Assessment of genetically modified soybean SYHT0H2 for food and feed uses, import and processing, under Regulation (EC) No 1829/2003 (application EFSA-GMO-DE- 2012-111)

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Assessment of genetically modified soybean SYHT0H2 for food and feed uses, import and processing, under Regulation (EC) No 1829/2003 (application EFSA-GMO-DE-2012-111)

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Abstract

The scope of application EFSA-GMO-DE-2012-111 is for food and feed uses, import and processing of genetically modified (GM) soybean SYHT0H2 in the European Union. Soybean SYHT0H2 was developed to confer tolerance to the herbicidal active substances mesotrione and other *p*-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides and glufosinate ammonium. The molecular characterisation data and bioinformatic analyses do not identify issues except for sequence similarity of AvHPPD-03 to bacterial haemolysins that was considered in food/feed safety assessment. The outcome of the comparative analysis (agronomic/phenotypic and compositional characteristics) did not need further assessment except for the changes in seed levels of α-tocopherol and γ-tocopherol that were assessed for food and feed relevance. The GMO Panel does not identify toxicological and allergenicity concerns for the AvHPPD-03 and PAT proteins expressed in soybean SYHT0H2 and finds no evidence that the genetic modification would change the overall allergenicity of soybean SYHT0H2. The nutritional impact of food/feed from soybean SYHT0H2 is expected to be the same as that of food/feed from the conventional counterpart and commercial non-GM soybean reference varieties. The GMO Panel concludes that soybean SYHT0H2 is as safe as and nutritionally equivalent to the conventional counterpart and the tested non-GM soybean reference varieties, and no post-market monitoring of food/feed is considered necessary. In the case of accidental release of viable soybean SYHT0H2 grains into the environment, soybean SYHT0H2 would not raise environmental safety concerns. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of soybean SYHT0H2. In conclusion, the GMO Panel considers that soybean SYHT0H2, as described in this application, 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.

Keywords: GMO, soybean (*Glycine max*), SYHT0H2, Regulation (EC) No 1829/2003, AvHPPD-03, PAT

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Summary

In the present scientific opinion, the scientific Panel on Genetically Modified Organisms of the European Food Safety Authority (hereafter referred to as the 'GMO Panel') reports the outcome of its risk assessment of soybean SYHT0H2 in the context of its scope as defined in application EFSA-GMO-DE-2012-111. The GMO Panel conducted the assessment of soybean SYHT0H2 in line with the principles described in Regulation (EC) No 1829/2003 and its applicable guidelines for the risk assessment of genetically modified (GM) plants.

The molecular characterisation data establish that soybean SYHT0H2 contains a single insert consisting one copy of the *avhppd-03* gene and four copies of the *pat* gene. Bioinformatic analyses of the sequences encoding the newly expressed proteins and other open reading frames (ORFs) present within the insert or spanning the junctions between the insert and genomic DNA indicate a ~ 30% sequence identity of AvHPPD-03 to some proteins of bacterial origin annotated as possible haemolysins. This finding was considered for its food and feed safety relevance and found not to raise concerns. In addition, an eight amino acid exact match between an ORF and a putative serine carboxylpeptidase from *Triticum aestivum* was identified. This ORF is found within the transcriptional unit of the AvHPPD-03 coding sequence but in a reverse orientation and does not contain any in-frame translational start codons (ATG). In conclusion, these analyses indicate that the expression of an ORF showing significant similarities to toxins or allergens is highly unlikely. The stability of the inserted DNA and introduced trait is confirmed over several generations. The methodology used to quantify the levels of the AvHPPD-03 and PAT proteins is considered adequate. The protein characterisation data comparing the structural, biochemical and functional properties of plant- and microbe-produced AvHPPD-03 and PAT proteins indicate that these proteins are equivalent, and the microbe-produced protein can be used in safety studies.

None of the agronomic/phenotypic and compositional differences identified between soybean SYHT0H2 and the conventional counterpart needed further assessment except for seed levels of α-tocopherol and γ-tocopherol. These differences were further assessed for their safety and nutritional relevance and found not to raise concerns.

The GMO Panel does not identify toxicological and allergenicity concerns regarding the AvHPPD-03 and PAT proteins expressed in soybean SYHT0H2 and finds no evidence that the genetic modification would change the overall allergenicity of soybean SYHT0H2. The nutritional impact of food/feed from soybean SYHT0H2 is expected to be the same as that of food/feed from the conventional counterpart and the tested commercial non-GM soybean reference varieties. The GMO Panel concludes that soybean SYHT0H2 is as safe as and nutritionally equivalent to the conventional counterpart and the tested non-GM soybean reference varieties, and no post-market monitoring of food/feed is considered necessary.

Considering the introduced traits, the outcome of the comparative analysis, and the routes and levels of exposure, the GMO Panel concludes that soybean SYHT0H2 would not raise safety concerns in the case of accidental release of viable GM soybean grains into the environment. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of soybean SYHT0H2.

In delivering its scientific opinion, the GMO Panel took into account application EFSA-GMO-DE-2012-111, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications. The GMO Panel concludes that soybean SYHT0H2, as described in this application, 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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1. Introduction

The scope of application EFSA-GMO-DE-2012-111 is for food and feed uses, import and processing of the genetically modified (GM) herbicide-tolerant soybean SYHT0H2 in the European Union (EU).

1.1. Background

On 8 August 2012, the European Food Safety Authority (EFSA) received from the Competent Authority of Germany application EFSA-GMO-DE-2012-111 for authorisation of soybean SYHT0H2 (Unique Identifier SYN-ØØØH2-5), submitted by Syngenta (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003[1].

Following receipt of application EFSA-GMO-DE-2012-111, EFSA informed EU Member States and the European Commission, and made the application available to them. Simultaneously, EFSA published the summary of the application on the EFSA website.[2]

EFSA checked the application for compliance with the relevant requirements of Regulation (EC) No 1829/2003, EFSA guidance documents and, when needed, asked the applicant to supplement the initial application. On 9 January 2013, EFSA declared the application valid and made the valid application available to MS and the European Commission.

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 6 months to issue a scientific opinion on application EFSA-GMO-DE-2012-111. Such time limit was extended whenever EFSA and/or its 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-DE-2012-111 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 SYHT0H2 in the context of its scope as defined in application EFSA-GMO-DE-2012-111.

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 including the opinions of the nominated risk assessment bodies of EU Member States.[4] In addition to the present scientific opinion, EFSA was also asked to report on the particulars listed under Articles 6(5) and 18(5) of Regulation (EC) No 1829/2003.

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 SYHT0H2 on the valid application EFSA-GMO-DE-2012-111, additional information provided by the applicant during the risk assessment, relevant scientific comments submitted by EU 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: http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2012-00753
[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] Opinions of the nominated risk assessment bodies of EU Member States can be found at the EFSA Register of Questions (http://registerofquestions.efsa.europa.eu/roqFrontend/login), querying the assigned Question Number
[5] http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2012-00753

2.2. Methodologies

The GMO Panel conducted its assessment in line with the principles described in Regulation (EC) No 1829/2003, its applicable guidelines (i.e. EFSA GMO Panel, 2010a,b,c, 2011a,b) and explanatory notes and statements (i.e. EFSA, 2017, 2019) for the risk assessment of GM plants.

In the frame of the contracts OC/EFSA/GMO/2013/01 and CFT-EFSA-AMU-2011-06 (specific call SC/EFSA/GMO/2012/01), 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. Molecular characterisation[6]

3.1.1. Transformation process and vector constructs

Soybean SYHT0H2 was developed by *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*)-mediated transformation. Immature seeds of soybean (*Glycine max* (L.) Merr.) variety 'Jack' were co-cultivated with the *A. tumefaciens* strain EHA101 containing the binary transformation vector pSYN15954.

The pSYN15954 vector contains three expression cassettes between the left and right borders of the transfer DNA (T-DNA): *avhppd-03*, the *pat-03-01* and *pat-03-02*.

The *avhppd-03* cassette contains the *Figwort Mosaic Virus* (FMV), the *Cauliflower mosaic virus* (CaMV) 35S transcriptional enhancer sequences and a synthetic minimal plant (SMP) promoter, the *Tobacco mosaic virus* (TMV) translational enhancer sequences, the *avhppd-03* gene originated from oat, conferring herbicide tolerance, and the terminator sequence from the nopaline synthase (*nos*) gene of *A. tumefaciens*.

The *pat-03-01* cassette contains the CaMV 35S promoter, the codon-optimised *pat-03-01* gene from *Streptomyces viridochromogenes* strain Tü494 and the *nos* terminator.

The *pat-03-02* cassette contains the *Cestrum yellow leaf curling virus* (CMP) promoter and the TMV translational enhancer sequence, the codon-optimised *pat-03-02* gene from *S. viridochromogenes* strain Tü494 and the *nos* terminator sequence. Both *pat-03-01* and *pat-03-02* genes encode for the same PAT protein.

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

3.1.2. Transgene constructs in the GM plant

Molecular characterisation of soybean SYHT0H2 was performed by Southern analysis, polymerase chain reaction (PCR), and DNA sequence analysis, in order to determine copy number, size and organisation of the inserted sequences and to confirm the absence of plasmid backbone sequences. The approach used was acceptable both in terms of coverage and sensitivity.

