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Assessment of genetically modified cotton GHB811 for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-ES-2018-154)

EFSA Panel on Genetically Modified Organisms (GMO),
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Abstract

Cotton GHB811 was developed to confer tolerance to glyphosate and HPPD inhibitor herbicides. 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 cotton GHB811 and its conventional counterpart needs further assessment, except for % lint, lint length and dihydrosterculic acid, which do not raise nutritional and safety concerns. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the 2mEPSPS and HPPD W336 proteins as expressed in cotton GHB811 and finds no evidence that the genetic modification would change the overall allergenicity of cotton GHB811. In the context of this application, the consumption of food and feed from cotton GHB811 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that cotton GHB811 is as safe as the conventional counterpart and non-GM cotton reference varieties tested, and no post-market monitoring of food/feed is considered necessary. In the case of accidental release of viable cotton GHB811 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 cotton GHB811. The GMO Panel concludes that cotton GHB811 is as safe as its conventional counterpart and the tested non-GM cotton reference varieties with respect to potential effects on human and animal health and the environment.

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Amendment: Editorial amendments were made to Sections 3.1, 3.2.2 and 3.4.3.1. The original version of the statement has been removed from the EFSA Journal, but is available on request, as is a version showing all the changes made.

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Summary

The scope of application EFSA-GMO-ES-2018-154 is for food and feed uses, import and processing of the genetically modified (GM) herbicide-tolerant cotton GHB811 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 cotton GHB811 according to the scope of the application EFSA-GMO-ES-2018-154. The GMO Panel conducted the assessment of cotton GHB811 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 cotton GHB811 contains a single insert consisting of one copy of the *hppdPfW336-1Pa* and the *2mepsps* expression cassettes. 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 HPPD W336 and 2mEPSPS proteins is considered adequate. The protein characterisation data comparing the biochemical, structural and functional properties of plant- and microbial-produced HPPD W336 and 2mEPSPS proteins, indicate that these proteins are equivalent and the microbial-derived proteins can be used in the safety studies.

Considering the selection of test materials, the field trial sites and the associated management practices and the agronomic-phenotypic characterisation as an indicator of the overall field trial quality, the GMO Panel concludes that the field trials are appropriate to support the comparative analysis.

None of the identified differences in the agronomic/phenotypic and compositional characteristics tested between cotton GHB811 and its conventional counterpart needed further assessment, except for % lint, lint length and dihydrosterculic acid, which do not raise nutritional and safety concerns. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the HPPD W336 and 2mEPSPS proteins as expressed in cotton GHB811 and finds no evidence that the genetic modification would change the overall allergenicity of cotton GHB811. In the context of this application, the consumption of food and feed from cotton GHB811 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that cotton GHB811 is as safe as the conventional counterpart and non-GM cotton 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, cotton GHB811 would not raise safety concerns in the case of accidental release of viable GM cotton seeds into the environment. The post-market environmental monitoring (PMEM) plan and reporting intervals are in line with the intended uses of cotton GHB811.

Based on the relevant publications identified through the literature searches, the GMO Panel does not identify any safety issues pertaining to the uses of cotton GHB811.

The GMO Panel concludes that cotton GHB811 is as safe as its conventional counterpart and the tested non-GM cotton reference varieties with respect to potential effects on human and animal health and the environment.

Table of contents

Abstract 1
Summary 3
1. Introduction 6
1.1. Background and Terms of Reference as provided by the requestor 6
1.2. Terms of Reference as provided by the requestor 6
2. Data and methodologies 6
2.1. Data 6
2.2. Methodologies 7
3. Assessment 7
3.1. Systematic literature review 7
3.2. Molecular characterisation 7
3.2.1. Transformation process and vector constructs 7
3.2.2. Transgene constructs in the GM plant 8
3.2.3. Protein characterisation and equivalence 8
3.2.4. Information on the expression of the insert 9
3.2.5. Inheritance and stability of inserted DNA 10
3.2.6. Conclusion on molecular characterisation 10
3.3. Comparative analysis 10
3.3.1. Overview of studies conducted for the comparative analysis 10
3.3.2. Experimental field trial design and statistical analysis 10
3.3.3. Suitability of selected test materials 11
3.3.3.1. Selection of the test materials 11
3.3.3.2. Seed production and quality 11
3.3.3.3. Conclusion on suitability 11
3.3.4. Representativeness of the receiving environments 11
3.3.4.1. Selection of field trial sites 11
3.3.4.2. Meteorological conditions 11
3.3.4.3. Management practices 11
3.3.4.4. Conclusion on representativeness 12
3.3.5. Agronomic and phenotypic endpoints 12
3.3.6. Compositional analysis 12
3.3.7. Conclusions on the comparative analysis 14
3.4. Food/feed safety assessment 14
3.4.1. Effects of processing 14
3.4.2. Stability of the newly expressed proteins 14
3.4.3. Toxicology 15
3.4.3.1. Testing of the newly expressed proteins 15
3.4.3.2. Testing of new constituents other than the newly expressed proteins 15
3.4.3.3. Information on altered levels of food/feed constituents 15
3.4.3.4. Testing of the whole genetically modified food/feed 15
3.4.4. Allergenicity 16
3.4.4.1. Assessment of allergenicity of the newly expressed proteins 16
3.4.4.2. Assessment of allergenicity of the whole GM plant or crop 17
3.4.5. Dietary exposure assessment to new constituents 17
3.4.5.1. Human dietary exposure 17
3.4.5.2. Animal dietary exposure 18
3.4.6. Nutritional assessment of endogenous constituents 18
3.4.6.1. Human nutrition 18
3.4.6.2. Animal nutrition 19
3.4.7. Post-market monitoring of GM food/feed 19
3.4.8. Conclusions on the food/feed safety assessment 19
3.5. Environmental risk assessment and monitoring plan 19
3.5.1. Environmental risk assessment 19
3.5.1.1. Persistence and invasiveness of the GM plant 19
3.5.1.2. Potential for gene transfer 20
3.5.1.3. Interactions of the GM plant with target organisms 21
3.5.1.4. Interactions of the GM plant with non-target organisms 21
3.5.1.5. Interactions with the abiotic environment and biogeochemical cycles 21
3.5.2. Post-market environmental monitoring 21

3.5.3. Conclusion of the environmental risk assessment and monitoring plan 21
4. Overall conclusions 22
5. Documentation as provided to EFSA (if appropriate) 22
References 23
Abbreviations 25
Appendix A – List of relevant publications identified by the applicant through systematic literature searches (1 April 2008–30 April 2020) 26
Appendix B – Additional studies 27
Appendix C – Statistically significant findings in 90-day study on GHB811 cottonseed in rats 28
Appendix D – Animal dietary exposure 29

1. Introduction

The scope of the application EFSA-GMO-ES-2018-154 is for food and feed uses, import and processing of cotton GHB811 and does not include cultivation in the European Union (EU). Cotton GHB811 was developed to confer tolerance to glyphosate and to HPPD inhibitor herbicides such as isoxaflutole. Glyphosate and isoxaflutole were the intended herbicides tested in this specific application.

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

On 11 October 2018, the European Food Safety Authority (EFSA) received from the Competent Authority of Spain application EFSA-GMO-ES-2018-154 for authorisation of cotton GHB811 (Unique Identifier BCS-GH811-4), submitted by BASF Agricultural Solutions Belgium NV (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003[1]. Following receipt of application EFSA-GMO-ES-2018-154, EFSA informed EU Member States and the European Commission, and made the application available to them. Simultaneously, EFSA published summary of the application.[2]

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 16 January 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-ES-2018-154. 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[3]. The EU Member States had three months to make their opinion known on application EFSA-GMO-ES-2018-154 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 cotton GHB811 in the context of its scope as defined in application EFSA-GMO-ES-2018-154.

