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SCIENTIFIC OPINION

Scientific Opinion on an application (EFSA-GMO-BE-2011-98) for the placing on the market of herbicide-tolerant genetically modified soybean FG72 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience[1]

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ABSTRACT

Soybean FG72 was developed by biolistic transformation to express the HPPD W336 and 2mEPSPS proteins, which confer tolerance to isoxaflutole- and glyphosate-based herbicides. The molecular characterisation of soybean FG72 did not give rise to safety issues. The agronomic and phenotypic characteristics of soybean FG72 tested under field conditions revealed no biologically relevant differences between soybean FG72 and its conventional counterpart that would give rise to any food and feed or environmental safety concerns. No differences in the compositional data requiring further safety assessment were identified. There were no concerns regarding the potential toxicity and allergenicity of the newly expressed proteins HPPD W336 and 2mEPSPS, and no evidence that the genetic modification might significantly change the overall allergenicity of soybean FG72. The nutritional characteristics of soybean FG72 is not expected to differ from that of non-GM soybean varieties. There are no indications of an increased likelihood of establishment and spread of feral soybean plants. Considering the scope of this application, interactions with the biotic and abiotic environment were not considered to be an issue. Risks associated with an unlikely but theoretically possible horizontal gene transfer from soybean FG72 to bacteria have not been identified. The monitoring plan and reporting intervals are in line with the scope of the application. In conclusion, the EFSA GMO Panel considers that the information available for soybean FG72 addresses the scientific comments raised by Member States and that soybean FG72, as described in this application, is as safe as its conventional counterpart and non-GM soybean reference varieties with respect to potential effects on human and animal health and the environment in the context of the scope of this application.

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[1] On request from the Competent Authority of Belgium, Question No EFSA-Q-2012-00692, adopted on 25 June 2015.

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KEY WORDS

GMO, soybean (Glycine max (L.) Merr.), FG72, herbicide tolerance, HPPD W336, 2mEPSPS, Regulation (EC) No 1829/2003

SUMMARY

Following the submission of an application (EFSA-GMO-BE-2011-98) under Regulation (EC) No 1829/2003 from Bayer CropScience, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) was asked to deliver a scientific opinion on the safety of herbicide-tolerant genetically modified (GM) soybean (*Glycine max* L.) FG72 (Unique Identifier MST-FGØ72-2). The scope of application EFSA-GMO-BE-2011-98 is for import, processing, and food and feed uses of soybean FG72 within the European Union (EU), but it excludes cultivation in the EU.

The EFSA GMO Panel evaluated soybean FG72 with reference to the scope and appropriate principles described in its guidelines for the risk assessment of GM plants. The evaluation addressed the following components of the risk assessment: the molecular characterisation of the inserted DNA and analysis of the expression of the corresponding proteins; the comparative analyses of compositional, agronomic and phenotypic characteristics; the safety of the newly expressed proteins and the whole food/feed with respect to potential toxicity, allergenicity and nutritional characteristics; and the environmental risk assessment and the post-market environmental monitoring plan.

Soybean FG72 was developed by biolistic transformation of callus cells. It expresses the HPPD W336 and 2mEPSPS proteins, which confer tolerance to isoxaflutole- and glyphosate-based herbicides. The molecular characterisation data established that soybean FG72 contains one functional insert consisting of two copies of the *hppdPfW336* and 2m*epsps* expression cassettes, which confer the intended traits. There is also a second, non-functional insert, consisting of a non-coding plasmid fragment. Bioinformatic analyses and genetic stability studies were performed and the results did not give rise to safety issues. The levels of the newly expressed proteins present in soybean FG72 were obtained and reported adequately.

The agronomic, phenotypic and compositional characteristics of soybean FG72 were compared with those of the conventional counterpart under field conditions. Differences were noted in soybean FG72 for two agronomic endpoints (i.e. plant health and days to maturity), but they did not give rise to any food and feed or environmental safety concerns. No differences requiring further assessment with regard to safety by the EFSA GMO Panel were identified at analyses of compositional data of forage or grains obtained from soybean FG72.

The safety assessment of the newly expressed proteins and the whole food/feed was performed. No concerns were identified regarding the potential toxicity or allergenicity of the newly expressed 2mEPSPS and HPPD W336 proteins in soybean FG72. There was no evidence that the genetic modification might significantly change the overall allergenicity of soybean FG72. Based on the comparative analysis, the nutritional characteristics of food and feed derived from soybean FG72 is not expected to differ from that of food and feed derived from non-GM soybean varieties. The EFSA GMO Panel concluded that soybean FG72 is as safe and nutritious as its conventional counterpart and non-GM soybean reference varieties.

Application EFSA-GMO-BE-2011-98 covers the import, processing, and food and feed uses of soybean FG72, and excludes cultivation. Therefore there is no requirement for a scientific assessment of possible environmental effects associated with the cultivation of this GM soybean. The EFSA GMO Panel concluded that there are no indications of an increased likelihood of establishment and spread of feral soybean FG72 plants in case of accidental release into the environment of viable GM soybean seeds. Potential interactions with the biotic and abiotic environment were not considered to be an issue by the EFSA GMO Panel. Risks associated with an unlikely but theoretically possible horizontal gene transfer from soybean FG72 to bacteria have not been identified. The PMEM plan provided by the applicant is in line with the scope of the application and the requirements of the EFSA GMO Panel for post-market environmental monitoring of GM plants. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the monitoring plan.

In delivering its scientific opinion, the EFSA GMO Panel took into account application EFSA-GMO-BE-2011-98, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications. In conclusion, the EFSA GMO Panel considers that the information available for soybean FG72 addresses the scientific comments raised by Member States and that soybean FG72, as described in this application, is as safe as its conventional counterpart and non-GM soybean reference varieties with respect to potential effects on human and animal health and the environment in the context of the scope of this application.