Southern analyses indicated that soybean SYHT0H2 contains a single insert consisting of two inverted partial copies of the T-DNA. The insert and copy number were confirmed by multiple restriction enzyme/probe combinations covering the T-DNA region and flanking regions. PCR analyses confirmed the results obtained by the Southern analyses. The absence of vector backbone sequences was demonstrated by Southern analysis using backbone-specific overlapping probes.

The nucleotide sequence of the entire insert of soybean SYHT0H2 together with 1,000 bp of both 5′ and 3′ flanking regions were determined. Sequence analysis indicated that the soybean SYHT0H2 insert contains one copy of the *avhppd-03* gene and four copies of the *pat* gene. Within the insert, two additional insertions of 44 bp (with identity to *avhppd-03*) and 17 bp respectively were found. The results indicated that 15 bp of the soybean genome 5′ to the SYHT0H2 insert were deleted as a result of the transformation and 7 bp were inserted 3′ to the insert. The possible interruption of known soybean genes by the SYHT0H2 insert was evaluated by bioinformatic analyses of the pre-insertion locus and of the genomic sequences flanking the insert. The results did not reveal the interruption of known endogenous genes in soybean SYHT0H2.

The results of segregation (see Section 3.1.5) and bioinformatic analyses established that the insert is located in the nuclear genome.

[6] Dossier: Part II – Section A.2; additional information: 17/7/2015, 1/9/16, 3/10/16, 31/5/2017, 5/4/2018 and 28/8/2019.

Updated bioinformatic analyses of the amino acid sequences of the two newly expressed proteins (AvHPPD-03 and PAT) revealed no significant similarities to known allergens. Sequence similarity search of the newly expressed proteins against known toxins indicated a ~ 30% sequence identity of AvHPPD-03 to some proteins of bacterial origin (e.g. from *Vibrio vulnificus* and *Legionella pneumophila*), which are annotated as possible haemolysins. The relevance for the safety assessment of these findings is further discussed in Section 3.3.3.1. 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 indicated the presence of an eight amino acid exact match between an ORF and a putative serine carboxylpeptidase from *Triticum aestivum.* This ORF is found within the transcriptional unit of the AvHPPD-03 coding sequence but in a reverse orientation and does not contain any in-frame translational start codons (ATG). In conclusion, these analyses indicate that the expression of an ORF showing significant similarities to toxins or allergens is highly unlikely, with the exception of the AvHPPD-03 protein (see above).

In order to assess the possibility for horizontal gene transfer by homologous recombination (HR), the applicant performed a sequence identity analysis of the inserted regions of bacterial origin in soybean SYHT0H2. The likelihood and potential consequences of the plant-to-bacteria gene transfer are described in Section 3.4.1.2.

3.1.3. Protein characterisation and equivalence

Soybean SYHT0H2 expresses two new proteins, AvHPPD-03 and PAT. 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 soybean- and *E. coli*-derived AvHPPD-03 and PAT proteins. Purified proteins from these two sources were characterised and compared in terms of their physicochemical, structural and functional properties.

3.1.3.1. AvHPPD-03 characterisation and equivalence

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and western blot analysis show that plant- and microbe-produced AvHPPD-03 proteins have the expected molecular weight of ~ 47 kDa and are comparably immunoreactive to AvHPPD-03 protein-specific antibodies. Glycosylation detection analysis demonstrates that none of the AvHPPD-03 proteins are glycosylated. Amino acid sequence analysis by mass spectrometry and N-terminal sequencing methods show that both proteins match the deduced sequence as defined by the *avhppd-03* gene. These data also show that the first four amino acids of the plant-derived protein were truncated. Functional equivalence is demonstrated by a biochemical *in vitro* activity assay which shows that both proteins have comparable activity for the intended substrate.

3.1.3.2. PAT characterisation and equivalence

SDS–PAGE and western blot analysis show that soybean- and microbe-produced PAT proteins have the expected molecular weight of ~ 20.5 kDa and are comparably immunoreactive to PAT protein specific antibodies. Glycosylation detection analysis demonstrated that none of these two PAT proteins are glycosylated. Amino acid sequence analysis by mass spectrometry and N-terminal sequencing methods show that both proteins match the deduced sequence as defined by the *pat* gene. These data also show that the N-terminal methionine of the plant-derived PAT was truncated. Such a modification is common in eukaryotic proteins (e.g. Moerschell et al., 1990; Polevoda and Sherman, 2000). Functional equivalence is demonstrated by a biochemical *in vitro* activity assay which shows that both proteins have comparable activity for the intended herbicide.

The protein characterisation data comparing the structural, biochemical and functional properties of plant- and microbe-produced AvHPPD-03 and PAT proteins, indicate that these proteins are equivalent. Therefore, the GMO Panel accepts the use of the AvHPPD-03 and PAT proteins expressed in bacteria for the safety studies.

3.1.4. Information on the expression of the insert

Protein levels of the AvHPPD-03 and PAT were analysed by enzyme-linked immunosorbent assay (ELISA) in material harvested from a field trials across four locations in Argentina during the 2011/2012 growing season. Samples analysed included leaves (V4, V8, V10, R6), root (V8, R6), forage (R6) and seeds (R8), both those treated and not treated with mesotrione and glufosinate ammonium. The mean

values and ranges of protein expression levels in seeds and forage (n = 20) of the AvHPPD-03 and PAT proteins are summarised in Table 1. High variability in protein expression data is observed for all newly expressed proteins in all tissues. This variability is taken into account for the dietary exposure estimations which is discussed in Section 3.3.3.1.

Table 1: Mean values (n = 20) and ranges of newly expressed protein in seeds [μg/g dry weight (DW) and μg/g fresh weight (FW)] and forage (μg/g DW) from soybean

	Mesotrione and glufosinate ammonium treatment	
	Not treated	**Treated**
Seeds (R8)		
AvHPPD-03	8.18/7.16 (ranges DW: 0.62–28.30; FW: 0.55–24.94)	7.91/6.96 (ranges DW: 0.39–28.36; FW: 0.34–24.84)
PAT	2.70/2.36 (ranges DW: 0.07–14.85; FW: 0.06–13.13)	3.89/ 3.42 (ranges DW: < LQQ–16.30; FW: < LOQ–14.27)
Forage (R6)		
AvHPPD-03	79.66 (range DW: 16.76–164.01)	92.99 (range DW: 34.73–171.79)
PAT	19.17 (range DW: 1.12–60.91)	29.73 (range DW: 1.27–68.10)

HPPD: *p*-hydroxyphenylpyruvate dioxygenase; LOQ: limit of quantification; PAT: phosphinothricin acetyltransferase.

3.1.5. Inheritance and stability of inserted DNA

Genetic stability of the soybean SYHT0H2 insert was assessed by Southern analysis of genomic DNA from five consecutive generations and segregation analysis of both herbicide tolerance traits of soybean SYHT0H2. For the Southern analysis, 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. The results support the presence of a single insertion, segregating in a Mendelian fashion.

3.1.6. Conclusions on molecular characterisation

The molecular characterisation data establish that soybean SYHT0H2 contains a single insert consisting of one copy of the *avhppd-03* gene and four copies of the *pat* gene.

Bioinformatic analyses of the sequences encoding the newly expressed proteins and other ORFs present within the insert or spanning the junctions between the insert and genomic DNA indicate a ~ 30% sequence identity of AvHPPD-03 to some proteins of bacterial origin annotated as haemolysins. The relevance for the safety assessment of this finding is further discussed in Section 3.3.3.1. In addition, an eight amino acid exact match between an ORF and a putative serine carboxylpeptidase from *Triticum aestivum* was identified. This ORF is found within the transcriptional unit of the AvHPPD-03 coding sequence but in a reverse orientation and does not contain any in-frame translational start codons (ATG). In conclusion, these analyses indicate that the expression of an ORF showing significant similarities to toxins or allergens is highly unlikely, with the exception of the AvHPPD-03 protein, which shows similarity to bacterial haemolysins. The stability of the inserted DNA and of the introduced herbicide tolerance traits is confirmed over several generations. The methodology used to quantify the levels of the AvHPPD-03 and PAT proteins is considered adequate. The protein characterisation data comparing the structural, biochemical and functional properties of the plant- and microbe-produced AvHPPD-03 and PAT proteins indicate that these proteins are equivalent and the microbe-produced proteins can be used in safety studies.

3.2. Comparative analysis[7]

3.2.1. Choice of comparator and production of material for the comparative analysis

Application EFSA-GMO-DE-2012-111 presents data on agronomic/phenotypic characteristics as well as on forage and seed composition of soybean SYHT0H2. In addition, the application contains data on characteristics of seed from soybean SYHT0H2 (Table 2).

[7] Dossier: Part II – Section A.3; additional information: 14/10/2013.