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 Open EFSA 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.[4]

2. Data and methodologies

2.1. Data

The GMO Panel based its scientific risk assessment of cotton GHB811 on the valid application EFSA-GMO-ES-2018-154, additional information provided by the applicant during the risk assessment, relevant scientific comments submitted by the Member States and relevant peer-reviewed scientific publications.

[1] 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.
[2] Available online: https://open.efsa.europa.eu/questions/EFSA-Q-2018-00808
[3] 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.
[4] https://open.efsa.europa.eu/questions/EFSA-Q-2018-00808

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, 2017), explanatory notes and statements (i.e. EFSA, 2017, 2019a,b) 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, 2019b). In the frame of the contracts OC/EFSA/GMO/2018/02 and OC/EFSA/GMO/2018/04, contractors performed preparatory work and delivered reports on the methods applied by the applicant in performing statistical analyses and literature search, respectively.

3. Assessment

3.1. Systematic literature review[5]

The GMO Panel assessed the applicant's literature searches on cotton GHB811, which include a scoping review, according to the guidelines given in EFSA (2010, 2019b).

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-ES-2018-154. Based on the outcome of the scoping review, the GMO Panel agrees that there is limited value of undertaking a systematic review for cotton GHB811 at present.

The GMO Panel considered the overall quality of the performed literature searches is acceptable. The literature searches identified six relevant publications on cotton GHB811.

Based on the relevant publications identified through the literature searches (Appendix A), the GMO Panel does not identify any safety issues pertaining to the intended uses of cotton GHB811.

3.2. Molecular characterisation[6]

3.2.1. Transformation process and vector constructs

Cotton GHB811 was developed by *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*)-mediated *transformation. Hypocotyl segments of cotton variety Coker 312 were co-cultured with a disarmed Agrobacterium tumefaciens* strain ABI containing the vector pTSIH09. The plasmid pTSIH09 used for the transformation contains two expression cassettes between the right and left border of the T-DNA, containing the following genetic elements:

- The *hppdPfW336-1Pa* expression cassette consists of the Pcsvmv promoter from Cassava Vein Mosaic Virus, the DNA sequence encoding TPotpY-1Pa transit peptide from the RuBisCO small subunit genes of *Zea mays* and *Helianthus annuus*, the hppdPfW336-1Pa coding sequence of the 4-hydroxyphenylpyruvate dioxygenase gene of *Pseudomonas fluorescens* strain A32 and the ThistonAt sequence including the 3′ untranslated region of the histone H4 gene of *Arabidopsis thaliana*.
- The *2mepsps* expression cassette consists of the Ph4a748 promoter region of the histone H4 gene of *Arabidopsis thaliana*, the intron1 h3At of the gene II of the histone H3.III variant of *Arabidopsis thaliana*, the TPotpC coding sequence of the optimized transit peptide containing sequence of the RuBisCO small subunit genes of *Zea mays* and *Helianthus annuus*, the 2me*psps* coding sequence of the double-mutant 5-enol-pyruvylshikimate-3-phosphate synthase gene of *Zea mays* and the ThistonAt sequence including the 3′ untranslated region of the histone H4 gene of *Arabidopsis thaliana*.

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

[5] Dossier: Part II – Section 7; additional information: 17/5/2019, 8/12/2019 and 7/6/2021.
[6] Dossier: Part II – Section 1.2; additional information: 5/2/2019, 17/9/2019, 17/7/2020, 4/12/2020 and 7/6/2021.

3.2.2. Transgene constructs in the GM plant

Southern blot analysis indicated that cotton GHB811 contains a single insert, consisting of a single copy of the T-DNA with no rearrangements as compared to the pTSIH09 transformation vector. The absence of vector backbone sequences was demonstrated by Southern analysis using three overlapping backbone-specific probes.

The nucleotide sequence of the entire insert of cotton GHB811 together with 1,217 bp of the 5′ and 1296 bp of the 3′ flanking regions were determined by Sanger sequencing. The insert of 6,681 bp is identical to the T-DNA of pTSIH09.

A comparison with the pre-insertion locus indicated that 13 bp were deleted from the cotton genomic DNA. The possible interruption of known endogenous cotton genes by the insertion in cotton GHB811 was evaluated by bioinformatics analyses of the pre-insertion locus and of the genomic sequences flanking the insert. The results of these analyses do not indicate the interruption of any known endogenous gene in cotton GHB811.

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

Updated bioinformatics analyses of the amino acid sequence of the newly expressed HPPD W336 and 2mEPSPS proteins reveal no significant similarities to toxins and allergens. In addition, updated bioinformatic analyses of the newly created open reading frames (ORFs) within the insert and spanning the junctions between the insert and genomic DNA also do not indicate significant similarities to toxins and allergens.

In order to assess the possibility for horizontal gene transfer (HGT) by homologous recombination (HR), the applicant performed a sequence identity analysis for cotton GHB811 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

Cotton GHB811 expresses two new proteins: 2mEPSPS and HPPD W336. 2mEPSPS is a modified 5-enol-pyruvylshikimate-3-phosphate synthase from *Zea mays* which confers tolerance to glyphosate. HPPD W336 is a modified 4-hydroxyphenylpyruvate dioxygenase from *Pseudomonas fluorescens* conferring tolerance to HPPD inhibiting herbicides. The GMO Panel has previously evaluated 2mEPSPS and other HPPD proteins (EFSA, 2009a; EFSA GMO Panel, 2015b). Given the technical restraints in producing large enough quantities from plants, these proteins were recombinantly produced in *Escherichia coli*. Prior to safety studies, a set of biochemical methods was employed to demonstrate the equivalence between cotton- and microbe-produced proteins. Purified proteins from these two sources were characterised and compared in terms of their biochemical, structural and functional properties.

2mEPSPS protein characterisation and equivalence

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) and western blot analysis showed that both plant- and microbe-produced 2mEPSPS proteins had the expected molecular weight of ~ 47.4 kDa and were comparably immunoreactive to 2mEPSPS protein-specific antibodies. Glycosylation detection analysis demonstrated that none of the 2mEPSPS proteins were glycosylated. Amino acid sequence of the plant-derived 2mEPSPS protein by mass spectrometry (MS) methods showed that the protein matched the deduced sequence and molecular weight as defined by the *2mepsps* gene. These sequence analysis data were consistent with the previously analysed microbe-produced 2mEPSPS. In addition, the MS data showed that the N-terminal methionine was truncated from both 2mEPSPS proteins and an additional variant of the plant-produced 2mEPSPS was identified with an N-terminal cysteinic sulfinic acid. 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 2mEPSPS proteins produced data consistent with those by MS. Functional equivalence was demonstrated by an *in vitro* assay which showed that plant- and microbe-derived 2mEPSPS proteins had comparable enzymatic activity.

HPPD W336 protein characterisation and equivalence

SDS–PAGE and western blot analysis showed that both plant- and microbe-produced HPPD W336 proteins had the expected molecular weight of ~ 40.3 kDa and were comparably immunoreactive to HPPD W336 protein-specific antibodies. Glycosylation detection analysis demonstrated that none of the

HPPD W336 proteins were glycosylated. Amino acid sequence of the plant-derived HPPD W336 protein by MS methods showed that the protein matched the deduced sequence and molecular weight as defined by the *hppd w336* gene. These sequence analysis data were consistent with the previously analysed microbe-produced HPPD W336. In addition, the MS data showed that the N-terminal methionine was truncated from both HPPD W336 proteins and an additional variant of the plant-produced HPPD W336 was identified with an N-terminal cysteinic sulfinic acid. 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 W336 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 W336 proteins had comparable enzymatic activity. Microbially produced HPPD W336 protein was also tested 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-dihydroxyphenylpyruvate (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 W336 has a metabolic impact within cotton GHB811 different from that of the native (endogenous) enzyme.

The protein characterisation data comparing the biochemical, structural and functional properties of plant- and microbe-produced 2mEPSPS and HPPD W336 proteins, indicate that these proteins are equivalent. Therefore, the GMO Panel accepts the use of the 2mEPSPS and HPPD W336 proteins produced in bacteria for the safety studies.