TABLE OF CONTENTS

Abstract 1
1. Introduction 6
1.1. Background 6
1.2. Terms of Reference as provided by requestor 7
2. Data and Methodologies 7
2.1. Data 7
2.2. Methodologies 7
3. Assessment 7
3.1. Molecular characterisation 7
3.1.1. Evaluation of relevant scientific data 7
3.1.2. Conclusion 10
3.2. Comparative analysis 10
3.2.1. Evaluation of relevant scientific data 10
3.2.2. Conclusion 13
3.3. Food/feed safety assessment 13
3.3.1. Evaluation of relevant scientific data 13
3.3.2. Conclusion 19
3.4. Environmental risk assessment and monitoring plan 19
3.4.1. Evaluation of relevant scientific data 19
3.4.2. Conclusion 24
4. Conclusions 25
Documentation provided to EFSA 25
References 27

1. Introduction

Soybean FG72 was developed to confer tolerance to isoxaflutole (5-cyclopropylisoxazol-4-yl 2-mesyl-4-trifluoromethylphenyl ketone)- and glyphosate (N-(phosphonomethyl)glycine)-based herbicides. Tolerance to isoxaflutole is provided by the expression of HPPD W336, a modified version of the HPPD protein (4-hydroxyphenylpyruvate dioxygenase), which has a low sensitivity to the herbicide. Tolerance to glyphosate is achieved by the expression of 2mEPSPS protein (5-enolpyruvyl-shikimate-3-phosphate synthase), which has a low binding affinity for glyphosate and maintains enzymatic activity in its presence.[4]

1.1. Background

On 24 June 2011, the European Food Safety Authority (EFSA) received from the Belgian Competent Authority an application (Reference EFSA-GMO-BE-2011-98) for authorisation of GM soybean FG72 (Unique Identifier MST-FGØ72-2), submitted by Bayer CropScience within the framework of Regulation (EC) No 1829/2003 on GM food and feed.[5]

After receiving the application EFSA-GMO-BE-2011-98 and in accordance with Articles 5(2)(b) and 17(2)(b) of the Regulation (EC) No 1829/2003, EFSA informed the Member States and the European Commission, and made the summary of the application publicly available on the EFSA website.[6] EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of the Regulation (EC) No 1829/2003. On 2 September 2011 and 5 October 2011, EFSA received additional information requested under completeness check (requested on 27 July 2011 and 22 September 2011). On 24 October 2011, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the European Commission, and consulted nominated risk assessment bodies of Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC[7] following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. Member States had three months after the date of receipt of the valid application (until 24 January 2012) to make their opinion known.

The EFSA GMO Panel requested additional information from the applicant On 8 February 2012, 4 April 2012, 6 December 2012, 29 August 2013, 27 May 2014, 4 September 2014, 19 February 2015, 3 March 2015 and 22 May 2015. The applicant provided the requested information on 8 May 2012, 13 June 2012, 2 April 2013, 29 April 2014, 17 July 2014, 27 October 2014, 23 March 2015, 1 April 2015 and 3 June 2015. The applicant also provided spontaneous additional information on 29 April 2014.

In giving its scientific opinion on soybean FG72 to the European Commission, Member States and the applicant, and in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003, EFSA has endeavoured to respect a time limit of six months from the acknowledgement of the valid application. As additional information was requested by the EFSA GMO Panel, the time limit of six months was extended accordingly, in line with Articles 6(1), 6(2), 18(1), and 18(2) of Regulation (EC) No 1829/2003.

According to Regulation (EC) No 1829/2003, this scientific opinion is to be seen as the report requested under Articles 6(6) and 18(6) of that Regulation and thus will be part of the EFSA overall opinion in accordance with Articles 6(5) and 18(5).

[4] Dossier: Part I—Section D1.
[5] 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.
[6] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2011-00848
[7] 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.

1.2. Terms of Reference as provided by requestor

The EFSA GMO Panel was requested to carry out a scientific assessment of soybean FG72 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003.

Where applicable, any conditions or restrictions which should be imposed on the placing on the market and/or specific conditions or restrictions for use and handling, including post-market monitoring requirements based on the outcome of the risk assessment and, in the case of GMOs or food/feed containing or consisting of GMOs, conditions for the protection of particular ecosystems/environment and/or geographical areas should be indicated in accordance with Articles 6(5)(e) and 18(5)(e) of Regulation (EC) No 1829/2003.

The EFSA GMO Panel was not requested to give an opinion on information required under Annex II to the Cartagena Protocol. Furthermore, the EFSA GMO Panel did not consider proposals for labelling and methods of detection (including sampling and the identification of the specific transformation event in the food/feed and/or food/feed produced from it), which are matters related to risk management.

2. Data and Methodologies

2.1. Data

In delivering its scientific opinion, the EFSA GMO Panel took into account application EFSA-GMO-BE-2011-98, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications.

2.2. Methodologies

The EFSA GMO Panel carried out an evaluation of the scientific risk assessment of soybean FG72 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. The EFSA GMO Panel took into account the appropriate principles described in its guidelines for the risk assessment of genetically modified (GM) plants and derived food and feed (EFSA, 2006a; EFSA GMO Panel, 2011a), the environmental risk assessment of GM plants (EFSA GMO Panel, 2010c) and on the post-market environmental monitoring of GM plants (EFSA, 2006b; EFSA GMO Panel, 2011b).

The comments raised by Member States are addressed in Annex G of the EFSA overall opinion[8] and were taken into consideration during the evaluation of the risk assessment.

3. Assessment

3.1. Molecular characterisation

3.1.1. Evaluation of relevant scientific data

3.1.1.1. Transformation process and vector constructs

Soybean FG72 was developed by biolistic transformation of callus cells, isolated from Jack soybean (*Glycine max* (L.) Merr.), with a *Sal*I fragment purified by high-performance liquid chromatography from plasmid pSF10.[9]

[8] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2015-00392
[9] Dossier: Part I—Section C1.

The *Sal*I fragment used for transformation contained two gene cassettes.[10]

The 2m*epsps* cassette, which confers tolerance to glyphosate-based herbicides, contains the following genetic elements between the right and left borders: Ph4a748 promoter of the histone H4 gene and the first intron of gene II of the histone H3.III variant, both from *Arabidopsis thaliana*, together providing high constitutive expression of 2mEPSPS; optimised TPotp C transit peptide-encoding sequence, derived from the RuBisCO small subunit genes of sunflower and maize, which targets the mature protein to the plastid; 2m*epsps* gene, a mutated version of maize *epsps* gene with an additional methionine codon in the N-terminus; and 3′histoneAt, the 3′ untranslated region of the histone H4 gene of *A. thaliana* as transcription terminator and polyadenylation signal.