Table 2: Main comparative analysis studies to characterise soybean SYHT0H2 in application EFSA-GMO-DE-2012-111

Study focus	Study details	Comparator	Commercial non-GM reference varieties
Agronomic and phenotypic analysis	Field study, USA, 2010, eight sites[(a)]	Jack	Six[(b)]
Compositional analysis			
Seed germination	F_1 seeds tested under controlled conditions		Three[(c)]

GM: genetically modified.
(a): The field sites were in Richland (IA), York (NE), Fisk (MO), Stewardson (IL), Mebane (NC), Hamburg (PA), Carlyle (IL) and Rockville (IN).
(b): The reference varieties used in the field trials were 03JR313108, S23-T5, 03RM89303, NE0800097, WN0800099 and 06RM934408.
(c): The reference varieties used in the seed germination study were 03JR313108, S23-T5 and 03RM89303.

The field trials were conducted in typical soybean growing areas of the USA, representing regions of diverse agronomic practices and environmental conditions. At each site, the following materials were grown in a randomised complete block design with four replicates: soybean SYHT0H2 not treated with the intended herbicides (not treated), soybean SYHT0H2 treated (sprayed) with the intended herbicides mesotrione and glufosinate ammonium (treated), a non-GM comparator and six commercial non-GM soybean reference varieties. All materials were treated with required maintenance pesticides (including conventional herbicides) according to local requirements.

The comparator used in the comparative analysis studies was the non-GM soybean variety (Jack) that was also transformed to establish the event SYHT0H2. As documented by the pedigree, Jack has the same genetic background as soybean SYHT0H2: hence, the GMO Panel considered that it is the appropriate conventional counterpart.

3.2.1.1. Statistical analysis of the field trials data

The agronomic, phenotypic and compositional data were analysed as specified by the EFSA GMO Panel (2010b, 2011a). This includes, for each of the two treatments of the GM soybean, the application of a difference test (between the GM soybean and the conventional counterpart) and an equivalence test (between the GM soybean 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]

3.2.2. Agronomic and phenotypic analysis

3.2.2.1. Agronomic and phenotypic characteristics tested under field conditions

Twelve agronomic and phenotypic endpoints plus information on abiotic stressors, disease incidence and arthropod damage were evaluated in the field trials (Table 2). Six of these endpoints were not analysed with formal statistical methods because of lack of variability in the data.[9] The remaining six endpoints[10] were analysed with the tests of difference and equivalence, with the following results:

- For soybean SYHT0H2 (not treated), no statistically significant differences were identified with respect to the conventional counterpart.
- For soybean SYHT0H2 (treated), statistically significant differences were identified for early and final stand count, both of which fell under equivalence category I.

The mean values of the endpoints that were not statistically analysed fell within the range of the non-GM reference varieties.

[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] Seedling vigour, lodging, days to 50% flowering, flower colour, days to maturity and pod shattering.
[10] Early stand count, plant height, seed test weight, seed moisture, seed yield and final stand count.

3.2.2.2. Agronomic and phenotypic characteristics tested under controlled conditions

The applicant reported data on seed characteristics of soybean SYHT0H2. Seeds were incubated at six temperature regimes. The endpoints analysed were the numbers of normal germinated seeds, abnormal germinated seeds, hard seeds, dead seeds, and firm swollen seeds. For all endpoints and temperature regimes, there were no statistically significant differences between soybean SYHT0H2 and its conventional counterpart.

Although the applicant refers to seed dormancy when discussing seed characteristics, specific data on induced seed dormancy were not supplied. Therefore, the GMO Panel considers that only the conclusions on seed germination are supported by the data available.

3.2.3. Compositional analysis

Soybean forage and seeds harvested from the field trials in the USA in 2010 were analysed for 66 different constituents (seven in forage and 59 in seeds[11]), including the key constituents recommended by the OECD (OECD, 2001a). Six seed constituents with more than 50% of the observations below the limit of quantification were excluded from the statistical analysis.[12]

The statistical analysis (Section 3.2.1.1) was applied to the remaining 60 constituents (seven in forage[13] and 53 in seeds[14]). A summary of the outcome of the test of difference and the test of equivalence is presented in Table 3.

- For soybean SYHT0H2 (not treated), the test of difference identified statistically significant differences with the conventional counterpart for 32 endpoints (one in forage and 31 in seeds). All these endpoints fell under equivalence category I/II, except for the levels of α-tocopherol and γ-tocopherol that fell under equivalence category III/IV.
- For soybean SYHT0H2 (treated), the test of difference identified statistically significant differences with the conventional counterpart for 27 constituents (two in forage and 25 in seeds). All these endpoints fell under equivalence category I/II, except for the levels of γ-tocopherol that fell under equivalence category III.

Table 3: Outcome of the comparative compositional analysis in forage and seeds of soybean SYHT0H2. 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	26	30[(d)]	31	26[(d)]
	Category III/IV	2[(e)]	2[(f)]	2[(e)]	1[(f)]
	Total endpoints	60		60	

(a): Comparison between soybean SYHT0H2 and the 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).
(c): Not treated/treated with the intended herbicides mesotrione and glufosinate ammonium.
(d): Endpoints with significant differences between soybean SYHT0H2 and the conventional counterpart and falling in equivalence category I-II. In forage, not treated only: ash. Treated only: fat and moisture. In seed, not treated only: ADF, NDF, calcium, magnesium, glycitein, serine, threonine, linolenic acid (C18:3) and folic acid. Treated only: methionine, valine, phytonadione and γ-tocopherol. Both treated and not treated: alanine, arginine, aspartic acid, glutamic acid, glycine,

[11] In addition to α-tocopherol, recommended by the OECD, the applicant analysed β-tocopherol, γ-tocopherol and δ-tocopherol and four tocotrienols (α-tocotrienol, β-tocotrienol, γ-tocotrienol and δ-tocotrienol). These compounds were included in the analysis because soybean SYHT0H2 expresses AvHPPD-03, an enzyme involved in the degradation of tyrosine and the biosynthesis of tocopherols.
[12] These were β-carotene, β-tocopherol, α-tocotrienol, β-tocotrienol, γ-tocotrienol and δ-tocotrienol.
[13] Ash, carbohydrates, fat, moisture, protein, acid detergent fibre (ADF) and neutral detergent fibre (NDF).
[14] Ash, carbohydrates, fat, moisture, protein, acid detergent fibre (ADF), neutral detergent fibre (NDF), alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, calcium, iron, magnesium, phosphorus, potassium, palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C21:0), behenic acid (C22:0), folic acid, phytonadione, riboflavin, thiamine, α-tocopherol, γ-tocopherol, δ-tocopherol, daidzein, genistein, glycitein, lectin, phytic acid, raffinose, stachyose and trypsin inhibitor.

histidine, leucine, lysine, phenylalanine, proline, tyrosine, iron, potassium, palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), arachidic acid (C20:0), behenic acid (C22:0) and δ-tocopherol.

(e): Endpoints falling in equivalence category III-IV and with no significant differences between soybean SYHT0H2 and the conventional counterpart. In seed, both treated and not treated: daidzein and stachyose.

(f): Endpoints with significant differences between soybean SYHT0H2 and the conventional counterpart and falling in equivalence category III-IV. Quantitative results for these endpoints are reported in Table 4.

The GMO Panel assessed all the significant differences between soybean SYHT0H2 and the conventional counterpart, taking into account the potential impact on plant metabolism and the natural variability observed for the set of non-GM reference varieties. Quantitative results for the endpoint showing significant differences between soybean SYHT0H2 and the conventional counterpart and falling under equivalence category III/IV are given in Table 4.

Table 4: Quantitative results (estimated means and equivalence limits) for endpoints with significant differences between soybean SYHT0H2 and the conventional counterpart and falling under equivalence category III/IV (see Table 3)

	Endpoint	Soybean SYHT0H2		Conventional counterpart	Non-GM reference varieties	
		Not treated[(a)]	Treated[(a)]		Mean	Equivalence limits
Seed	α-tocopherol (mg/g DM)	0.0206*	0.0212*	0.0229	0.0262	0.0221–0.0312
	γ-tocopherol (mg/g DM)	0.225*	0.213*	0.199	0.174	0.141–0.214

For the GM soybean, significantly different values are marked with an asterisk, while the outcomes of the test of equivalence are differentiated by grey scale backgrounds. Light and dark grey backgrounds correspond to equivalence category III and IV, respectively. A white background is used for equivalence category I/II.

Means and equivalence limits were calculated on a log-transformed scale; the values shown in the table are back-transformed to the original scale.

DM: dry matter.

(a): Not treated: treated only with conventional herbicides. Treated: treated with the intended herbicides (mesotrione and glufosinate ammonium).

Together with the increase in the levels of γ-tocopherol in soybean SYHT0H2 (equivalence category III), a statistically significant increase of δ-tocopherol as compared to the conventional counterpart was also observed in both treated and not treated plants, although mean values fell within the equivalence limits of the non-GM reference varieties. This increase in the levels of γ-tocopherol and δ-tocopherol are expected due to the integration in soybean SYHT0H2 of the *avhppd-03* gene originated from oat. The *avhppd-03* gene encodes a HPPD enzyme that catalyses the formation of homogentisic acid, the aromatic precursor in plastoquinone and tocopherol biosynthesis (Kramer et al., 2014). The statistically significant increase in the levels of γ-tocopherol and δ-tocopherol without a corresponding increase in the α- and β-isoforms is consistent with published literature reporting tocopherol methyltransferase as a possible rate-limiting enzyme in the tocopherol biosynthetic pathway (Shintani and DellaPenna, 1998; Collakova and DellaPenna, 2003).

