2

bw: body weight.

Table B.3: Dietary exposure to Cry14Ab-1 and HPPD-4 proteins (µg/kg bw per day) in livestock, based on the consumption of soybean hull

	Dietary exposure (µg/kg bw per day)	
	Cry14Ab-1	**HPPD-4**
Beef	2,750	140
Dairy	NA	NA
Ram/ewe	7,650	390
Lamb	9,750	497
Breeding	2,650	135
Finishing	3,440	176
Broiler	4,050	206
Layer	3,920	200
Turkey	NA	NA

NA: not applicable; bw: body weight.

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The GMO Panel considers the factor of 13-fold applied by the applicant highly conservative to estimate the mean Cry14Ab-1 and HPPD-4 levels in hulls. An appropriate factor could be derived based on the ratio between the protein content of soybean hulls relative to soybean seed (e.g. OECD, 2012), assuming that no losses of these proteins occur during processing.

Table B.4: Dietary exposure to Cry14Ab-1 and HPPD-4 proteins (μg/kg bw per day) in livestock, based on the consumption of soybean forage, hay and silage

	Dietary exposure (μg/kg bw per day)[(a)]	
	Cry14Ab-1	**HPPD-4**
Forage		
Laying Hen[(a)]	361	883
Dairy Cow[(b)]	406	992
Hay		
Laying Hen[(a)]	541	1,320
Dairy Cow[(b)]	608	1,489
Silage		
Laying Hen[(a)]	180	441
Dairy Cow[(b)]	203	496

bw: body weight.
(a): estimations provided by the applicant.
(b): estimations provided by EFSA.

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