The *hppdPfW336* cassette, conferring tolerance to isoxaflutole-based herbicides, contains the following genetic elements: Ph4a748 ABBC sequence from *A. thaliana*, which includes the H4 histone gene promoter with an internal duplication, providing increased expression of the *hppdPfW336* gene; *TEV*, 5′ enhancer, leader sequence of the *Tobacco etch virus*, involved in the regulation of gene expression; TPotp Y transit peptide-encoding sequence derived from the RuBisCO small subunit genes of sunflower and maize; *hppdPfW336* gene, obtained by site-directed point mutation of a wild-type *hppd* gene from *Pseudomonas fluorescens*, coding for the HPPD W336 protein, which confers tolerance to isoxaflutole; and 3′ *nos* terminator from *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*) as a transcription terminator and polyadenylation signal.

Outside the *Sal*I fragment used for transformation, the original plasmid pSF10 included two origins of replication and the *bla* beta-lactamase gene that confers resistance to the antibiotic ampicillin.

3.1.1.2. Transgene constructs in the GM plant

Molecular characterisation of soybean event FG72 was performed by Southern analysis and polymerase chain reaction (PCR) amplification to determine copy number, size and organisation of the inserted sequences, and to confirm the absence of plasmid backbone sequences in soybean FG72.[11] The approach used was acceptable in terms of both coverage and sensitivity.

Southern analysis indicated that the soybean event FG72 contains two inserts. The main insert consists of two partial 3′histoneAt sequences in a tail-to-tail orientation, followed by two complete copies of the *Sal*I fragment, arranged in a head-to-tail orientation. The organisation and integrity of the insert were confirmed by Southern analysis using 10 restriction enzymes in combination with nine probes. The absence of all three genetic elements of the vector backbone was demonstrated by Southern analysis (see Section 3.1.1.1).[12]

Sequencing of the soybean FG72 main insert and the 5′ and 3′ flanking regions confirmed the results obtained by Southern analyses. A comparison with the pre-insertion locus indicated that 24 base pairs of filler DNA were introduced and 27 base pairs were deleted from soybean genomic DNA during the integration process.[13]

The second insert consists of 158 base pairs of the Ph4a748 promoter linked to the 3′ end of a translocated soybean genomic fragment.

To determine the possible disruption of known endogenous soybean genes by the two inserts in FG72, bioinformatic analyses were carried out on the genomic flanking sequences.[14] A BLASTN search was performed against the National Center for Biotechnology Information (NCBI) reference genomic sequences *Glycine max*, Nucleotide collection (nr/nt) and Expressed Sequence Tags (EST) databases,

[10] Dossier: Part I—Section C2.
[11] Dossier: Part I—Section D2.
[12] Dossier: Part I—Section D2 (a).
[13] Dossier: Part I—Section D2 (b).
[14] Dossier: Part I—Section D3 (d); additional information: 23/3/2015.

and a BLASTX search was performed against NCBI non-redundant protein sequences. The results did not reveal the interruption of known endogenous genes in soybean FG72. The results also confirmed the location of the insert within the nuclear genome.

In order to assess whether the sequences encoding the newly expressed proteins or any other open reading frames (ORFs) present within the insert and spanning the junction sites between the insert and genomic DNA give rise to any safety issues, their putative translation products were compared with known allergens and toxins by using suitable algorithms and appropriate databases.[15] Bioinformatic analysis of the HPPD W336 amino acid sequence against known toxins showed significant identity (~50 %) to some proteins of bacterial origin (e.g. from *Vibrio vulnificus* and *Legionella pneumophila*), which are annotated as possible haemolysins. Detailed sequence analysis suggested that this match was due to the high sequence conservation of the homologous HPPD structural fold found among these proteins. The relevance for the safety assessment of these findings is further discussed in Section 3.3.1.2. None of the newly expressed proteins showed significant similarities with known allergens. None of the other potential ORF-derived amino acid sequences identified within the insert or spanning the junctions showed biologically relevant similarities with known toxins or allergens; the bioinformatic analyses support the conclusion that, even in the unlikely event that any of these ORFs were translated, they would not give rise to a safety issue.

3.1.1.3. Information on the expression of the insert

Levels of HPPD W336 and 2mEPSPS proteins in soybean FG72 were analysed by enzyme-linked immunosorbent assay (ELISA). Considering the scope of the application, data from seed are considered the most relevant. Seeds were harvested from field trials performed in the USA in 2008 (ten field trials) and 2009 (six field trials).[16] Each site contained plots both treated and not treated with the intended herbicides (Table 1).

Table 1: Mean, standard deviation (upper row) and ranges (lower row) of HPPD W336 and 2mEPSPS protein levels in soybean FG72 (µg/g dry weight)

	HPPD W336		**2mEPSPS**	
	Treated	**Not treated**	**Treated**	**Not treated**
Seed (2008)	0.887 ± 0.195	0.936 ± 0.171	150 ± 23.68	150 ± 22.82
(n = 30)	0.609–1.4	0.622–1.26	100–250	100–200
Seed (2009)	1.77 ± 0.228	1.37 ±0.231	142 ± 23.5	132 ± 20.8
(n = 18)	1.29–2.31	0.844–1.72	101–176	79.5–171

3.1.1.4. Inheritance and stability of inserted DNA

Genetic and phenotypic stability of the FG72 insert was studied by Southern analysis and segregation analysis. Samples from 159 plants, representing three genetic backgrounds and three generations, were tested in order to confirm the stability of the FG72 transgenic locus (functional and non-functional insert). The restriction enzyme–probe combinations used were sufficient to conclude that all the generations tested retained the functional and non-functional insert, which were genetically linked and stably inherited.

Phenotypic analysis (glyphosate tolerance) of 172 T_2 seeds, and PCR-based zygosity analysis of 901 F2 progeny plants (result of selfing of F_1 progeny from a cross between a non-GM soybean and a homozygous FG72 line) demonstrated Mendelian segregation of the FG72 transgenic locus.

[15] Additional information: 23/3/2015.
[16] Dossier: Part I—Section D3 (a), (b) and (d).

3.1.2. Conclusion

The molecular characterisation data establish that soybean FG72 contains one functional insert, consisting of two copies of the 2m*epsps* and *hppdPfW336* expression cassettes, conferring the intended traits, and a non-functional insert, consisting of a non-coding plasmid fragment. Bioinformatic analyses of the sequences encoding the newly expressed proteins and of other ORFs present within the insert or spanning the junction sites between the insert and genomic DNA did not give rise to safety issues. The stability of the inserted DNA and of the herbicide tolerance traits was confirmed over several generations.