3.2.4. Conclusion on 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 in agronomic and phenotypic characteristics between soybean SYHT0H2 and the conventional counterpart needs further assessment for potential environmental impact.
- None of the compositional differences between soybean SYHT0H2 and the conventional counterpart needs further assessment for food/feed safety except for seed levels of α-tocopherol and γ-tocopherol, which are discussed in Section 3.3.

3.3. Food/feed safety assessment[15]

3.3.1. Effects of processing

Soybean SYHT0H2 will undergo existing production processes used for conventional soybean. Based on this, the characteristic of the intended trait and the outcome of the comparative assessment, processing of soybean SYHT0H2 is not expected to result in food and feed products being different from those of conventional non-GM soybean varieties.

3.3.2. Influence of temperature and pH on newly expressed proteins

The applicant provided information on the effects of temperature on the immunoreactivity and enzymatic activity of the AvHPPD-03 protein. At temperatures of 37°C for 30 min, the AvHPPD-03 protein lost ~ 25% of its immunoreactivity. Whereas, at temperatures of 65°C for 30 min, the protein immunoreactivity lost was of ~ 97%. In line with this outcome, enzymatic activity studies showed that at temperatures of 65°C for 30 min, the AvHPPD-03 activity was below the limit of detection. In relation to the effect of pH on the AvHPPD-03 protein, the molecular mass (~ 47 kDa) and immunoreactivity of the protein was unchanged at pH 1.2 and 7.5. Effects of temperature and pH on the PAT protein have been previously evaluated by the GMO Panel in the context of other applications (e.g. EFSA GMO Panel, 2011c).

3.3.3. Toxicology

3.3.3.1. Testing of the newly expressed proteins

The two proteins AvHPPD-03 and PAT newly expressed in soybean SYHT0H2 have been extensively characterised (Section 3.1.3).

The **AvHPPD-03 protein** is a *p*-hydroxyphenylpyruvate dioxygenase (HPPD) enzyme. HPPD proteins are members of a sub class of Fe-dependent, non-heme oxygenases and are present in almost all aerobic organisms, where they play a key role in the degradation of aromatic compounds (Fritze et al., 2004). HPPDs show a similar overall structural fold and domain organisation with a highly conserved active site architecture (Moran, 2005).

The GMO Panel assessed the toxicological profile of AvHPPD-03 protein taking into account the source of the gene (oat) and the history of safe consumption of native HPPD proteins from oat and other plant species commonly consumed as food and feed. In the assessment, following a weight of evidence approach, the GMO Panel considered dietary exposure estimations for AvHPPD-03 protein and the native HPPD proteins, bioinformatic search for homology of AvHPPD-03 to known toxins, available toxicological studies, and information from *in vitro* digestibility studies.

Source of the gene and homology analysis to other HPPD proteins

The *avhppd-03* gene encoding for the AvHPPD-03 protein newly expressed in soybean SYHT0H2 is originated from oat (*Avena sativa*). The AvHPPD-03 protein differs by one amino acid from the native oat HPPD. Taking into account the amino acid sequence and *in silico* structural analysis performed by the applicant on the AvHPPD-03 protein as well as available literature (e.g. Fritze et al., 2004), this change is not considered critical for AvHPPD-03 folding and function. Therefore, the AvHPPD-03 protein expressed in soybean SYHT0H2 can be considered structurally and functionally similar to the native oat HPPD.

HPPD proteins are widely expressed in plants, where they are involved in the synthesis of plastoquinones (required for carotenoid biosynthesis), and tocopherols (Fritze et al., 2004). Detailed sequence homology analysis showed sequence identity between AvHPPD-03 and HPPDs in plants that are sources of staple foods and other plants that are also highly consumed as food and feed (~ 80% identity in monocotyledons such as wheat, barley, rice, maize; ~ 60% in soybean, tomato, potato, carrot, banana).[16]

[15] Dossier: Part II – Sections A.4, A.5, A.6, B, C; additional information: 12/9/2014, 5/4/2015, 3/6/2015, 3/10/2016, 23/12/2016, 1/12/2017, 3/4/2018, 3/9/2018, 2/5/2019, 16/5/2019, 2/8/2019, 30/9/2019 and 21/10/2019.

[16] Top 100 alignment reported by the applicant (UniprotKB).

Dietary exposure to HPPD proteins (AvHPPD-03 and native HPPD proteins)

Upon the GMO Panel's request, the applicant compared the dietary exposure to AvHPPD-03 protein through the consumption of food and feed from soybean SYHT0H2 with the dietary exposure to native HPPD proteins present in food and feed from selected conventional crops.

The levels of AvHPPD-03 protein in soybean SYHT0H2 were derived from replicated field trials across four locations in Argentina during the 2011/2012 growing season (Table 1, Section 3.1.4). Based on sequence homology analysis, six different conventional crops were selected by the applicant: soybean, wheat, barley, maize, rice and oat (sequence identity ranging between 61% and > 99.7%). Expression levels of native HPPD proteins in seeds and forage from these crops and used in the dietary exposure assessment are shown in Table 5.

Table 5: Summary statistics of native HPPD proteins analysed in seeds and forages (μg/g fresh weight (FW)) from selected conventional crops

Concentration[(a)] of HPPD protein (μg/g FW)			
	Seed		Forage
	Mean	Average of the samples above the third quartile	Mean
Barley	1.21	1.72	0.66
Maize	0.45	0.64	0.29
Oat	1.25	1.49	0.52
Rice	0.60	0.74	0.17[(b)]
Wheat	0.98	1.30	0.63
Soybean	1.57	2.04	0.23

HPPD: *p*-hydroxyphenylpyruvate dioxygenase.

(a): Concentration of HPPD proteins was estimated by liquid chromatography–tandem mass spectrometry (LC–MS/MS) in ten different varieties for each crop sourced from the United States Department of Agriculture (USDA); each of the varieties were analysed in duplicate.

(b): No measurements available; assumed to be equal to the lowest cereal straw concentration.

Human dietary exposure

Concentration data

When expression levels are analysed in raw primary commodities commonly consumed as processed blended commodities, mean concentrations are most adequate to estimate not only chronic but also acute dietary exposure. However, when relatively high variability is observed in the expression levels as reported for AvHPPD-03 protein in soybean SYHT0H2 (Section 3.1.4), it is adequate, for acute dietary exposure estimations, the use of a concentration value that represents a hypothetical worst-case scenario where processed foods are produced from GM crops with high levels of AvHPPD-03 protein (EFSA, 2019). Therefore, together with the mean value (6.96 μg/g FW) to be used for estimating chronic dietary exposure (Table 1, Section 3.1.4), the average of the expression levels[17] above the third quartile (Q3) of the distribution (17.83 μg/g FW) was used to estimate acute dietary exposure to AvHPPD-03 protein. The same approach was used to estimate chronic and acute dietary exposure to native HPPDs, although the variability in the expression levels was much lower than that observed for AvHPPD-03.

Consumption data

Summary statistics from the EFSA Comprehensive European Food Consumption Database (EFSA consumption database)[18,19] were used as a source of consumption data when estimating dietary exposure to AvHPPD-03 and HPPD proteins. Since no specific consumption data are available for commodities containing, consisting of or obtained from soybean SYHT0H2, a conservative scenario

[17] In dietary exposure assessment, different statistical estimations are used to represent high concentration when estimating worst-case scenarios (typically 95th/97.5th percentiles). When the number of samples is lower than 60, these percentiles are not considered statistically robust (EFSA,2011), and the average of the samples above the third quartile (Q3) can be used as an alternative.

[18] http://www.efsa.europa.eu/en/data/food-consumption-data

[19] Accessed on 20 December 2018 (.xlsx format).

with 100% replacement of conventional soybean by the GM soybean SYHT0H2 was considered. Food commodities where proteins are expected to be absent or in negligible amounts were excluded from the assessment (e.g. soybean oil).

Different processed foods (soya milk, tofu, protein concentrate and protein isolates) produced from SYHT0H2 soybean were analysed by the applicant for their content in AvHPPD-03 protein. Overall, there was a significant decrease of the levels quantified for AvHPPD-03 protein when compared to those reported in the seeds. Most probably processing conditions cause the denaturation of the proteins hampering their detection by ELISA even if the proteins might still be present in the processed foods. Following a conservative approach, it was assumed that no losses of AvHPPD-03 and HPPD proteins occur during processing, therefore these processed samples were not considered during the assessment. Before estimating dietary exposure, the levels of AvHPPD-03 and HPPD proteins in the consumed processed commodities were derived using diverse recipes and factors to estimate the amount of raw primary commodities present in each food.[20]

Methodology

Acute and chronic dietary exposure estimates for high consumers were provided for different European countries. Dietary exposure estimations were based on the methodology described for the use of the summary statistics of the EFSA consumption database (EFSA, 2015, 2019). The assumption taken is that an individual could be a high consumer of up to two food commodities and an average consumer of the remaining food commodities containing HPPD/AvHPPD-03 proteins. Briefly, for acute and chronic dietary exposure, the two commodities leading to the highest exposure were assumed to be highly consumed (95th percentile consumption, consumers only) and to contain high and average levels, respectively, of HPPD/AvHPPD-03 proteins, while the remaining commodities (average consumption, whole population) would contain average levels of these proteins.