3.2.4. Information on the expression of the insert

Protein levels of 2mEPSPS and HPPD W336 were analysed by an enzyme-linked immunosorbent assay (ELISA) in material harvested in a field trial across three locations in USA during the 2015 growing season. Samples analysed included leaves (BBCH 14-16, BBCH 51-55, BBCH 60-67), root (BBCH 14-16), pre-candle squares (BBCH 60-67), immature bolls (BBCH 60-67), pollen (BBCH 60-69), whole plant (BBCH 60-67) and fuzzy seeds (BBCH 83-97) from plants treated and not treated with the combination of isoxaflutole and glyphosate. The mean values and standard deviations of protein expression levels in seeds (n = 12) and pollen (n = 12) of the 2mEPSPS and HPPD W336 proteins used to estimate human and animal dietary exposure (see Section 3.4.5) are reported in Table 1.

Table 1: Mean values, standard deviation and ranges of newly expressed proteins in fuzzy seeds [μg/g dry weight (dw) and μg/g fresh weight (fw)] and pollen (μg/g fw) from cotton GHB811 (n = 12)

	Isoxaflutole and glyphosate treatment			
	Not treated		**Treated**	
	μg/g dry weight (dw)	**μg/g fresh weight (fw)**	**μg/g dry weight (dw)**	**μg/g fresh weight (fw)**
Fuzzy seeds (BBCH 83-97)				
2mEPSPS	166.79[(a)] ± 43.52[(b)] (87.77—254.51)[(c)]	149.18 ± 44.15 (74.79–236.65)	173.43 ± 32.04 (99.62–228.66)	152.80 ± 23.42 (92.91–187.08)
HPPD W336	31.67 ± 16.00 (11.67–66.66)	28.29 ± 14.60 (9.95–59.85)	28.89 ± 10.46 (11.78–46.89)	25.47 ± 9.05 (10.98–42.20)
Pollen (BBCH 60-69)				
2mEPSPS	–	34.77 ± 9.30 (18.11–47.14)	–	38.98 ± 4.89 (30.17–46.69)
HPPD W336	–	v< LOQ[(d)] (< LOQ-0.69)[(e)]	–	< LOQ[(d)] (< LOQ-0.68)[(e)]

(a): Mean value.
(b): Standard deviation.
(c): Ranges.
(d): HPPD W336 levels were below the limit of quantification (LOQ = 0.6 μg/g) in 11 out of 12 pollen samples.
(e): HPPD W336 levels in pollen were not adjusted for extraction efficiency. The extraction efficiency could not be determined because the analyte expression was too low.
–: not applicable, pollen samples were analysed on fresh tissue only.
LOQ: limit of quantification.

3.2.5. Inheritance and stability of inserted DNA

Genetic stability of cotton GHB811 insert was assessed by Southern analysis of genomic DNA from five generations (T1, T3, T4, BC1F2 and BC2F3) and polymerase chain reaction (PCR)-based segregation analysis from five generations (BC1F3, BC2F2, BC2F3, BC2F4 and BC2F5). The restriction enzyme/probe combinations used were sufficient to conclude 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 presence of 2mEPSPS and HPPD W336 proteins in leaves collected from five generations. The expression of the 2mEPSPS and HPPD W336 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 cotton GHB811 contains a single insert consisting of one copy of the *hppdPfW336-1Pa* and *2mepsps* expression cassettes. Bioinformatics analyses of the sequences encoding the newly expressed proteins and ORFs within the insert or spanning the junctions between the insert and genomic DNA do not raise concerns. The stability of the inserted DNA and of the introduced traits is confirmed over several generations. The methodology used to quantify the levels of the HPPD W336 and 2mEPSPS proteins is considered adequate. The protein characterisation data comparing the biochemical, structural and functional properties of plant- and microbe-produced HPPD W336 and 2mEPSPS proteins indicate that these proteins are equivalent and the microbial-produced protein can be used in safety studies.

3.3. Comparative analysis[7]

3.3.1. Overview of studies conducted for the comparative analysis

Application EFSA-GMO-ES-2018-154 presents data on agronomic and phenotypic characteristics as well as on seed composition of cotton GHB811 (Table 2).

Table 2: Overview of the comparative analysis studies to characterise cotton GHB811 in application EFSA-GMO-ES-2018-154

Study focus	Study details	Comparator	Non-GM reference varieties
Agronomic and phenotypic analysis	Field study, USA, 2014 and 2015, 15 sites[(a)]	Coker 312	Seven[(b)]
Compositional analysis	Field study, USA, 2014 and 2015, 8 sites[(a)]		

(a): Eight field trials were used for both the agronomic/phenotypic characterisation and the compositional analysis and were at the following locations: in 2014, one each in California, Georgia, Louisiana, Mississippi and South Carolina; in 2015, one in North Carolina and two in Texas. Seven field trials were used only for the agronomic/phenotypic characterisation and were at the following locations: in 2014, one each in Texas and Arkansas; in 2015, one each in Georgia, Arkansas and Mississippi and two in Texas.

(b): The non-GM cotton varieties used in the agronomic, phenotypic and compositional field trials were Acala Maxxa, DP399, FM958, FM966, FM989, ST457 and ST468.

3.3.2. Experimental field trial design and statistical analysis

The materials grown at each field trial site were: cotton GHB811 exposed to the intended isoxaflutole- and glyphosate-containing herbicides (treated), cotton GHB811 not exposed to the intended herbicides (untreated), the comparator Coker 312 and three commercial non-GM cotton reference varieties (hereafter '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 cotton GHB811, the application of a difference test (between the GM cotton and the comparator) and an equivalence test (between

[7] Dossier: Part II – Section 1.3; Additional information: 30/4/2019, 17/9/2019, 27/3/2020 and 4/12/2021.

the GM cotton and the set of non-GM reference varieties). The results of the equivalence test are categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence).[8]

3.3.3. Suitability of selected test materials

3.3.3.1. Selection of the test materials

Cotton GHB811 was obtained using Coker312 as non-GM recipient line. For the field trial study, two different generations directly derived from the original transformant, were used to conduct the agronomic and phenotypic and the compositional assessment. The comparator selected in the field trials is cotton Coker312 that is considered the conventional counterpart.

Seven non-GM reference varieties (see Table 2) were selected by the applicant and at each selected site three of them were tested. On the basis of the information provided the GMO Panel considers the selected non-GM reference varieties appropriate for the comparative assessment.

Cotton GHB811 and its conventional counterpart and the selected non–GM reference varieties, are appropriate for growing in a range of environments across North America.

3.3.3.2. Seed production and quality

The seeds of the cotton GHB811 and the conventional counterpart used in the 2014 and the ones used in the 2015 field trials (see Table 2) were produced in different seasons. The seed lots used in 2014 and 2015 were verified for their identity via lateral flow strip testing and via event-specific PCR analysis, respectively. The germination of cotton GHB811 and the 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 adequate quality.

3.3.3.3. Conclusion on suitability

The GMO Panel is of the opinion that cotton GHB811, the conventional counterpart and the non-GM reference varieties were properly selected and are of adequate quality. Therefore, the test materials are considered appropriate for the comparative analysis.

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 cotton-growing regions of the United States. Climate and soil characteristics of the selected fields were diverse,[9] corresponding to optimal, near optimal and sub-optimal conditions for cotton cultivation (Sys et al., 1993). The GMO Panel considers that the selected sites, including the subset chosen for the compositional analysis, reflect commercial cotton-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.[10] However, due to the lack of major impacts on plant growth at these sites, 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 cotton GHB811, plots with the conventional counterpart and plots with non-GM reference varieties, mostly managed according to local agricultural practices. In addition, the field trials included plots containing cotton GHB811 managed following the same agricultural practices, plus exposed to an HPPD inhibitor– and glyphosate-containing herbicides that were applied, respectively, at BBCH 0-13 and BBCH 16-19 growth stages. Isoxaflutole was the intended herbicide

[8] 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).