3.2. Comparative analysis

3.2.1. Evaluation of relevant scientific data

3.2.1.1. Choice of comparator and production of material for the comparative assessment[17]

Field trials for the comparative assessment of soybean FG72 were performed in the USA at 10 locations in 2008[18] and six locations in 2009.[19] In both field trials the variety Jack, which was originally transformed to generate soybean FG72, was used as the conventional counterpart. As differences in seed quality were reported by the applicant,[20] the EFSA GMO Panel considered these field trials not appropriate for the comparative analysis of compositional, agronomic and phenotypic endpoints. Upon request of the EFSA GMO Panel, additional field trials for the comparative analysis of soybean FG72 (BC_1F_5) were performed in the USA in 2011.[21] The additional field trials did not fulfil the requirements laid down by EFSA (EFSA GMO Panel, 2011a), because soybean FG72 exposed to the intended herbicides was not included in the experimental design. The 2011 field trials were considered to be complementary data for the agronomic and phenotypic characterisation of soybean FG72.

At EFSA GMO Panel's request, the applicant performed new field trials in the USA in 2013.[22] For the new field trials, the event FG72 was introduced into the non-transgenic soybean line MST39 (BC_3F_6), which was used as the comparator. The EFSA GMO Panel considered MST39 to be a suitable conventional counterpart. The field trials were performed at 10 sites within soybean cultivation areas in the USA appropriate for soybeans of the maturity groups 2-4.[23] At each site the following six test materials were grown in a randomised complete block design with four replicates: soybean FG72 (treated and not treated with the intended herbicides); the conventional counterpart (soybean MST39); and three non-GM soybean reference varieties. Overall the field trials included six non-GM soybean reference varieties.[24] Except for the location Doniphan (Kansas), all test materials at all other locations were treated with conventional herbicides. The intended herbicides were Balance Pro (isoxaflutole) and Roundup PowerMAX (glyphosate, in the form of its ammonium salt).

3.2.1.2. Statistical analysis of field trial data

The statistical analysis of the agronomic, phenotypic and compositional data followed the recommendations of the EFSA GMO Panel (EFSA GMO Panel, 2010a, 2011a). This includes a test of difference to determine whether the GM plant is different from its comparator/conventional counterpart, and a test of equivalence to determine whether the GM plant falls within the range of

[17] Dossier: Part I—Section D7.1-7.2; additional information: 13/6/2012, 2/4/2013, 29/4/2014 and 27/10/2014.
[18] Cherokee (IA), Hardin (IA), Greene (IA), Dallas (IA), Dallas (IA), Madison (IA), Clinton (MO), Vermillion (IL), Tipton (IN) and Des Moines (IA).
[19] Dallas (IA), Dallas (IA), Madison (IA), Vermillion (IA), Tipton (IN) and Des Moines (IA).
[20] Additional information: 13/6/2012.
[21] Additional information: 13/6/2012 and 2/4/2013.
[22] Additional information: 29/4/2014 and 27/10/2014.
[23] York (NE), Jefferson (IA), Shelby (MO), Butler (MO), Adair (MO), Doniphan (KS), Clinton (IL), Shelby (IL), Montgomery (IN) and Rush (IN).
[24] The non-GM reference materials were Stine varieties 35E23, 29E22, 35E32, 33E22, 31E22 and 30E32.

natural variation estimated from the non-GM soybean reference varieties. As described by EFSA (EFSA GMO Panel, 2011a), the result of the equivalence test is categorised into four possible outcomes to facilitate drawing conclusions with respect to the presence or absence of equivalence. These four categories are category I, indicating full equivalence; category II, indicating that equivalence is more likely than non-equivalence; category III, indicating that non-equivalence is more likely than equivalence; and category IV, indicating non-equivalence.

3.2.1.3. Agronomic and phenotypic characteristics[25]

For the analysis of agronomic and phenotypic characteristics of soybean FG72, its conventional counterpart and the non-GM soybean reference varieties, 14 quantitative endpoints[26] and 7 qualitative endpoints[27] were recorded. For categorical endpoints,[28] the difference between soybean FG72 and the conventional counterpart was tested using a Cochran–Mantel–Haenszel (CMH) test.

The test of difference for phenotypic and agronomic characteristics of soybean FG72 (sprayed only with the conventional herbicides) identified no significant differences for any of the endpoints analysed. A statistically significant difference was identified between soybean FG72 (sprayed with the intended herbicides) and its conventional counterpart for only one endpoint (days to maturity). The equivalence test indicated that the endpoint 'days to maturity' fell into equivalence category I.

The CMH test identified no significant differences between soybean FG72 and its conventional counterpart, except for plant health[29] at the R1 growth stage for FG72 sprayed with the intended herbicide; however, the average plant health ratings were within the range of the non-GM reference varieties.

Agronomic and phenotypic characteristics tested under controlled conditions[30]

(a) Seed germination

The applicant also reported data on seed characteristics, germination and dormancy of soybean FG72 (100-seed weight, hilum colour, mottling score, seed coat colour, seed coat lustre, seed quality and seed shape). The seeds were harvested from soybean FG72 (T_8) and its conventional counterpart (Jack) from the 10 field trial locations in the USA in 2008.[31] The measurement was done using four independent samples of 100 seeds from each of the 10 locations. The seeds used for the germination study were produced under similar conditions, therefore this material was considered suitable by the EFSA GMO Panel.

The endpoints analysed were the number of normal germinated seeds, abnormal germinated seeds, hard seeds, dead seed and dormant seeds. The seeds were incubated for five days under controlled conditions; germinated seeds were scored on day 6 and (in the case of hard seeds) again on day 13. Among all characteristics, only differences smaller than one standard deviation were observed between soybean FG72 (94 % germination) and Jack (96% germination) on day six. Thirteen days of incubation resulted in germination rates of 95 % (FG72) vs. 96 % (Jack).

[25] Additional information: 29/4/2014 and 27/10/2014.
[26] Days to 50 % emergence, stand count, plant vigour, plant health (at growth stages R1, V4, V5 and maturity), days to 50 % flowering, days to 90 % maturity, plant lodging, final stand count, pod shattering (at maturity and at two weeks after maturity), yield and plant height.
[27] Flower colour, leaf shape, canopy architecture, pubescence colour, pod colour, hilum colour and growth habit.
[28] Plant vigour, plant health ratings (at R1, V4, V5, and maturity), plant lodging and pod shattering.
[29] Plant health assessment included a record of phytotoxic effects of plots with FG72 soybean sprayed with isoxaflutole-glyphosate as compared to plots with conventionally-treated FG72 and plots with its conventional counterpart.
[30] Dossier: Part I—Section D7.4.
[31] The 2008 and 2009 field trials were considered not appropriate for the comparative analysis of compositional, agronomic and phenotypic endpoints because of the differences in seed quality among the tested materials. The EFSA GMO Panel considered the material collected from these field trials suitable for pollen and seed characteristics, since it was produced under similar conditions.