Dietary exposure estimates

Overall, for both AvHPPD-03 and HPPD proteins, dietary exposure estimates in the adult population (adults, elderly and very elderly) were lower than those in the young population (infants, toddlers, other children and adolescents) as reflected by the median estimates (Table 6). Although the range of dietary exposure estimations across dietary surveys and populations was larger for AvHPPD-03 protein as compared to the estimations for HPPD proteins, the median estimates were rather similar for HPPD proteins from conventional crops and for AvHPPD-03 protein, or even higher for HPPD proteins in the case of chronic exposure estimations (Table 6).

Table 6: Median and maximum dietary exposure estimations [μg/kg bw per day] across European dietary surveys in adults and children for AvHPPD-03 and HPPD proteins from conventional crops

	Adults[a]				Children[b]			
	Acute		Chronic		Acute		Chronic	
	Median	Maximum	Median	Maximum	Median	Maximum	Median	Maximum
HPPD[c]	12.9	45.2	6.4	31.9	26.2	106	12.3	43.6
AvHPPD-03	15.4	283[d]	3.2	120[d]	29.7	245[e]	6.2	41.1

bw: body weight; HPPD: *p*-hydroxyphenylpyruvate dioxygenase.
(a): Adults: adults, elderly and very elderly.
(b): Children: infants, toddlers, other children and adolescents.
(c): Combined dietary exposure estimates for native HPPD proteins present in conventional foods from oat, barley, rice, maize, soybean and wheat.
(d): The highest exposure estimates for AvHPPD-03 protein in adults (i.e. 283 μg/kg bw per day and 120 μg/kg bw per day) derive from the acute/chronic consumption of protein-based supplements.
(e): The acute exposure estimate of 245 μg/kg bw per day refers to one dietary survey with just one subject reporting the consumption of 'Follow-on formula, soya-based, powder'.

Box-plots were used to show the distribution of the dietary exposure estimations to AvHPPD-03 and HPPD proteins (acute and chronic) across young and adult population (Appendix A). In the young population, the acute and chronic dietary exposure estimates to AvHPPD-03 protein are, overall, in the

[20] Example: 100 g of corn bread are made with approximately 75 g of corn flour that are produced from approximately 90 g of corn grains.

same range as those for HPPD proteins in conventional crops. In the adult population, chronic dietary exposures estimates are also largely in the same range for AvHPPD-03 and HPPD proteins with just few exceptions resulting from the consumption of protein-based supplements, that are generically reported as 'Protein and protein components for sports people' or 'Protein and amino acids supplements' without further details. The reported consumption of protein-based supplements also leads to relatively high acute dietary exposure estimates. This is particularly evident for AvHPPD-03 protein due to the high initial concentration value used (17.83 μg/g FW in seeds) derived from the average of the expression levels above the Q3 of the distribution. In fact, all exposure estimates for AvHPPD-03 protein above 100 μg/kg body weight (bw) are related to dietary surveys where the top contributing commodity are protein-based supplements (Appendix A). Detailed uncertainties linked to these exposure estimates are further considered below.

When comparing the dietary exposure to AvHPPD-03 protein to that to the native HPPD proteins, all the assumptions taken, their associated uncertainties and the limitations of the available data need to be considered. The high variability in the protein expression data and the relatively high expression levels reported for AvHPPD-03 in seeds (~ 75 fold; range: 0.34–24.84 μg/g FW, n = 20) had a considerable impact on the dietary exposure estimations. The upper part of the range contains expression levels for AvHPPD-03 protein that are considerably higher than those reported for HPPD proteins analysed in the seeds selected from conventional crops. This is particularly impacting the estimations of acute dietary exposure where to cover a worst-case scenario the average of the expression levels above the Q3 of the distribution was used. Regarding consumption, the lack of specific consumption data on GM-food obliged to assume that the consumption of all soybean related foods refers to soybean SYHT0H2 when estimating dietary exposure to AvHPPD-03 protein (100% replacement scenario). On the other hand, accepting the representativeness of the varieties analysed in the different conventional crops for the presence of HPPDs, the exposure estimates to the native HPPDs represents a realistic scenario against the hypothetical scenario for AvHPPD-03 protein (as supported by the knowledge of the current use of GM-soybean in human consumption in Europe). For the consumption of protein-based supplements, the assumption is that all of them are made of soybean protein and they all contain ~ 90% protein. This likely results in an overly overestimation of the dietary exposure to AvHPPD-03 protein considering that nowadays animal proteins (in particular whey proteins) are still the most commonly used protein supplements, and that protein supplements include a large array of products with diverse protein content (e.g. protein bars) and not only protein isolates with 90% protein content. Furthermore, the limited number of consumers of protein supplements in the EFSA consumption database adds uncertainty to the representativeness of the data. Together with the most likely overestimation of the dietary exposure to AvHPPD-03, there are also evidences that the current dietary exposure to native HPPD proteins is underestimated. Sequence homology data show that many other plants which are typically consumed (mainly their fruits) contain HPPD protein that shares high similarity with the AvHPPD-03 protein (e.g. potato, banana, chickpea, tomato, mango, pineapple, with sequence identity between 62% and 71%). The inclusion of these plants into the dietary exposure estimations to HPPD proteins would result in much higher exposure as compared to that obtained considering only oat, barley, rice, maize, soybean and wheat.

Therefore, it can be considered that consumers are exposed to AvHPPD-03 protein in soybean SYHT0H2 at similar extent as to native HPPD proteins naturally present in different crops.

Animal dietary exposure

Dietary exposure estimates across livestock animal species (i.e. beef cattle, dairy cattle, rams and ewes, lambs, breeding and finishing swine, broiler and layer hens, and turkeys) were conducted following the consumption of different feed materials which commonly enter the feed chain (e.g. seeds[21] and by-products, green or dried forages); estimates for animal body weight, daily feed intake and inclusion rates (percentage) of feed materials in animal diets are those referred to OECD Guidance Document on residues in livestock (OECD, 2013). A conservative scenario with 100% replacement of conventional soybean derived feed by the soybean SYHT0H2 feed materials was considered.

The applicant estimated dietary exposures using the EFSA (2017, 2019) dietary burden calculator[22]; mean and average of the samples above the Q3 concentrations of AvHPPD-03 and native HPPD proteins, respectively, in soybean SYHT0H2 and conventional crops, were used for seeds and green forages (Table 1, Section 3.1.4 and Table 5, Section 3.3.3.1). In the current assessment, the

[21] In this section, seed refers to grain and beans.
[22] Full description of the model was provided in the study report McClain (2019) #SSB-131-19 A2, Annex 1.

calculation relying on mean values was used by the GMO Panel. To calculate levels concentration in by-products and dried forages, correction factors were applied[23] based on the ratio between their crude protein content relative to content in respective seeds and forages, assuming that no losses of NEP occur during processing.

Estimates of dietary exposure to native HPPD proteins based on rations containing feed materials (seeds, by-products and forages) from aggregated crops were compared with estimates to AvHPPD-03 protein based on rations containing soybean SYHT0H2 seeds (soybean A), seeds and by-products (soybean B) or seeds, by-products and forages (soybean C). This comparison showed that ruminants (cattle and sheep) are similarly exposed to AvHPPD-03 and HPPD proteins, as detailed in Table 7.

Table 7: Comparison of exposure to native HPPD and AvHPPD-03 proteins (mean concentration scenario)

NEP	Crops	Dietary exposure (mg/kg per bw)			
		Cattle		Sheep	
		Beef	Dairy	Ram/Ewe	Lamb
	Body weight kg	500	650	75	40
	Feed intake kg dry matter/day	12	25	2.5	1.7
HPPD	Aggregate	0.091	0.168	0.204	0.263
AvHPPD-03	soybean A	0.019	0.030	0.026	0.066
	soybean B	0.068	0.128	0.111	0.175
	soybean C	0.068	0.128	0.111	0.175

bw: body weight; HPPD: *p*-hydroxyphenylpyruvate dioxygenase.
Aggregate: seed + by-products + forages from oat, maize, rice, barley, wheat or triticale and conventional soybean.
Soybean A: seed from soybean SYHT0H2.
Soybean B: seed + by-products from soybean SYHT0H2.
Soybean C: seed + by-products + forages from soybean SYHT0H2.

Following the same approach used for ruminants, exposure to native HPPD and AvHPPD-03 proteins in pigs and poultries was similar, with the exception of layers, where higher estimates to AvHPPD-03 were reported (1.027 mg AvHPPD-03/kg per bw; 0.201 mg HPPD/kg per bw).

Because the use of forages in diets of swine and poultry is uncommon, the GMO Panel estimated dietary exposures to native HPPD and AvHPPD-03 proteins based on rations containing feed materials (seeds, by-products) with exclusion of forages, respectively, from conventional crops (Aggregate*) and soybean SYHT0H2 (Soybean B). This comparison confirmed that pigs and poultry, including layers, are similarly exposed to AvHPPD-03 and HPPD proteins, as detailed in Table 8.