[9] Soil types of the field trials were sandy clay loam, clay loam, loamy sand, sandy loam, clay, loam, silt loam and sand; soil organic matter ranged from 0.7% to 1.6% except for the site in North Carolina (38.0%); soil pH ranged from 5.5 to 8.0. Average temperatures and sum of precipitations during the usual crop-growing season ranged, respectively, from 23.4°C to 27.4°C and from 25 to 622 mm.

[10] Excessive rain events reported at sites in Cheneyville (LA), West Memphis (AR) and Waller (TX), hail at the site in Groom (TX).

selected as representative of the HPPD-inhibitor group of herbicides. At some field trial sites, sowing occurred later than usual, resulting in a shorter growing cycle. The additional information indicated that at each field trial site, where late sowing happened, the required heat units were accumulated at the time of harvest. Therefore, the late sowing did not affect the quality of the field trials. The GMO Panel considers that the management practices were acceptable for the field trials.

3.3.4.4. Conclusion on representativeness

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

3.3.5. Agronomic and phenotypic endpoints

Forty-five agronomic and phenotypic endpoints were collected from the field trials (Table 2). Of those, 14 endpoints (including information on biotic and abiotic stressors) were measured on a categorical scale and were analysed with the Cochran–Mantel–Haenszel (CMH) test.[11]

The remaining 31 endpoints[12] were analysed as described in Section 3.3.2, with the following outcome:

- For cotton GHB811 (not treated with the intended herbicide), statistically significant differences with the comparator were identified for 15 endpoints.[13] All those endpoints fell under equivalence category I or II except for % lint and lint length which fell under equivalence category III or IV.[14]
- For cotton GHB811 (treated with the intended herbicide), statistically significant differences with the comparator were identified for seven endpoints.[15] All those endpoints fell under equivalence category I or II except for lint length which fell under equivalence category IV.[13]

For disease stressor rating (BBCH 61-69), the CMH test identified statistically significant differences between both treatments of cotton GHB811 and the comparator; however, the mean values for cotton GHB811 were within the range of the non-GM reference varieties.

For % lint and lint length, statistically significant differences were identified between cotton GHB811 and the conventional counterpart and the endpoints fell under equivalence category III/IV. The GMO Panel considered that the differences were small in magnitude. The % lint and lint length are endpoints related to the latest developmental stages of cotton plants that (taken alone) are not indicators of the quality of the field trials, with lint not included in the compositional analysis (Section 3.3.6). For these reasons, the GMO Panel considered that these results do not indicate issues in the materials used for the comparative analysis.

3.3.6. Compositional analysis

Fuzzy seeds of cotton GHB811 harvested from the field trials (Table 2) were analysed for 73 constituents, including those recommended by OECD (2009). The statistical analysis was not applied to 17 constituents[16] because more than half of the samples were below the limit of quantification.

[11] These included plant lodging, boll type and rating of abiotic, disease and insect stressors; stressor ratings were evaluated at four different stages (at BBCH 12-52, 54-65, 61-69 and 81-89).

[12] Early stand count (measured at two different stages: BBCH 10-13 and 13-18), % ground cover, days to 10% flower, heat units to 10% flower, days to first open bolls, heat units to first open bolls, % open bolls, final stand count, plant height, number of bolls, number of nodes, height to node ratio, first fruiting branch, number of vegetative branches, number of fruiting branch bolls, number of vegetative bolls, number of potential fruiting sites, % fruit retention, % harvestable fruiting branch bolls, hundred seed weight, total seedcotton yield, lint yield, % lint, seeds per boll, boll weight and fibre properties (lint micronaire, length, uniformity index, strength and elongation).

[13] Early stand count (BBCH 13-18), % open bolls, final stand count, boll weight, total seedcotton yield, plant height, number of bolls per plant, number of vegetative bolls per plant, % harvestable fruiting branch bolls, height to node ratio, lint yield, % lint, lint micronaire, lint length and lint uniformity index.

[14] The estimated mean values for % lint were 38.3 (untreated GM cotton), 38.4 (treated GM cotton), 39.1 (conventional counterpart) and 41.4 (reference varieties); equivalence limits: 38.3–44.4. The estimated mean values for lint length (inches) were 1.24 (untreated GM cotton), 1.24 (treated GM cotton), 1.23 (conventional counterpart) and 1.16 (reference varieties); equivalence limits: 1.12–1.20.

[15] Early stand count (BBCH 13-18), days to first open bolls, heat units to first open bolls, % open bolls, boll weight, % lint and lint length.

[16] 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), eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3), arachidonic acid (C20:4), eicosapentaenoic acid (C20:5), erucic acid (C22:1), docosapentaenoic acid (C22:5n-3), docosapentaenoic acid (C22:5 n-6) and docosahexaenoic acid (C22:6), β-tocopherol and δ-tocopherol.

The statistical analysis was applied to a total of 56 constituents[17]; a summary of the outcome of the test of difference and the test of equivalence is presented in Table 3:

- For cotton GHB811 not treated with the intended herbicide, significant differences between the GM cotton and the comparator were found for 11 endpoints, all of which fell under equivalence category I or II.
- For cotton GHB811 treated with the intended herbicide, significant differences between the GM cotton and the comparator were found for 18 endpoints; all those endpoints fell under equivalence category I or II except for dihydrosterculic acid which fell under equivalence category IV (Table 4).

Table 3: Outcome of the comparative compositional analysis in fuzzy seeds for cotton GHB811. The table shows the number of endpoints in each category

		Test of difference [(a)]			
		Not treated [(c)]		**Treated** [(c)]	
		Not different	**Significantly different**	**Not different**	**Significantly different**
Test of equivalence[(b)]	Category I/II	40	11[(d)]	34	17[(d)]
	Category III/IV	1[(e)]	–	–	1[(f)]
	Not categorised	4[(g)]	–	4[(g)]	–
	Total endpoints	56		56	

(a): Comparison between cotton GHB811 and its comparator.
(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 cotton GHB811 and its comparator and falling under equivalence category I-II. Both treated and not treated: carbohydrates, crude protein, neutral detergent fibre, total dietary fibre, palmitoleic acid (C16:1), stearic acid (C18:0), arachidic acid (C20:0), manganese, α-tocopherol, free gossypol and total gossypol. Only not treated: none. Only treated: cystine, methionine, myristic acid (C14:0), γ-tocopherol and calcium.
(e): Endpoint with no significant differences between cotton GHB811 and its comparator and falling under equivalence category IV: dihydrosterculic acid (not treated).
(f): Endpoint with significant differences between cotton GHB811 and its comparator and falling in equivalence category IV: dihydrosterculic acid (treated). Estimated means for this endpoint are reported in Table 4.
(g): Endpoints not categorised for equivalence and without significant differences between cotton GHB811 (both treated and not treated) and its comparator: tyrosine, linolenic acid (C18:3), lignoceric acid (C24:0) and sodium.

The GMO Panel assessed all the significant differences between cotton GHB811 and its comparator, taking into account the potential impact on plant metabolism and the natural variability observed for the set of non-GM reference varieties. A significant difference between cotton GHB811 and its comparator was found for levels of dihydrosterculic acid, which also fell under equivalence category IV; mean estimates for this endpoint are given in Table 4.

Following a request from the GMO Panel, additional information was provided on the levels of β-, γ-, δ-tocopherol and total tocopherols in cotton fuzzy seeds to complement the initial information reported on α-tocopherol. This request was based on the involvement of HPPD protein in the tyrosine metabolic pathway which is directly linked to tocopherol synthesis in plants (Kramer et al., 2014). As described above, the outcome of the comparative assessment indicated that no further assessment regarding food and feed safety was required for the different tocopherols.

[17] Moisture, crude protein, crude fat, ash, total carbohydrates (calculated), total dietary fibre, acid detergent fibre, neutral detergent fibre, alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), 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 (C22:0), lignoceric acid (C24:0), calcium, copper, iron, sodium, magnesium, manganese, phosphorus, potassium, zinc, α-tocopherol, γ-tocopherol, total tocopherol, total gossypol, free gossypol, dihydrosterculic acid, malvalic acid and sterculic acid.