As no data on induced seed dormancy were supplied, the EFSA GMO Panel concluded that only the conclusions on seed germination of soybean FG72 are substantiated by the provided data.

(b) Seed morphology, pollen morphology and viability

In addition, seed morphology as well as pollen morphology, viability and germination were evaluated. The materials were grown in 2009 in the USA under controlled (greenhouse) conditions. For these studies, soybean FG72 was compared with the conventional counterpart Jack. To illustrate the seed characteristics, photographs were taken: the EFSA GMO Panel considered this approach not suitable to identify differences in morphology. For pollen morphology and viability, pollen grains were sampled from flowers (10 flowers per genotype), stained and photographed. For germination tests, a drop of pollen was incubated in a germination medium containing the harvested pollen (around 600 pollen grains analysed per genotype). No differences were identified in germination between the pollen of the two genotypes. Intact flowers and characteristic flower elements (including stamens and pistils) were also morphologically compared. For further details, see Section 3.4.1.1.

3.2.1.4. Compositional analysis[32]

Forage and seeds harvested from 8 of the 10 field trial locations[33] were analysed for 93 constituents (86 in seed[34] and seven in forage[35]), including the key constituents recommended by OECD (OECD, 2001). Eight seed constituents having more than 50% of the observations below the limit of quantification were excluded from the analysis.[36]

Statistically significant differences between soybean FG72 and its conventional counterpart (both sprayed with conventional herbicides only) were found for 22 constituents in seeds.[37] The test of equivalence indicated that the level of 19 of the 22 constituents fell under equivalence category I or II. For the three remaining seed constituents (γ-tocopherol, β-tocotrienol and total tocotrienols), the test of equivalence could not be performed because of the small variation among the non-GM soybean reference varieties (Table 2).

Statistically significant differences between soybean FG72 sprayed with glyphosate and isoxaflutole herbicides (in addition to required conventional herbicides) and its conventional counterpart (sprayed with conventional herbicides only) were found for 20 constituents in seeds.[38] The test of equivalence indicated that the level of 17 of the 20 constituents fell into equivalence category I or II. For the three

[32] Additional information: 29/4/2014 and 27/10/2014.

[33] York (NE), Jefferson (IA), Shelby (MO), Butler (MO), Adair (MO), Clinton (IL), Shelby (IL) and Montgomery (IN) were randomly selected out of the pool of ten field trial locations.

[34] Proximates (protein, fat, ash, and moisture and carbohydrate), fibre fractions (acid detergent fibre (ADF) and neutral detergent fibre (NDF)), minerals (calcium, potassium, phosphorus, manganese, sodium and iron), amino acids (alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine), fatty acids (caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), myristoleic acid (C14:1), pentadecanoic acid (C15:0), pentadecenoic acid (C15:1), palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), heptadecenoic acid (C17:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), octadecatetraenoic acid (C18:4), arachidic acid (C20:0), eicosenoic acid (C20:1), eicosadienoic acid (C20:2), arachidonic acid (C20:3), eicosatrienoic acid (C20:4), eicosapentaenoic acid (C20:5), behenic acid (C22:0), erucic acid (C22:1), docosapentaenoic acid (C22:5), docosahexaenoic acid (C22:6), lignoceric acid (C24:0)), β-carotene, thiamine, riboflavin, vitamin K1, tocopherols (α, β, γ, δ and total), tocotrienols (α, β, γ, δ and total), folic acid, anti-nutrients (phytic acid, trypsin inhibitor, lectins, stachyose and raffinose) and other secondary metabolites (daidzin, genistin, glycitin, daidzein, genistein, glycitein, and total isoflavones).

[35] Moisture, protein, fat, ash, ADF, NDF and carbohydrates.

[36] These were β carotene, γ-tocotrienol, δ-tocotrienol, glycitein, heptadecenoic acid (C17:1), octadecatetraenoic acid (C18:4), eicosadienoic acid (C20:2) and erucic acid (C22:1).

[37] Protein, fat, ADF, NDF, alanine, arginine, histidine, isoleucine, leucine, methionine, magnesium, stearic acid (C18:0), linolenic acid (C18:3), arachidic acid (C20:0) eicosenoic acid (C20:1), α-tocopherol, γ-tocopherol, total tocopherols, β-tocotrienol, total tocotrienol, stachyose and raffinose.

[38] Fat, ADF, NDF and carbohydrates; the amino acid lysine, the minerals potassium and magnesium; the fatty acids palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linolenic acid (C18:3), arachidic acid (C20:0) and eicosenoic acid (C20:1); and α-tocopherol, γ-tocopherol, total tocopherol, β-tocotrienol, total tocotrienol, stachyose and raffinose.

remaining seed constituents (γ-tocopherol, β-tocotrienol and total tocotrienols), the test of equivalence could not be performed because of the small variation among the non-GM soybean reference varieties (Table 2).

Table 2: Compositional endpoints that are further discussed based on the results of the statistical analysis: means (for the conventional counterpart and the GM soybean) estimated from field trials data collected in the USA in 2013. The test of equivalence could not be performed on any of the three endpoints.

Endpoint	Conventional counterpart (MST39 untreated)	Soybean FG72	
		Untreated [(a)]	Treated [(b)]
γ-Tocopherol (seed) (mg/kg)	237	262*	268*
β-Tocotrienol (seed) (mg/kg)	2.96	3.41*	3.40*
Total tocotrienols (seed) (mg/kg)	2.96	3.41*	3.44*

* Significantly different entry.
(a): Untreated: soybean FG72 not sprayed with the intended herbicides (glyphosate and isoxaflutole).
(b): Treated: soybean FG72 sprayed with the intended herbicides (glyphosate and isoxaflutole).

The EFSA GMO Panel concluded that no further assessment was needed for γ-tocopherol, β-tocotrienol and total tocotrienols in seed, as the reported differences would have no measurable nutritional impact and are not relevant to food and feed safety.