Table 8: Comparison of exposure to native HPPD and AvHPPD-03 proteins (mean concentration scenario)

NEP	Crops	Dietary exposure (mg/kg per bw)				
		Pigs		Poultry		
		Breeding	Finishing	Broiler	Layer	Turkey
	Body weight kg	260	100	1.7	1.9	7
	Feed intake kg dry matter/day	6	3	0.12	0.13	0.5
HPPD	Aggregate*	0.062	0.080	0.181	0.193	0.163
AvHPPD-03	Soybean B	0.088	0.139	0.399	0.255	0.412

bw: body weight; HPPD: *p*-hydroxyphenylpyruvate dioxygenase.
Aggregate*: seed + by-products from oat, maize, rice, barley, wheat or triticale and conventional soybean.
Soybean B: seed + by-products from soybean SYHT0H2.

[23] Correction factors: Barley: brewer's grain (2.19); silage (0.93); straw (0.35). Maize: milled by-pdts (0.89); hominy meal (1.06); gluten feed (2.13); gluten meal (6.38); stover (0.47). Oat: hay (0.87); straw (0.34). Rice: bran/pollard (2). Triticale: hay; straw (extrapolation from wheat). Wheat: gluten meal (6.79); milled by-pdts (1.3); distiller's grain dried (2.96); hay (0.49); straw (0.38). Soybean (conventional and SYHT0H2): meal (1.35); hulls (0.33); hay (1.01); silage (1.36).

The GMO Panel recognises that the model proposed by the applicant based on the use of the EFSA (2017, 2019) dietary burden calculator to compare dietary exposures to avHPPD-03 and HPPD proteins from soybean SYHT0H2 and conventional crops, is designed to identify the worst-case scenario in term of 'highest exposure'.

In addition, the original assumption of a conservative scenario with 100% replacement of conventional soybean derived feed by the soybean SYHT0H2 feed materials, likely results in an overly overestimation of the dietary exposure to avHPPD-03 protein, compared to exposure estimates to the native HPPDs, based on a diversified and more realistic scenario, accepting the representativeness of the varieties analysed in the different conventional crops for the presence of HPPDs.

Therefore, it can be considered that animals are exposed to AvHPPD-03 protein in soybean SYHT0H2 at similar extent as to native HPPD proteins naturally present in different crops.

Bioinformatic search for homology to known toxins

The AvHPPD-03 protein showed around 30% identity to bacterial haemolysins (see Section 3.1.2). Some bacterial HPPD proteins are annotated as haemolysins in publicly available protein databases (Dreesen et al., 2018) because these catalyse the production of a haemolytic substance (a melanin-like pigment), consisting of polymers of homogentisate. This is a metabolite produced by HPPD during tyrosine catabolism under specific *in vitro* experimental conditions (Dreesen et al., 2018). The applicant provided an *in vitro* haemolysis study on the AvHPPD-03 protein. Under the tested experimental conditions, the protein was shown not to induce haemolysis.

Toxicological studies

The potential acute oral toxicity of an *E. coli*-produced AvHPPD-03 protein was investigated in Crl: CD-1 mice in a study based on OECD TG 420 (OECD, 2001b) and in accordance with the principles of Good Laboratory Practice (GLP). No adverse effects related to the AvHPPD-03 protein were observed up to 2000 mg/kg bw.

In a 28-day toxicity study based on OECD TG 407 (OECD, 2008) and in accordance with the GLP principles, an *E.coli*-produced AvHPPD-03 protein was administered orally by gavage to rats (Han Wistar Crl:WI(Han), 5/sex per group) at the doses of 2, 10 or 51 mg/kg per day. No effects on body weight, food consumption, clinical condition (including FOB assessments), ophthalmoscopy, haematology, coagulation, blood chemistry or macroscopic and microscopic pathology were observed in AvHPPD-03 treated rats when compared to the vehicle receiving animals.

In vitro degradation studies

The resistance to degradation by pepsin of the AvHPPD-03 protein from a microbial recombinant system was investigated in solutions at pH ~ 1.2. The integrity of the test protein in samples of the incubation mixture taken at various time points was analysed by SDS-PAGE gel electrophoresis followed by protein staining or by Western blotting. The AvHPPD-03 protein was degraded by pepsin within 1 min.

The GMO Panel notes that the resistance to degradation by standalone simulated intestinal fluids (SIF) is currently not specifically required by either EFSA GMO Panel (2011a) or Codex Alimentarius (2009). Due to the intrinsic limitations of such standalone SIF degradation study for food and feed safety of the newly expressed protein, it was not considered in the overall safety assessment.

Conclusions on AvHPPD-03 protein

The GMO Panel assessed the toxicological profile of AvHPPD-03 protein taking into account: the source of the gene (oat); the fact that AvHPPD-03 protein is part of a large family of HPPD proteins expressed in plant species with a history of safe consumption as food and feed, complemented by dietary exposure estimations; bioinformatic search for homology of AvHPPD-03 to known toxins; available toxicological studies; and information from in vitro digestibility studies. Based on the above, the GMO Panel concludes that there are no indications of toxicological concerns for the AvHPPD-03 protein expressed in soybean SYHT0H2.

The **PAT protein** was previously assessed by the GMO Panel (e.g. EFSA GMO Panel, 2011c) and no safety concerns for humans and animals were identified. Updated bioinformatics analysis does not reveal similarities of PAT protein to known toxins. The GMO Panel is not aware of any new information that would change the previous conclusion of the risk assessment.

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

No new constituents other than the newly expressed proteins were identified in soybean SYHT0H2. Therefore, no further safety assessment of food and feed components other than the newly expressed proteins is required.

3.3.3.3. Information on altered levels of food/feed constituents

Levels of α-tocopherol and γ-tocopherol in seeds from soybean SYHT0H2 are significantly different from those of the conventional counterpart and show a lack of equivalence with the set of non-GM reference varieties (Section 3.2.3). Considering the biological relevance of these compounds the GMO Panel considers that no further toxicological assessment is needed. Further considerations on the safety of tocopherols are presented in Section 3.3.6.

3.3.3.4. Testing of the whole genetically modified food/feed

No animal studies on the whole food and feed from soybean SYHT0H2 were provided by the applicant No substantial modifications in the composition of soybean SYHT0H2 and no indication of possible unintended effects relevant for food and feed safety were identified. Therefore, animal studies on food and feed from soybean SYHT0H2 are not necessary (EFSA GMO Panel, 2011a).

3.3.4. Allergenicity

The strategies to assess the potential risk of allergenicity focus on the source of the recombinant protein, on the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in already sensitised persons and on whether the transformation may have altered the allergenic properties of the modified plant.

3.3.4.1. 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 evidence to predict allergenicity (Codex Alimentarius 2009; EFSA GMO Panel, 2011a).

The *pat* gene originates from *S. viridochromogenes* which is not considered an allergic source. The *avhppd-03* gene originates from *A. sativa* (common oat). Oat was listed in Commission Directive 2007/68/EC[24] and subsequently in Regulation 1169/2011[25], which include a list of most common allergenic foods.

Updated bioinformatic analyses of the amino acid sequences of the AvHPPD-03 and PAT proteins, using the criterion of 35% identity in a sliding window of 80 amino acids, revealed no significant similarities to known allergens. In addition, the applicant performed analyses searching for matches of eight contiguous identical amino acid sequences between these newly expressed proteins and known allergens, which confirmed the outcome of the previous bioinformatic analyses.

Following a request from the EFSA GMO Panel in line with its guidance document applicable to this dossier (EFSA GMO Panel, 2011a), the applicant provided serum screening data assessing the allergenic potential of the AvHPPD-03 protein originated from oat. In general, a critical issue for such testing is the availability of human sera from sufficient numbers of individuals (Codex Alimentarius, 2009). The GMO Panel acknowledges the difficulty in obtaining sufficient number, quantity and quality of oat-reactive sera for screening purposes. To overcome such limitations, the applicant performed two studies in two different laboratories using oat-reactive sera, which were wheat-positive sera. It has been shown that oat can exhibit immunoglobulin E (IgE)-cross reactivity to wheat even in the absence of clinical allergy (e.g. Martens et al., 2011). The serum samples were tested against oat extract and the recombinant *E. coli* derived-AvHPPD-03 protein described in Section 3.1.3. To determine potential IgE binding specific to the AvHPPD-03 protein, western blot with capillary electrophoresis system (WES) was used employing oat reactive sera from nine allergic individuals in one of the studies. In the other study, the Immune-CAP method was used employing oat reactive sera from 37 allergic individuals.

[24] Directive 2007/68/EC of the European Parliament and of the Council of 27 November 2007 amending Annex IIIa to Directive 2000/13/EC of the European Parliament and of the Council as regards certain food ingredients. OJ L 310, 27.11.2007, p. 11–14.

[25] 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.