Table 4: Estimated means for dihydrosterculic acid

Endpoint	Cotton GHB811		Comparator	Non-GM reference varieties
	Not treated[(a)]	Treated[(a)]		
Dihydrosterculic acid (% FA)	0.179	0.163*	0.192	0.261

For cotton GHB811, significantly different values are marked with an asterisk. Both treated and not treated GM, the endpoint fell under equivalence category IV.
% FA: percentage total fatty acids.
(a): Treated: treated with the intended herbicide; not treated: treated only with conventional herbicides (see Section 3.3.4.3).

3.3.7. Conclusions on the comparative analysis

Considering the selection of test materials, the field trial sites and the associated management practices and the agronomic-phenotypic characterisation as an indicator of the overall field trial quality, the GMO Panel concludes that the field trials are appropriate to support the comparative analysis.

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

- None of the differences identified in agronomic and phenotypic characteristics between cotton GHB811 and the comparator needs further assessment except for % lint and lint length. These differences are further discussed for potential environmental impact in Section 3.5.
- None of the differences identified in fuzzy seed composition between cotton GHB811 and its comparator needs further assessment regarding food and feed safety, except for the levels of dihydrosterculic acid, which are further assessed in Sections 3.4.3.3 and 3.4.6.

3.4. Food/feed safety assessment

3.4.1. Effects of processing

Cotton GHB811 will undergo existing production processes used for conventional cotton. No novel production process is envisaged. Based on the outcome of the comparative assessment, processing of the GM cotton into food and feed products is not expected to result in products being different from those of conventional non-GM cotton varieties.

Information was provided on the process to produce cottonseed oil, the main and almost only cottonseed-derived food commodity consumed by humans.[18] The presence of HPPD W336 and 2mEPSPS newly expressed proteins was investigated in both the crude oil and the refined bleached deodorised (RBD) oil. No newly expressed proteins were quantified in either type of oil.[19] Additionally, the applicant refers to cottonseed linters, a by-product produced during cottonseed processing and used for human consumption. These cottonseed linters, with more than 99% fibre content, are used in different food products such as baked goods, dressings, snacks and processed meat, among others.

3.4.2. Stability of the newly expressed proteins

Protein stability is one of several relevant parameters to consider in the weight-of-evidence approach in protein safety (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, a prominent trait 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 effects of temperature and pH on newly expressed proteins 2mEPSPS and HPPD W336 have been previously evaluated by the GMO Panel (EFSA, 2009a; EFSA GMO Panel, 2014, 2015b). Additional studies addressing heat stability were provided by the applicant (Appendix B). The outcome of these

[18] Main dossier and study report M-215504-03-2.
[19] Study report M-582366-01-1. Limit of quantification (LOQ) for HPPD W336 = 0.0125 mg/g; LOQ for 2mEPSPS protein = 0.00625 mg/g.

studies is consistent with the previous analogous studies assessed by the GMO Panel (EFSA, 2009a; EFSA GMO Panel, 2014, 2015b).

In vitro protein degradation by proteolytic enzymes

In vitro protein degradation studies on 2mEPSPS and HPPD W336 proteins have been previously evaluated by the EFSA GMO Panel (EFSA, 2009a; EFSA GMO Panel, 2014, 2015b, 2018). Additional *in vitro* degradation studies proteins were provided by the applicant (Appendix B). The outcome of these studies is consistent with the previous studies assessed by the GMO Panel (EFSA, 2009a; EFSA GMO Panel, 2015b, 2018).

3.4.3. Toxicology

3.4.3.1. Testing of the newly expressed proteins

Cotton GHB811 expresses two new proteins, 2mEPSPS and HPPD W336. The GMO Panel assessed the safety of these proteins considering molecular characterisation and bioinformatic analyses (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 cotton GHB811 are expected. The 2mEPSPS and HPPD W336 plant proteins have been extensively characterised and their equivalence to *E. coli*-produced proteins used in safety studies was demonstrated (Section 3.2.3).

The 2mEPSPS and HPPD W336 proteins were previously assessed by the GMO Panel in the context of other applications (e.g. EFSA 2009a; EFSA GMO Panel 2015b) and no safety concerns for humans and animals were identified.

Bioinformatic analyses of the amino acid sequences of the 2mEPSPS and HPPD W336 proteins revealed no significant similarities to known toxins.

Two additional studies addressing subacute toxicity of the HPPD W336 protein were provided by the applicant and assessed by the GMO Panel (Appendix B). A 28-day toxicity study (M-368158-01-1) was previously assessed in the frame of application EFSA-GMO-BE-2011-98; the GMO Panel concluded on the absence of adverse effects in mice after administration of the HPPD W336 protein at a target dose of 1,000 mg/kg body weight per day (EFSA GMO Panel 2015b). A new 28-day toxicity study (M-514143-01-1) was provided in the context of this application to further confirm the safety assessment of the HPPD W336 protein, and assessed by the GMO Panel; the outcome of this study is consistent with the previous conclusions.

The GMO Panel is not aware of any new information that would change previous conclusion on the safety of the 2mEPSPS and HPPD W336 proteins.

3.4.3.2. Testing of new constituents other than the newly expressed proteins

No new constituents other than newly expressed proteins have been identified in seeds from cotton GHB811. 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/feed constituents

Dihydrosterculic acid levels in seeds were significantly different (lower) in cotton GHB811 treated with the intended herbicide when compared with its conventional counterpart and showed a lack of equivalence with the non-GM reference varieties (Section 3.3.7). Taking into account the biological characteristics and functions of this compound, the observed difference is considered of no toxicological concern. Further information on safety is provided in Section 3.4.6.

3.4.3.4. Testing of the whole genetically modified food/feed

Based on the outcome of molecular characterisation and comparative analysis assessment, no compositional modifications, or indication of possible unintended effects relevant to food and feed safety have been identified for cotton GHB811. Therefore, animal feeding studies with food/feed derived from cotton GHB811 are not considered necessary by the GMO Panel (EFSA GMO Panel, 2011a). In accordance with Regulation (EU) No 503/2013, the applicant provided a 90-day feeding study in rats receiving diets derived from cotton GHB811 (study number 0021232). In this study, pair-housed Crl:CD(SD) rats (16 per sex per group; 2 rats per cage) were allocated to three groups using a randomised complete block design with eight replications per sex. Groups were fed diets containing 10% of incorporation rate of toasted cottonseed meal either from cotton GHB811 plants treated with

the intended herbicides[20] (test material), from the conventional counterpart (control material) or a non-transgenic commercial reference cotton (FM966, reference material). The upper level of 10% was justified based on the content of gossypol in cottonseed. The study was adapted from OECD test guideline 408 (1998), aligned with EFSA Scientific Committee guidance (EFSA Scientific Committee, 2011) and complied with the principles of good laboratory practice (GLP) with some minor deviations not impacting the study results and interpretation (i.e. test item stability, homogeneity and concentration), which are detailed below. Event-specific PCR analysis confirmed the presence of the event GHB811 in both the GM toasted cottonseed meals and diets and excluded the presence of the event in the respective controls. Both the GM toasted cottonseed meals and diets were analysed for nutrients, antinutrients and potential contaminants (e.g. selected heavy metals, mycotoxins and pesticides). Balanced diets were formulated based on the specifications for PMI Certified Rodent LabDiet® 5002. The stability of the test and control materials was not verified; however, in accordance to product expiration declared by the diet manufacturer, the constituents of the diets are considered stable for the duration of the treatment. The GMO Panel considered this justification acceptable. Diet preparation procedures and regular evaluations of the mixing methods guaranteed the homogeneity and the proper concentration of the test or control substances in them. Feed and water were provided ad libitum. In-life procedures and observations and terminal procedures were conducted in accordance to OECD TG 408 (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; in case a significant sex-by-diet interaction was identified (and for sex-specific endpoints) the results of a sex-specific analysis 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. One control male died on day 64; post-mortem investigations identified a malignant lymphoma. 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.