3.2.2. Conclusion

The differences in agronomic and phenotypic characteristics observed in plant health at growth stage R1 and days to maturity in the 2013 field trials between soybean FG72 and the conventional counterpart are further assessed for their potential environmental impact in Section 3.4.1.

The EFSA GMO Panel concluded that none of the differences identified in the composition of grain and forage between soybean FG72 and the conventional counterpart necessitated further assessment regarding food and feed safety.

3.3. Food/feed safety assessment

3.3.1. Evaluation of relevant scientific data

3.3.1.1. Effects of processing[39]

Soybean FG72 will be used for production and manufacturing of food and feed products like any other commercial soybean variety. Taking into account the compositional analysis, providing no indication of biologically relevant compositional changes except for the expression of the 2mEPSPS and HPPD W336 proteins in soybean FG72, the EFSA GMO Panel has no reason to assume that the characteristics of soybean FG72 and derived processed products would be different from those products derived from conventional soybean varieties, except for the presence of the 2mEPSPS and HPPD W336 proteins. The three major processed fractions produced from whole soybean are oil, protein-rich meal, and lecithin.

[39] Dossier: Part I—Section D7.6.

3.3.1.2. Toxicology[40]

Soybean FG72 expresses two new proteins, HPPD W336 and 2mEPSPS. The EFSA GMO Panel has previously assessed the 2mEPSPS protein (e.g. EFSA 2009a; EFSA GMO Panel 2014).

Proteins used for safety assessment[41]

Given the technical limitations in obtaining sufficient amounts of purified proteins in large enough quantities from soybean FG72, recombinant HPPD W336 produced in *Escherichia coli* was used for safety studies.

Prior to safety studies, a set of biochemical methods was carried out to demonstrate the equivalence between the soybean (leaves) and *E. coli*-derived HPPD W336. Purified proteins from these two sources were characterised and compared in terms of their physicochemical, structural and functional properties.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis showed that both plant and microbially derived HPPD W336 proteins migrated close to the expected molecular weight of approximately 40 kDa, and were immunoreactive to a specific polyclonal antibody, as shown by western blot analysis. Amino acid sequence analysis by liquid chromatography/mass spectrometry suggested that both proteins matched their expected sequence. These data also showed that only the N-terminal methionine of both proteins was truncated from the N-termini. In contrast, the C-termini were identical and fully matched the theoretical sequences. Sequence analysis and an experimental glycosylation assay showed that neither plant nor microbial HPPD W336 proteins were glycosylated. Functional equivalence was demonstrated by a biochemical *in vitro* activity assay that showed that both proteins had comparable activity for the intended herbicide.[42] Microbially produced HPPD W336 protein was also screened for its ability to utilise certain endogenous plant substrates.[43] The data demonstrated that it is unlikely that HPPD W336 has a metabolic impact within soybean FG72, as also indicated by the compositional analyses.

Based on these data, the EFSA GMO Panel accepts the use of the HPPD W336 protein produced in *E. coli* for the safety studies.

Toxicological assessment of the newly expressed proteins

The EFSA GMO Panel has assessed the 2mEPSPS protein in the context of previous applications for the placing on the EU market of GM crops and did not identify safety concerns (e.g. EFSA, 2009a; EFSA GMO Panel 2014). No scientific data have emerged which call for the EFSA GMO Panel to change its opinion. Updated bioinformatic analysis[44] of the amino acid sequence of the 2mEPSPS protein did not detect significant similarities to known toxic proteins.

The EFSA GMO Panel has not previously assessed the HPPD W336 protein. The applicant provided a number of studies made with the bacterial-derived protein to characterise and support the safety assessment of HPPD W336.

(a) Heat and pH stability

The thermal stability of HPPD W336 protein was assessed at different temperatures (up to 95 °C) over 60 minutes. Data showed that all activity was lost at ≥ 60 °C after 2.5 minutes and half of the activity was lost after 20 minutes at 45 °C. However there was no evidence of the thermal degradation of the

[40] Dossier: Part I—Section D7.8.
[41] Dossier: Part I—Section D7.8.1
[42] Additional information: 2/6/2015
[43] 4-Hydroxyphenylpyruvate, phenyl pyruvate, 3,4-dihydroxyphenylpyruvate, α-ketoisocaproate, α-keto-γ-(methyltio)-butyrate.
[44] Additional information: 23/3/2015.

protein itself, which remained intact when heated up to 90 °C for 60 minutes as shown by SDS-PAGE and western blot analysis.

The optimum temperature for activity was 25 °C and the optimum pH between 7.5 and 8.5. There was no activity under acidic conditions (pH < 7.0).

(b) *In vitro* degradation studies

The resistance to degradation by pepsin of the bacterial HPPD W336 protein was investigated in solutions at ~pH 1.2. The integrity of the test protein in probes taken at various time points was analysed by SDS-PAGE followed by protein staining or western blotting. The HPPD W336 protein was degraded by pepsin within 30 seconds.

(c) Bioinformatic studies

Bioinformatic analysis of the HPPD W336 amino acid sequence against known toxins showed significant identity to some proteins of bacterial origin, which are annotated as possible haemolysins (see Section 3.1.1.2). The HPPD W336 protein was tested for haemolytic potential *in vitro* and the protein, incubated with whole blood, was shown not to induce haemolysis.[45]

(d) Acute oral toxicity testing

No adverse effects were observed in a study on acute oral toxicity after administration of the HPPD W336 protein produced in *E. coli* at a single dose of 2 000 mg/kg body weight to female mice.

The EFSA GMO Panel is of the opinion that acute toxicity testing of the newly expressed proteins is of little value for the risk assessment of the repeated consumption of food and feed from GM plants by humans and animals.

(e) 28-day repeated dose oral toxicity study

The applicant provided a repeated-dose oral toxicity study using a protocol adapted from OECD Technical Guideline 407 (OECD, 1995). In this study the bacterial-derived HPPD W336 protein was administered by gavage to groups of individually-housed C57BLl/6J mice (five animals/sex/group) for 28 consecutive days. As no toxicity was anticipated, only one dose level, i.e. 1 000 mg/kg body weight per day, was applied, which is in accordance with the OECD Technical Guideline 407 (Limit test). The control group received bovine serum albumin (BSA) at the same dose level. A similarly designed 28-day study was provided by the applicant[46] showing that there were no relevant differences in parameters after administration by gavage of BSA or ovalbumin (both at 1 000 mg/kg body weight per day) or vehicle (aqueous solution) to C57BLl/6J mice, which confirmed that BSA is an appropriate control material for the testing of the HPPD W336 protein.