The GMO Panel requested additional information on the Immuno-CAP-based method because several sera produced measurable levels of IgE-binding to the AvHPPD-03-loaded Immuno-CAP. The applicant provided more data on the specificity of the AvHPPD-03-binding which resulted in the finding that confounding factors could contribute to the variation in the potential IgE-binding to the recombinant *E. coli* AvHPPD-03 protein. First, it was observed that most of the sera used contained high levels of total IgE antibodies with a lack of consistent linearity after dilution. Second, the applicant developed *E. coli* preparations lacking the AvHPPD-03 protein, which provided a comparable signal as noted in the original experiments. These observations likely indicate that the initial positive response to AvHPPD-03 was caused by non-specific proteins other than the recombinant AvHPPD-03 protein. In addition to the human sera data provided, the GMO Panel also considered that the current clinical evidence supporting oat as a strong source of allergy is limited (EFSA NDA Panel, 2014; Burman et al., 2019). Moreover, the AvHPPD-03 protein in soybean SYHT0H2 is almost identical to that expressed in oat (a single amino acid difference) and highly similar to HPPD proteins expressed in other crops (see Section 3.3.3.1), evidencing an existing exposure to proteins highly similar to AvHPPD-03 with no indications of allergenicity.

Considering the data provided and evidence from literature described above, the GMO Panel is of the opinion that there are not indications that the AvHPPD-03 protein would raise safety concerns for IgE-mediated allergy under the conditions of use.

Because oat, together with wheat, barley and rye, is included in Regulation 1169/2011 as a gluten-containing food, the GMO Panel also assessed the potential of the AvHPPD-03 protein's capacity to cause celiac disease.[26] Although the consumption of non-contaminated (with other cereal grains) oat by celiac disease patients is generally considered safe, exceptions exist owing to the presence of avenins – gluten-like proteins (Lundin et al., 2003; EFSA GMO Panel, 2017). The amino acid sequence of the AvHPPD-03 protein contains relatively very few prolines with no proper spaces and relevant amino acids flanking these prolines that could raise a safety concern. The AvHPPD-03 amino acid sequence is not an avenin-like protein in oat, with no relevant DQ2- and DQ8-restricted T-cell epitopes. Overall, the amino acid sequence of the AvHPPD-03 protein does not resemble gluten. Moreover, HPPD proteins highly similar to the AvHPPD-03 have been consumed (see Sections 3.3.1 and 3.3.5) without safety issues on celiac disease described.

The studies on resistance of the AvHPPD-03 and PAT proteins to degradation by pepsin have been described in Section 3.3.3.1 or previously assessed by the Panel (EFSA GMO Panel, 2011c).

The EFSA GMO Panel has previously evaluated the safety of the PAT protein and no concerns on allergenicity were identified (EFSA GMO Panel, 2011c).

In addition, no information available on the structure or function of the newly expressed AvHPPD-03 and PAT proteins would suggest an adjuvant effect of these proteins in soybean SYHT0H2, resulting in or increasing an eventual IgE response to a bystander protein.

In the context of the present application, the GMO Panel considers that there are no indications that the newly expressed AvHPPD-03 and/or PAT proteins in soybean SYHT0H2 may be allergenic.

3.3.4.2. Assessment of allergenicity of the whole GM plant

Soybean is considered a common allergenic food[26,27] (OECD, 2012). Therefore, any potential change in the endogenous allergenicity of the GM plant when compared with that of the non-GM comparator should be assessed in line with the applicable guidance document (EFSA GMO Panel, 2011a). For such assessment, the applicant performed *in vitro* allergenicity studies with extracts from soybean SYHT0H2 and relevant comparators, and a comparative analysis of specific endogenous allergens.

Specifically, the applicant performed one-dimensional (1-D) and two-dimensional (2D) electrophoresis of extracts of soybean SYHT0H2, its conventional counterpart and two commercial non-GM soybean varieties, followed by Coomassie blue staining or Western blot using single serum from five allergic individuals to soybean. These studies showed no meaningful differences in the IgE-binding patterns between extracts of proteins derived from soybean SYHT0H2 and the comparators used. The applicant also provided a measurement of specific known allergens in soybean by mass spectrometry approaches which has previously been considered as an alternative/complementary acceptable approach for the assessment of endogenous allergenicity under the applicable guidance document (EFSA GMO Panel,

[26] It is pointed out that the requirements laid down in the recent EFSA guidance on allergenicity (EFSA GMO Panel, 2017) are not applicable to this dossier, as described in Section 1.5 'Transition period' of the guidance document. The GMO Panel performed a safety assessment of the AvHPPD-03 protein's potential to cause celiac disease following the principles of such guidance.

2011a; Fernandez et al., 2013; Selb et al., 2017). The outcome of such analysis showed that the genetic modification did not induce relevant changes in the levels of any of the five natural endogenous allergens tested (including various isoforms).

In the context of this application, the GMO Panel considers that there is no evidence that the genetic modification might change the overall allergenicity of soybean SYHT0H2 when compared with that of the conventional counterpart.

3.3.5. Nutritional assessment of endogenous constituents

The intended trait of soybean SYHT0H2 is herbicide tolerance, with no intention to alter nutritional parameters. However, levels of α-tocopherol (in not treated plants) and γ-tocopherol (in treated and not treated plants) in seeds were significantly different from the conventional counterpart and showed a lack of equivalence with the set of non-GM reference varieties (Section 3.2.3). 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.

Human nutrition

α-Tocopherol is part of the antioxidant defence system and is a peroxyl radical scavenger that especially protects polyunsaturated fatty acids (PUFAs) within membrane phospholipids and plasma lipoproteins. Currently, vitamin E is considered as α-tocopherol only, and adequate intakes (AIs) have been proposed for α-tocopherol for all population groups (EFSA NDA Panel, 2015). The main dietary sources of α-tocopherol include vegetable oils, fat spreads from vegetable oils, nuts and seeds, some fatty fish, egg yolk and whole grain cereals (Traber, 2012; EFSA NDA Panel, 2015). Considering the extent of the decrease (~ 10%) and, above all, the levels of α-tocopherol in soybean oil as compared to other vegetable oils such as sunflower oil, corn oil and rapeseed oil (Gliszczyńska-Świgło et al., 2007; Shahidi and de Camargo, 2016), the nutritional impact of soybean oil from soybean SYHT0H2 will be similar to that expected from soybean oil from its conventional counterpart and non-GM commercial varieties.

An increase of up to 13% in the levels of γ-tocopherol in soybean SYHT0H2 as compared to its conventional counterpart was also observed. γ-tocopherol is not considered an essential nutrient and no dietary reference values have been derived. No toxicological studies are available for γ-tocopherol, although results from toxicity studies on α-tocopherol are representative of the other tocopherols as a worst case (EFSA NDA Panel, 2015). The European Union's Scientific Committee on Food derived a tolerable upper intake level (UL) for α-tocopherol of 300 mg/day for adults in food supplements (SCF, 2003); no evidence of adverse effects from the consumption of vitamin E naturally occurring in foods exists. Therefore, the changes in γ-tocopherol levels in soybean SYHT0H2 are considered not to be nutritionally relevant.

Animal nutrition

Vitamin E (of which tocopherols are constituents) is not stored in the animal body in large amounts for any length of time and consequently a regular dietary provision is important. Main sources of vitamin E for animals are green forages, in particular leaves and cereal grains, while products of animal origin are relatively poor sources. The importance of vitamin E in animal nutrition is mainly related to its antioxidant and immunomodulatory properties (Baldi et al., 2000; Politis, 2012), and recently an effect of vitamin E on hepatic metabolism has been proposed in dairy cows (Pilotto et al., 2016). For the above reasons, integration of animal diets (farmed and companion) with vitamin E is always provided. Therefore, the changes in tocopherol levels in soybean SYHT0H2 are considered not to have nutritional impact.

3.3.6. Post-market monitoring of GM food/feed

The GMO Panel concludes that soybean SYHT0H2, as described in this application, is as safe as and nutritionally equivalent to the conventional counterpart and the non-GM reference varieties tested. No post-market monitoring (EFSA GMO Panel, 2011a) of food/feed from soybean SYHT0H2 is considered necessary.

3.3.7. Conclusion on the food/feed safety assessment

The GMO Panel did not identify toxicological and allergenicity concerns regarding the AvHPPD-03 and PAT proteins expressed in soybean SYHT0H2 and finds no evidence that the genetic modification might significantly change the overall allergenicity of soybean SYHT0H2.

Based on the outcome of the comparative and nutritional assessments, the GMO Panel concludes that the nutritional impact of soybean SYHT0H2 derived food and feed is expected to be the same as that from food and feed from the conventional counterpart and non-GM commercial reference varieties.

The GMO Panel concludes that soybean SYHT0H2, as described in this application is as safe as and nutritionally equivalent to the conventional counterpart and the non-GM reference varieties tested.

3.4. Environmental risk assessment and monitoring plan[27]

3.4.1. Environmental risk assessment

Considering the scope of application EFSA-GMO-DE-2012-111, which excludes cultivation, the environmental risk assessment (ERA) of soybean SYHT0H2 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 SYHT0H2 seeds during transportation and/or processing (EFSA GMO Panel, 2010b).

3.4.1.1. 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 proper 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 SYHT0H2 will provide a selective advantage to soybean plants, except when they are exposed to glufosinate ammonium containing- and/or mesotrione and other HPPD-inhibiting herbicides. However, this fitness advantage will not allow the GM plant to overcome other biological and abiotic factors (described above). 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 very unlikely that soybean SYHT0H2 will differ from conventional soybean 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 SYHT0H2 seeds.