Detailed description of statistically significant findings identified in rats given a diet-containing GHB811 cotton is reported in Appendix C.

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 or 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 10% toasted meal from GHB811 cottonseed 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

A weight-of-evidence approach was followed, taking into account all the information obtained on the newly expressed protein, as no single piece of information or experimental method yield sufficient

[20] Isoxaflutole- and glyphosate-containing herbicides.

evidence to predict allergenicity (Codex Alimentarius, 2009; EFSA GMO Panel, 2011a, 2017; Regulation (EU) No 503/2013).

The *2mepsps* gene encoding for the 2mEPSPS protein originates from *Zea mays*, and the *hppd* gene encoding for the HPPD W336 protein originates from *P. fluorescens*, none of which are considered allergenic sources.

Updated bioinformatic analyses of the amino acid sequences of the 2mEPSPS and HPPD W336 proteins, using the criterion of 35% identity in a sliding window of 80 amino acids, revealed no relevant similarities to known allergens. The studies on protein stability of the 2mEPSPS and HPPD W336 proteins have been described in Section 3.4.2.

The GMO Panel has previously evaluated the safety of the 2mEPSPS and HPPD W336 proteins and no concerns on allergenicity were identified (e.g. EFSA, 2009a; EFSA GMO Panel, 2015b). In addition, the GMO Panel did not find an indication that the newly expressed proteins 2mEPSPS and HPPD W336 at the levels expressed in cotton GHB811 might be adjuvants.

Furthermore, the applicant provided information on the safety of the 2mEPSPS and HPPD W336 proteins regarding their potential hazard to cause a celiac disease response.[21] For such assessment, the applicant followed the principles described in the EFSA GMO Panel guidance document (EFSA GMO Panel, 2017). The assessment of the 2mEPSPS and HPPD W336 proteins 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 2mEPSPS and/or HPPD W336 proteins in cotton GHB811 may be allergenic.

3.4.4.2. Assessment of allergenicity of the whole GM plant or crop

The GMO Panel regularly reviews the available publications on food allergy to cottonseed-derived products. However, cotton is not considered a common allergenic food[22] (OECD, 2009). Therefore, the GMO Panel does not request experimental data to analyse the allergen repertoire of GM cotton.

In the context of this application and considering the data from the molecular characterisation, the compositional analysis and the assessment of the newly expressed proteins (see Sections 3.2, 3.3 and 3.4), the GMO Panel identifies no indications of a potentially increased allergenicity of food and feed derived from cotton GHB811 with respect to that derived from the non-GM comparator.

3.4.5. Dietary exposure assessment to new constituents

In line with Regulation (EU) No 503/2013, the applicant provided dietary exposure estimates to HPPD W336 and 2mEPSPS newly expressed in cotton GHB811. Dietary exposure was estimated based on protein expression levels reported in this application for cotton GHB811 treated with the intended herbicides (see Table 1), 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

The applicant considered the dietary exposure to HPPD W336 and 2mEPSPS newly expressed proteins as negligible in the European population. No consumption data of cottonseed and cottonseed-derived products were available in the EFSA Comprehensive European Food Consumption Database.[23]

The GMO Panel identified different cottonseed-derived products such as flour and oil as well as by-products (cottonseed linters) used for human consumption, with the RBD oil being currently the most relevant. The GMO Panel confirmed that no consumption data of cottonseed oil or other cottonseed-derived products were available in the EFSA consumption database. However, cottonseed oil can be typically consumed as an ingredient in the production of a wide variety of food products such as dressings, mayonnaise, fine bakery wares, chocolate spreads and chips. As described in Section 3.4.1, HPPD W336 and 2mEPSPS proteins were not found in RBD oil from cotton GHB811 and, therefore, no dietary exposure to the newly expressed proteins is expected from the consumption of this oil.

[21] Technical dossier Section 1.5, additional information July 2020 and December 2020.

[22] 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.

[23] https://www.efsa.europa.eu/en/food-consumption/comprehensive-database. Data accessed February 2021.

Although dietary exposure to HPPD W336 and 2mEPSPS proteins cannot be excluded via the consumption of cottonseed flour, the presence of these products is currently minor in the European market. Consequently, the GMO Panel confirmed that, at present, the dietary exposure to HPPD W336 and 2mEPSPS can be considered as negligible.

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 cotton GHB811. HPPD W336 and 2mEPSPS levels in pollen as described in Table 1 were used to derive concentrations in pollen supplements considering around 6% moisture content in these products. HPPD W336 was only quantified in one sample out of twelve (0.69 mg/g fw); half of the LOQ (0.6 g/kg fw) was considered for the other eleven samples when estimating dietary exposure. Consumption data on pollen supplements are available for few consumers across eight different European countries.[23] The low number of consumers available adds uncertainty to the exposure estimations which should be carefully interpreted, and it prevents from estimating exposure for high consumers of pollen supplements. In average consumers of pollen supplements, the highest acute dietary exposure would be 64.0 and 0.54 mg/kg bw per day in the elderly population for 2mEPSPS and HPPD W336 proteins, respectively. Similarly, the highest chronic dietary exposure in average consumers would be 42.6 and 0.36 mg/kg bw per day also in the elderly population for 2mEPSPS and HPPD W336 proteins, respectively.

3.4.5.2. Animal dietary exposure[24]

Dietary exposure to HPPD W336 and 2mEPSPS proteins in cotton GHB811 was estimated across different animal species as below described, assuming the consumption of cotton products commonly entering the feed supply chain (i.e. undelinted seed and meal). A conservative scenario with 100% replacement of conventional cotton products by the cotton GHB811 products was considered.

Mean levels (dry weight) of HPPD W336 and 2mEPSPS proteins in undelinted seeds from cotton GHB811 plant treated with the intended herbicide used for dietary exposure are listed in Table 1.

To estimate the mean HPPD W336 and 2mEPSPS protein levels in cottonseed meal, a factor of 1.3 was applied based on default Processing Factors (PFs) retrieved from the 'Default PF by products' table of the 'Pesticides MRL Guidelines Animal Model 2017' excel table accessed on June 3, 2020 from the EU Commission website, assuming that no losses of proteins occur during processing.

The applicant estimated dietary exposure to HPPD W336 and 2mEPSPS proteins via the consumption of cottonseed meal in broiler, layer and turkey, breeding and finishing swine, beef and dairy cattle, ram/ewe and lamb, and cotton undelinted seed only in dairy cow, based on estimates for animal body weight, daily feed intake and inclusion rates (percentage) of cotton undelinted seed and cottonseed meal in diets (OECD, 2013).

Estimated dietary exposure in livestock animals is reported in Appendix D (Table D.1 and Table D.2).

3.4.6. Nutritional assessment of endogenous constituents

The intended trait of cotton GHB811 is herbicide tolerance, with no intention to alter nutritional parameters. However, dihydrosterculic acid in cotton seeds was significantly different (in plants treated with the intended herbicide) from its conventional counterpart and showed a lack of equivalence with the set of non-GM reference varieties (Section 3.3.7). The biological relevance of dihydrosterculic acid, the role of cotton as contributor to its total intake and the magnitude and direction of the observed change were considered during the nutritional assessment.

3.4.6.1. Human nutrition

Dihydrosterculic acid is a cyclopropenoid fatty acid (CPFA) that together with other CPFAs (sterculic acid and malvalic acid) and gossypol are considered natural toxicants in cottonseeds. CPFAs inhibit the enzyme Δ9-desaturase preventing the conversion of stearic acid to oleic acid and potentially causing significant health problems for organisms which consume them.[25] Deodorization seems to reduce the CPFA content of the oil due to extreme pH and temperature conditions, although CPFAs are still present in RBD cottonseed oil (Obert et al., 2007). Based on the observed decrease (7–15%) in the content of dihydrosterculic acid in cotton GHB811 as compared to its conventional counterpart, the

[24] Dossier Part II, Section 2 and additional information 17/9/2019 and 17/7/2020.
[25] Study report M-215504-03-2.