Analytical determinations indicated a mean concentration of 42.5 or 46.6 mg/mL of HPPD W336 or BSA respectively. The mean actual doses were 850 mg/kg body weight per day for HPPD W336 and 931 mg/kg body weight per day for BSA.

Both feed and water were provided *ad libitum*, except feed before blood sampling on study day 22 and prior to sacrifice when animals were diet fasted overnight. During the study, all animals were checked at least daily for mortality or general clinical signs and weekly for body weight and feed consumption. Detailed clinical observations were conducted on all animals pre-exposure and then weekly. Ophthalmoscopy was carried out before the start and at the end of the treatments. At the end of the treatment period, haematological[47] and serum chemistry analyses[48] were performed. Coagulation analysis was not performed. All animals were sacrificed and underwent a detailed necropsy

[45] Additional information: 3/6/2015.
[46] Additional information: 13/6/2012.
[47] Red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular haemoglobin concentration, white blood cell count and differential count evaluation and platelet count.
[48] Total bilirubin, urea, creatinine, total protein, albumin and total cholesterol concentrations, and aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase activities.

examination. A selection of organs and tissues from all animals were collected, weighed[49] and subjected to histopathological examination.[50]

One female animal of the HPPD W336-treated group showing reduced motor activity and laboured respiration was killed for humane reasons on day 6 of the treatment period. Lesions were noted in the thorax and in the lungs of this animal which indicate a gavage error and are not considered related to the treatment with HPPD W336. Apart from this, no clinically relevant effects were noted in the regular observations of the animals. Ophthalmoscopic examinations revealed no differences between the groups. Body weights, body weight gains and feed consumption were comparable. No statistically significant differences between the test and the control group were noted in haematology and urine analyses. In clinical chemistry plasma aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) activities were significantly lower in males administered the test substance (–22% and –31% respectively). A lower ASAT or ALAT activity is not considered to be an indication of an adverse effect. Significantly lower mean liver weights (10% absolute and 12% in relation to body weight but not in relation to brain weight) and higher mean spleen weights (25% absolute and in relation to brain weight but not in relation to body weight) were noted in males given HPPD W336 in comparison with controls. In the absence of relevant changes in the available related parameters, these are considered not adverse.

The EFSA GMO Panel notes that the study deviated from OECD Test Guideline 407 in that coagulation analysis was not performed and relevant organ weight determinations (e.g. thymus, heart) and histopathology (e.g. heart, brain) were not done. Regarding the assessed endpoints, no adverse effects were noted after administration of the HPPD W336 protein to mice for 28 days at a target dose of 1 000 mg/kg body weight per day (mean actual dose 850 mg/kg body weight per day).

Considering that no adverse effects were noted in the assessed parameters in a 28-day oral toxicity study and that issues identified by bioinformatics analysis were addressed (see Section 3.3.1.2.c), the EFSA GMO Panel concludes that there are no safety concerns with regard to the HPPD W336 protein expressed in soybean FG72.

Toxicological assessment of components other than newly expressed proteins

No new constituents other than 2mEPSPS and HPPD W336 proteins are expressed in soybean FG72 and no relevant changes in the composition of GM soybean were detected in the comparative compositional analysis (see Section 3.2.1.3).

3.3.1.3. Animal studies with the food/feed derived from GM plants

Subchronic feeding study in rats[51]

The applicant provided a 90-day feeding study with Wistar Rj:WI (IOPS HAN) rats, which was performed using a protocol adapted from OECD Technical Guideline 408 (OECD, 1998). Groups of 10 male and 10 female animals (housed five per sex per cage) received *ad libitum* diets containing 5 % or 15 % (w/w) toasted meal derived from seed of soybean FG72 for the treatment period. The diet containing 5 % meal from the GM soybean was supplemented with meal from the conventional counterpart (Jack) in order to achieve a total meal content of 15 %. The control group was administered a diet containing 15 % meal from the conventional counterpart, and an additional control group received feed with 15 % meal from a commercial non-GM soybean variety (Stine 3000-0). Animals were housed in cages with five rats of the same sex per cage, but the data analysis considered the individual animal as the experimental unit (EFSA Scientific Committee, 2011). As the cage should

[49] Adrenal gland, brain, kidney, liver, ovary, spleen, testis, uterus (including the cervix).
[50] Adrenal glands, bone marrow (sternum), epididymidis, intestine (duodenum, jejunum, ileum with Peyer's patches, caecum, colon, rectum), kidney, liver, lymph node (mesenteric), macroscopic findings, ovary, pancreas, spleen, stomach, testis, thymus, thyroid glands (and parathyroids), uterus (including cervix), vagina.
[51] Dossier: Part I—Section D7.8.4; additional information: 13/6/2012.

be considered the experimental unit and because of the low number of experimental units per treatment (two per sex), an appropriate statistical analysis of the data is not possible. Consequently, the GMO Panel is unable to draw relevant conclusions from this study. The EFSA GMO Panel considers that the study was not needed on the basis of molecular characterisation and comparative assessment.

42-day feeding study in chickens for fattening

A 42-day feeding study with a total of 420 male and female chickens for fattening (two-day old Ross 308) was provided.[52] The birds were randomly allocated to three dietary treatments with 140 chickens per treatment (seven pens/treatment per gender, 10 birds per pen). Soybean FG72 (verified by PCR in seeds and diets) was compared with its conventional counterpart and with one non-GM commercial variety (Stine 3000-0). Soybeans were harvested from the 2008 field trial. The starter, grower and finisher diets consisted of about 20 % toasted meal from the tested soybeans. The other components were mainly corn (55-63%) and a commercial 48 % crude protein (CP) soybean meal (9-17% added in addition to the tested soybean meal). Before feed formulation, all soybean seeds were analysed for proximates, amino acids, minerals, vitamin E, antinutrients and pesticides. The diets were isonitrogenous, isocaloric and balanced for limiting amino acids (confirmed by analysis). The starter diets (about 22 % CP, 3050 kcal metabolisable energy (ME)/kg) were given until day 7, grower diets (about 21 % CP, 3100 kcal ME/kg) from day 8 to day 21, and finisher diets (about 19 % CP, 3190 kcal ME/kg) from day 22 until the end. Feed in pelleted form and water were provided for *ad libitum* intake.