3.4.1.2. Potential for gene transfer

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through horizontal gene transfer (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 obtained 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

[27] Dossier: Part II – Section E; additional information: 14/10/2013 and 23/12/2016.

from plants to bacteria) is not likely to occur at detectable frequencies under natural conditions (for further details, see EFSA, 2009).

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, 2009). Independently of the transfer mechanism, the GMO Panel did not identify a selective advantage that a HGT would provide to bacterial recipients in the environment.

Bioinformatic analysis of soybean event SYHT0H2 identified sufficient length and sequence identity with a same, single *A. tumefaciens* nopaline Ti plasmid region. Because of its limited length (~ 250 bp) no increased potential for facilitated HGT by double homologous recombination was identified.

In summary, there is no indication for an increased likelihood of horizontal transfer of DNA from soybean SYHT0H2 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 SYHT0H2 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 SYHT0H2 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 so far (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 3.4.1.1). 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 3.4.1.1.

3.4.1.3. Interactions of the GM plant with target organisms

Taking the scope of application EFSA-GMO-DE-2012-111 (no cultivation) and the absence of target organisms into account, potential interactions with target organisms of occasional feral soybean SYHT0H2 plants arising from seed import spills are not considered a relevant issue.

3.4.1.4. 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 GM seeds is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM soybean, potential interactions of the GM plant with non-target organisms are not considered to raise any environmental safety concern.

3.4.1.5. Interactions with the abiotic environment and biogeochemical cycles

Given that environmental exposure to spilled seeds or occasional feral soybean SYHT0H2 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 to raise any environmental safety concern.

3.4.2. Post-market environmental monitoring

The objectives of a 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 SYHT0H2, no case-specific monitoring is required.

The PMEM plan proposed by the applicant for soybean SYHT0H2 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 newly established by EuropaBio for the collection of information recorded by the various operators; and (4) 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.

3.4.3. Conclusions on the environmental risk assessment and monitoring plan

The GMO Panel concludes that it is unlikely that soybean SYHT0H2 would differ from conventional soybean varieties in its ability to persist under European environmental conditions. Considering the scope of application EFSA-GMO-DE-2012-111, interactions of occasional feral soybean SYHT0H2 plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from soybean SYHT0H2 to bacteria does not indicate a safety concern. Therefore, considering the introduced traits, the outcome of the comparative analysis and the routes and levels of exposure, the GMO Panel concludes that soybean SYHT0H2 would not raise safety concerns in the event of accidental release of viable GM soybean seeds into the environment.

The GMO Panel considers that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of soybean SYHT0H2. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

4. Conclusions

The GMO Panel was asked to carry out a scientific assessment of soybean SYHT0H2 for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003.

The molecular characterisation data establish that soybean SYHT0H2 contains a single insert consisting of one copy of the *avhppd-03* gene and four copies of the *pat* gene. Bioinformatic analyses of the sequences encoding the newly expressed proteins and other ORFs present within the insert or spanning the junctions between the insert and genomic DNA indicate a ~ 30% sequence identity of AvHPPD-03 to bacterial haemolysins. This finding was assessed for its relevance for food and feed safety assessment. In addition, these analyses indicate that the expression of all other ORFs showing significant similarities to allergens and toxins is highly unlikely. The stability of the inserted DNA and of the introduced herbicide tolerance traits was confirmed over several generations. The methodology used to quantify the levels of the AvHPPD-03 and PAT proteins is considered adequate. The protein characterisation data comparing the structural, biochemical and functional properties of the plant- and microbe-produced AvHPPD-03 and PAT proteins indicate that these proteins are equivalent and the microbe-produced protein can be used in the safety studies.

None of the agronomic/phenotypic and compositional differences identified between soybean SYHT0H2 and the conventional counterpart needed further assessment except for seed levels of α-tocopherol and γ-tocopherol. These differences were further assessed for their safety and nutritional relevance and found not to raise safety concerns.

The GMO Panel did not identify toxicological and allergenicity concerns regarding the AvHPPD-03 and PAT proteins expressed in soybean SYHT0H2 and found no evidence that the genetic modification might significantly change the overall allergenicity of soybean SYHT0H2. Based on the outcome of the comparative and nutritional assessments, the GMO Panel concludes that the nutritional impact of soybean SYHT0H2 derived food and feed is expected to be the same as that from food and feed from the conventional counterpart and non-GM commercial reference varieties. The GMO Panel concludes that soybean SYHT0H2, as described in this application, is as safe as and nutritionally equivalent to the conventional counterpart and the non-GM reference varieties tested. Post-market monitoring of GM food/feed is not 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 soybean SYHT0H2 into the environment. The PMEM plan and reporting intervals are in line with the intended uses of soybean SYHT0H2.

In conclusion, the GMO Panel considers that soybean SYHT0H2, as described in this application, 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.

Documentation as provided to EFSA

- Letter from the Competent Authority of Germany received on 08 August 2012 concerning a request for authorization of the placing on the market of soybean SYHT0H2 submitted in accordance with Regulation (EC) No 1829/2003 by Syngenta Crop Protection AG.
- Application EFSA-GMO-DE-2012-111 validated by EFSA, 09 January 2013.
- Request for supplementary information to the applicant, 06 August 2013.
- Request for supplementary information to the applicant, 13 August 2013.
- Receipt of supplementary information from the applicant, 14 October 2013.
- Request for supplementary information to the applicant, 25 July 2014.
- Receipt of supplementary information from the applicant, 12 September 2014.
- Request for supplementary information to the applicant, 05 December 2014.
- Request for supplementary information to the applicant, 10 February 2015.
- Receipt of spontaneous information from the applicant, 08 April 2015.
- Receipt of supplementary information from the applicant, 03 June 2015.
- Receipt of supplementary information from the applicant, 17 July 2015.
- Request for supplementary information to the applicant, 14 September 2015.
- Request for supplementary information to the applicant, 09 November 2015.
- Receipt of supplementary information from the applicant, 01 September 2016.
- Receipt of spontaneous information from the applicant, 27 September 2016.
- Receipt of supplementary information from the applicant, 03 October 2016.
- Request for supplementary information to the applicant, 11 October 2016.
- Receipt of supplementary information from the applicant, 23 December 2016.
- Receipt of spontaneous information from the applicant, 23 December 2016.
- Request for supplementary information to the applicant, 16 January 2017.
- Request for supplementary information to the applicant, 08 February 2017.
- Receipt of supplementary information from the applicant, 31 May 2017.
- Request for supplementary information to the applicant, 19 July 2017.
- Receipt of supplementary information from the applicant, 01 December 2017.
- Request for supplementary information to the applicant, 28 March 2018.
- Receipt of supplementary information from the applicant, 03 April 2018.
- Receipt of supplementary information from the applicant, 05 April 2018.
- Receipt of spontaneous information from the applicant, 05 April 2018.
- Request for supplementary information to the applicant, 30 April 2018.
- Request for supplementary information to the applicant, 08 May 2018.
- Receipt of supplementary information from the applicant, 16 May 2018.
- Request for supplementary information to the applicant, 18 May 2018.
- Receipt of supplementary information from the applicant, 03 September 2018.
- Receipt of supplementary information from the applicant, 03 December 2018.
- Request for supplementary information to the applicant, 25 January 2019.
- Receipt of supplementary information from the applicant, 02 May 2019.
- Request for supplementary information to the applicant, 21 June 2019.

- Receipt of supplementary information from the applicant, 28 August 2019.
- Request for supplementary information to the applicant, 11 September 2019.
- Receipt of supplementary information from the applicant, 30 September 2019.
- Request for supplementary information to the applicant, 09 October 2019.
- Receipt of supplementary information from the applicant, 21 October 2019.

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Abbreviations

ADF	acid detergent fibre
AI	adequate intake
bp	base pair
bw	body weight
CaMV	cauliflower mosaic virus
CMP	*Cestrum yellow leaf curling virus*
DM	dry matter
DW	dry weight
EFSA	GMO Panel EFSA Panel on Genetically Modified Organisms
ELISA	enzyme-linked immunosorbent assay
ERA	environmental risk assessment
FMV	*Figwort Mosaic Virus*
FW	fresh weight
GLP	Good Laboratory Practice
GM	genetically modified
GMO	genetically modified organism
HGT	horizontal gene transfer
HPPD	*p*-hydroxypheylpyruvate dioxygenase
HR	homologous recombination
IgE	immunoglobulin E
LC–MS/MS	liquid chromatography–tandem mass spectrometry
LOQ	limit of quantification
NDF	neutral detergent fibre
nos	nopaline synthase
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PAT	phosphinothricin acetyltransferase
PCR	polymerase chain reaction

PMEM	post-market environmental monitoring
PUFA	polyunsaturated fatty acid
SIF	simulated intestinal fluids
SMP	synthetic minimal plant
TMV	*Tobacco mosaic virus*
UL	Tolerable Upper Intake Level
USDA	United States Department of Agriculture
UTR	untranslated region
WES	Western blot with capillary electrophoresis system

Appendix A – Dietary exposure to AvHPPD-03 and HPPD proteins across European countries

Box-plots of acute and chronic dietary exposure (high consumers, μg/kg bw per day) to AvHPPD-03 and HPPD proteins in the young population (infants, toddlers, other children and adolescents) and adult population (adults, elderly and very elderly) across European countries. See Section 3.3.3 for appropriate interpretation.