GMO concludes that the levels of dihydrosterculic acid in cotton GHB811 do not represent a nutritional concern.

3.4.6.2. Animal nutrition

Cyclopropenoid fatty acids are inhibitors of several fatty acid desaturases in animals, and this implies an increase of saturated fatty acids in animal fat (Phelps et al., 1965; Page et al., 1997) observed that feeding whole cottonseeds containing CPFA, affect lipogenesis, but does not influence the activity of stearoyl coenzyme desaturase in liver and adipose tissue. Therefore, a reduction in dihydrosterculic acid content in cottonseed GHB811 is not expected to affect animal health.

3.4.7. Post-market monitoring of GM food/feed

The GMO Panel concluded that cotton GHB811, 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 cotton, as described in this application, is not necessary.

3.4.8. Conclusions on the food/feed safety assessment

The proteins HPPD W336 and 2mEPSPS newly expressed in cotton GHB811 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 cotton GHB811, or regarding the overall allergenicity of this GM cotton. Based on the outcome of the comparative assessment and the nutritional assessment, the GMO Panel concludes that the consumption of cotton GHB811 does not represent any nutritional concern, in the context of the scope of this application. The GMO Panel concludes that cotton GHB811, 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[26]

3.5.1. Environmental risk assessment

Considering the scope of the application EFSA-GMO-ES-2018-154, which excludes cultivation, the environmental risk assessment (ERA) of cotton GHB811 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 cotton GHB811 seeds during transportation and/or processing (EFSA GMO Panel, 2010a).

3.5.1.1. Persistence and invasiveness of the GM plant

In Southern Europe, *Gossypium herbaceum*, *G. barbadense* and *G. hirsutum* have been grown since the 19th century and led to transient or locally naturalised cotton plants in the same area (Davis, 1967; Tutin et al., 1992; Sarno et al., 1993; Celesti-Grapow et al., 2010). However, survival of cottonseeds outside cultivation areas in Europe is limited due to the absence of a seed dormancy phase. Even if seeds from spillage germinate, the resulting cotton plants are unlikely to survive due to factors such as cold climatic conditions, the susceptibility to diseases and their low competitiveness (Eastick and Hearnden, 2006). For example, after the end of cotton cultivation in Italy in 1950s, no feral cotton was reported in southern Italy, except in some restricted areas (Sarno et al., 1993; Celesti-Grapow et al., 2010). Also, in other cotton-growing regions, such as in Australia, surveys showed that feral GM cotton established infrequently along transportation routes and mostly as transient populations (Addison et al., 2007). Field observations indicate that cottonseed may survive and overwinter in some EU regions, resulting in volunteers in subsequent crops (e.g., Charles et al., 2013). However, cotton volunteers have been shown to rarely yield as well as newly planted seeds due to seedling diseases and early emergence in cool conditions. Thus, the establishment and survival of feral and volunteer cotton plants in the EU is currently limited and transient.

[26] Dossier: Part II – Sections 5 and 6; spontaneous information: 18/6/2021.

It is unlikely that the intended traits of cotton GHB811 and the observed differences in %lint and lint length (see Section 3.3.5) will provide a selective advantage to cotton plants, except when they are exposed to isoxaflutole- and/or glyphosate-containing herbicides.

The GMO Panel considers that the fitness advantage provided by the intended traits, and the observed differences in %lint and lint length 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 and other observed differences will not affect the persistence and invasiveness of the GM plant.

In conclusion, the GMO Panel considers it unlikely that cotton GHB811 will differ from conventional cotton 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 cotton GHB811 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.

Plant to microorganism gene transfer

Genomic DNA can be a component of food and feed products derived from cotton. 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 domesticated 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, 2009b).

The only mechanism known to facilitate horizontal transfer of non-mobile, chromosomal DNA fragments to bacterial genomes is homologous recombination. 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, 2009b). 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 GHB811 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 cotton GHB811 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 GM cotton GHB811 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 cotton seeds need to germinate and develop into plants in areas containing sympatric wild relatives and/or cultivated cotton with synchronous flowering and environmental conditions favouring cross-pollination.

Cotton is an annual predominantly self-pollinating crop, although cross-pollination can occur at low frequencies in the presence of insect pollinators (such as wild bees, honeybees, bumblebees) (OECD, 2008). For cotton, no wild relatives have been reported in Europe; therefore, any vertical gene transfer is limited to *G. hirsutum*, *G. barbadense* and *G. herbaceum* cotton plants. However, gene transfer to *G. herbaceum* is considered unlikely due to the difference in ploidy level.

The potential of spilled cotton 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 cotton plants resulting from seed spillage, and weedy or cultivated *Gossypium* plants is considered extremely low. Even if cross-pollination would occur, the GMO Panel is of the opinion that environmental effects as a consequence of the spread of genes from occasional feral GM cotton plants in Europe will not differ from that of conventional cotton 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-ES-2018-154 (no cultivation) and thus the absence of target organisms into account, potential interactions of occasional feral cotton GHB811 plants arising from seed import spills with the target organisms are not considered a relevant issue.

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 cotton plants arising from spilled cotton GHB811 seeds is limited and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM cotton, potential interactions of cotton GHB811 with non-target organisms are not considered by the GMO Panel to raise any environmental safety concern.

3.6.1.5. Interactions with the abiotic environment and biogeochemical cycles

Given that environmental exposure to spilled seeds or occasional feral cotton GHB811 plants arising from seed import spills is limited and because most proteins are degraded before entering the environment through faecal material of animals fed GM cotton, 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 PMEM plan, according to Annex VII of Directive 2001/18/EC, are to: (1) 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) 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 post-market environmental monitoring plan provided by the applicant (EFSA GMO Panel, 2011b).

As the ERA did not identify potential adverse environmental effects from the cotton GHB811, no case-specific monitoring is required.

The PMEM plan proposed by the applicant for cotton GHB811 includes: (1) the description of an approach involving operators (federations involved in cotton 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 newly established by CropLife Europe for the collection of the 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 cotton GHB811. 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 the cotton GHB811 would differ from conventional cotton varieties in its ability to persist under European environmental conditions. Considering the scope of application EFSA-GMO-ES-2018-154, interactions of occasional feral cotton GHB811 plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from cotton GHB811 to bacteria does not indicate a safety concern. Therefore, considering the introduced traits, the outcome of the agronomic and phenotypic analysis, the routes and levels of exposure, the GMO Panel concludes that cotton GHB811 would not raise safety concerns in the event of accidental release of viable GM cotton 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 cotton GHB811.

4. Overall conclusions

The GMO Panel was asked to carry out a scientific assessment of cotton GHB811 for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003.

The molecular characterisation data establish that cotton GHB811 contains a single insert consisting of one copy of the *hppdPfW336-1Pa* and the *2mepsps* 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 HPPD W336 and 2mEPSPS proteins is considered adequate. The protein characterisation data comparing the biochemical, structural and functional properties of plant- and microbe-produced HPPD W336 and 2mEPSPS proteins, indicate that these proteins are equivalent and the microbial derived proteins can be used in the safety studies.

None of the identified differences in the agronomic/phenotypic and compositional characteristics tested between cotton GHB811 and its conventional counterpart needed further assessment, except for % lint, lint length and dihydrosterculic acid, which do not raise nutritional and safety concerns. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the HPPD W336 and 2mEPSPS proteins as expressed in cotton GHB811, and finds no evidence that the genetic modification would change the overall allergenicity of cotton GHB811. In the context of this application, the consumption of food and feed from cotton GHB811 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that cotton GHB811 is as safe as the conventional counterpart and non-GM cotton reference varieties tested, and no post-market monitoring of food/feed is considered necessary.

The GMO Panel concludes that there is a very low likelihood of environmental effects resulting from the accidental release of viable seeds from cotton GHB811 into the environment. The PMEM plan and reporting intervals are in line with the intended uses of cotton GHB811. Based on the relevant publications identified through the literature searches, the GMO Panel does not identify any safety issues pertaining to the uses of cotton GHB811.

The GMO Panel concludes that cotton GHB811 is as safe as its conventional counterpart and the tested non-GM cotton reference varieties with respect to potential effects on human and animal health and the environment.

5. Documentation as provided to EFSA (if appropriate)

1) Letter from the Competent Authority of Spain received on 11 October 2018 concerning a request for authorization of the placing on the market of cotton GHB811 submitted in accordance with Regulation (EC) No 1829/2003 by BASF Agricultural Solutions Belgium.
2) Application EFSA-GMO-ES-2018-154 validated by EFSA, 16 January 2019.
3) Request for supplementary information to the applicant, 21 January 2019.
4) Receipt of supplementary information from the applicant, 05 February 2019.
5) Request for supplementary information to the applicant, 13 February 2019.
6) Request for supplementary information to the applicant, 28 February 2019.
7) Request for supplementary information to the applicant, 18 March 2019.
8) Receipt of supplementary information from the applicant, 30 April 2019.
9) Receipt of supplementary information from the applicant, 02 May 2019.
10) Receipt of supplementary information from the applicant, 17 May 2019.
11) Request for supplementary information to the applicant, 21 June 2019.
12) Receipt of supplementary information from the applicant, 17 September 2019.
13) Request for supplementary information to the applicant, 08 October 2019.
14) Receipt of supplementary information from the applicant, 08 November 2019.
15) Request for supplementary information to the applicant, 15 November 2019.
16) Receipt of supplementary information from the applicant, 27 March 2020.
17) Request for supplementary information to the applicant, 19 May 2020.
18) Request for supplementary information to the applicant, 27 May 2020.
19) Receipt of supplementary information from the applicant, 17 July 2020.

20) Request for supplementary information to the applicant, 05 November 2020.
21) Request for supplementary information to the applicant, 05 November 2020.
22) Receipt of supplementary information from the applicant, 04 December 2020.
23) Receipt of supplementary information from the applicant, 05 February 2021.
24) Request for supplementary information to the applicant, 08 February 2021.
25) Request for supplementary information to the applicant, 18 February 2021.
26) Receipt of supplementary information from the applicant, 19 March 2021.
27) Receipt of supplementary information from the applicant, 23 March 2021.
28) Request for supplementary information to the applicant, 07 April 2021.
29) Receipt of supplementary information from the applicant, 07 June 2021.
30) Receipt of spontaneous information from the applicant, 18 June 2021.

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Abbreviations

3,4-dHPP	3,4-dihydroxyphenylpyruvate
bp	base pair
bw	body weight
dw	dry weight
ELISA	enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate
ERA	environmental risk assessment
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	hydroxyphenylpyruvate dioxygenase
LOQ	limit of quantification
MS	mass spectrometry
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

Appendix A – List of relevant publications identified by the applicant through systematic literature searches (1 April 2008–30 April 2020)

References
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Herouet-Guicheney C, David R, Martine F, Thomas C, Aris M, Junguo Z, Bates Elizabeth EM, Jean-Marc F, Koen H and Dominique R, 2009. Safety evaluation of the double mutant 5-enol pyruvylshikimate -3- phosphate synthase (2mEPSPS) from maize that confers tolerance to glyphosate herbicide in transgenic plants. Regulatory Toxicology and Pharmacology, 54, 143–153.
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Schafer BW, Embrey SK and Herman RA, 2016. Rapid simulated gastric fluid digestion of in-seed/grain proteins expressed in genetically engineered crops. Regulatory Toxicology and Pharmacology, 81, 106–112.
Gottula J, Chapman K, Gao Y, Gillikin N, Beale J, Dharmasri C and Privalle L; 2018. Molecular biology and physiology. Agronomic performance and crop composition of genetically engineered cotton tolerant to HPPD inhibiting herbicides. Journal of Cotton Science 22, 75–85.

Appendix B – Additional studies

List of additional studies performed by or on behalf of the applicant with regard to the evaluation of the safety of cotton GHB811 for humans, animal or the environment

Study identification	Title
M-368158-01-1	HPPD W336 protein 28-day toxicity study in the mouse by gavage
M-514143-01-1	HPPD W336 protein 28-day toxicity study in the mouse by gavage
M-275371-02-1	2mEPSPS protein *in vitro* digestibility study in simulated intestinal fluid
M-406126-01-1	2mEPSPS protein In vitro digestibility study in human simulated gastric fluid
M-447994-01-1	The effect of temperature on microbially produced HPPD W336 assessed by ELISA
M-549236-01-1	The effect of temperature on 2mEPSPS as assessed by the EPSPS quantitative activity assay
M-535903-01-1	The effect of temperature on 2mEPSPS as assessed by SDS-PAGE and Western blot

Appendix C – Statistically significant findings in 90-day study on GHB811 cottonseed in rats

Statistically significant parameter/endpoint	Finding	GMO Panel interpretation
Body weight, body weight gain, food consumption	Statistically significant Increases and decreases at different time periods (± 50%)	Sporadic changes at individual time points. No significant impact on body weight or body weight gain over the entire study period. Not an adverse effect of treatment.
Mean corpuscular haemoglobin concentration	Statistically significant decrease in females (1%)	Low magnitude, not adverse in isolation, no other significant changes in red cell parameters, not present in males, within normal variation. Not an adverse effect of treatment.
Lymphocyte and basophil as % of WBC count	Statistically significant decrease in males (10–50%).	No significant change in absolute counts. Likely secondary to a high value for neutrophils (see below). Not an adverse effect of treatment.
Neutrophil % and absolute count	Statistically significant increases in males (40–60%)	Within the normal variation seen in rats of the same supplier, strain, age and basal diet, as shown by in depth evaluation of HCD from the test facility supplied (additional information 7/6/2021). No evidence of histopathology changes in myeloid/lymphoid organs (e.g. thymus, spleen, lymph nodes, Peyer's patches) or of inflammation. Not an adverse effect of treatment.
ALT activity	Statistically significant decrease in males (15%).	Decrease is not adverse in isolation, no consistent pattern in other liver marker enzymes and no liver pathology findings. Not an adverse effect of treatment.
Calcium	Statistically significant increase in females (3%).	Low magnitude, values for all 3 groups (11.3, 11.5 and 11.6) are near or above the upper end of the historic control range (11.4). Not an adverse effect of treatment.
Chloride	Statistically significant decrease in males (1%).	Low magnitude, within normal variation. Not an adverse effect of treatment.
Liver weights (absolute and relative to brain weights)	Statistically significant increase in males (10–20%)	Partially related to a higher body weight, not significant relative to body weight and with no associated clinical chemistry or pathological findings. Not an adverse effect of treatment.
Spleen weights (absolute and relative to brain weights)	Statistically significant increase in males (10–15%).	Low magnitude, no associated haematology or pathological findings. Not an adverse effect of treatment.

Appendix D – Animal dietary exposure

Table D.1: Dietary exposure to HPPD W336 and 2mEPSPS proteins (μg/kg bw per day) in livestock, based on the consumption of cotton meal

	Dietary exposure (μg/kg bw per day)	
	HPPD W336	**2mEPSPS**
Beef	45.1	271
Dairy	72.2	434
Ram/ewe	188	1,130
Lamb	160	958
Breeding	86.7	520
Finishing	56.3	338
Broiler	133	796
Layer	128	771
Turkey	268	1,610

bw: body weight.

Table D.2: Dietary exposure to HPPD W336 and 2mEPSPS proteins (μg/kg bw per day) in dairy cows based on the consumption of cotton seed

	Dietary exposure (μg/kg bw per day)	
	HPPD W336	**2mEPSPS**
Beef	111	667

bw: body weight.