Chickens were observed daily for clinical signs; deaths were recorded and necropsy performed on all birds found dead. Body weight was measured at the start and on days 7, 21, 35 and 42. Feed intake was determined weekly for each pen. On day-43 and day-44 three birds per pen were taken for carcass evaluation (dressing percentage weight of thighs, breast, wings, drums and abdominal fat). On day-43 and day-44, males and females were processed for carcass evaluation (dressing percentage weight of thighs, breast, wings, legs and abdominal fat). Data were statistically analysed by comparing the soybean FG72 group to its conventional counterpart, and comparing the two non-GM groups.

Overall mortality was high (10.7 %) with no significant difference between the groups. No significant treatment × sex interaction was detected for performance characteristics. Overall no significant difference was seen in final body weight (about 2.7 kg), feed intake (about 4.7 kg), or feed:gain ratio (about 1.75) between the soybean FG72 and the conventional counterpart, or the conventional counterpart and the non-GM variety. No significant differences were observed in carcass parameters.

The EFSA GMO Panel is able to draw only a limited conclusion from this study, because of the high mortality observed and the low inclusion rate of the test items. However, the performance data would suggest that the meal derived from GM soybean FG72 is as nutritious as those derived from the conventional counterpart and the single commercial non-GM soybean tested.

3.3.1.4. 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.

[52] Dossier: Part I—Section D7.8.4; additional information: 12/6/2012.

Assessment of allergenicity of the newly expressed proteins[53]

A weight-of-evidence approach was followed, taking into account all of the information obtained on the newly expressed proteins, as no single piece of information or experimental method yield sufficient evidence to predict allergenicity (Codex Alimentarius, 2009; EFSA, 2006a).

The *hppdPfW336* gene originates from *P. fluorescens*, an ubiquitous microorganism that is not considered to be a common allergenic source. The *2mepsps* gene originates from *Zea mays* L., which is not considered to be a common allergenic food.

Updated bioinformatic analyses[54] of the amino acid sequences of the HPPD W336 and 2mEPSPS 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 also performed analyses searching for matches of eight contiguous identical amino acid sequences between the HPPD W336 and 2mEPSPS proteins and known allergens, which confirmed the outcome of the previous bioinformatic analysis.

The study on resistance to degradation of the HPPD W336 protein by pepsin has been described in Section 3.3.1.2.

The EFSA GMO Panel has previously evaluated the safety of the 2mEPSPS protein in the context of several other applications and no concerns about allergenicity were identified (e.g. EFSA, 2009a; EFSA GMO Panel, 2014).

There is no information available on the structure or function of the newly expressed HPPD W336 and 2mEPSPS proteins that would suggest an adjuvant effect of the individual proteins or their mixture in soybean FG72 resulting in or increasing an eventual IgE response to a bystander protein.

In the context of the present application, the EFSA GMO Panel considers that there are no indications that the newly expressed HPPD W336 and 2mEPSPS proteins, individually or their mixture, in soybean FG72 may be allergenic.

Assessment of allergenicity of the whole GM plant[55]

Soybean is considered to be a common allergenic food[56] (OECD, 2012). Therefore, any potential change in the endogenous allergenicity of the GM plant when compared with that of its comparator(s) should be assessed (EFSA, 2006a). The applicant performed *in vitro* allergenicity studies with extracts from soybean FG72, its conventional counterpart and non-GM reference soybean varieties.

Specifically, the applicant performed two-dimensional (2D) electrophoresis of extracts of soybean FG72, its conventional counterpart and two non-GM reference soybean varieties followed by western blotting using individual sera from six humans allergic to soybean. This study showed no meaningful differences in the IgE-binding patterns between the extracts of proteins derived from soybean FG72, its conventional counterpart and non-GM soybean reference varieties.

In addition, Rouquié et al. (2010) published a study in which the endogenous allergenicity of soybean FG72, its conventional counterpart and three commercial non-GM soybean lines was assessed using a 2D gel electrophoresis approach (Coomassie blue-stained). The authors concluded that no significant differences between allergen content in soybean FG72 and its non-GM comparators were identified.

[53] Dossier: Part I—Section A5; additional information: 23/3/2015.
[54] Additional information: 23/3/2015.
[55] Dossier: Part I—Section A5.
[56] 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.

The applicant also performed one-dimensional (1D) electrophoresis of extracts of soybean FG72, its conventional counterpart and three reference non-GM soybean varieties, followed by western blot analysis using individual sera from eight allergic humans to soybean. Inhibition ELISA studies were also carried out using pooled sera from four humans allergic to soybean,[57] or using one individual serum.[58] The EFSA GMO Panel has previously indicated the limitations of the 1D-PAGE gels and the use of pooled sera for the allergenicity assessment (see Annex 4 and Annex 5 of EFSA GMO Panel, 2010b).

Considering all available information, the EFSA GMO Panel considers that there is no evidence that the genetic modification might significantly change the overall allergenicity of soybean FG72.

3.3.1.5. Nutritional assessment of GM food/feed[59]

The intended trait of soybean FG72 is herbicide tolerance, with no intention of altering the nutritional parameters. Comparison of the nutrients and anti-nutrients of this GM soybean with its conventional counterpart did not identify differences that would require further safety assessment (see Section 3.2.1.3). Compositional data indicate that soybean FG72 would be expected to deliver the same nutrition as its conventional counterpart and other non-GM reference varieties.

3.3.1.6. Post-market monitoring of GM food/feed[60]

Given the absence of safety concerns identified in this assessment, the EFSA GMO Panel considers that post-market monitoring of GM food/feed produced from soybean FG72 is not necessary.

3.3.2. Conclusion

The safety assessment identified no concerns regarding the potential toxicity or allergenicity of the newly expressed 2mEPSPS and HPPD W336 protein in soybean FG72, and found no evidence that the genetic modification might significantly change the overall allergenicity of soybean FG72. Based on the comparative analysis, the nutritional characteristics of food and feed derived from soybean FG72 is not expected to differ from that of food and feed derived from non-GM soybean varieties. The EFSA GMO Panel concludes that soybean FG72 is as safe and nutritious as its conventional counterpart and non-GM reference soybean varieties.

3.4. Environmental risk assessment and monitoring plan

3.4.1. Evaluation of relevant scientific data

Considering the scope of application EFSA-GMO-BE-2011-98 (which excludes cultivation), the environmental risk assessment of soybean FG72 is concerned mainly with ingestion by animals and their manure and faeces leading to exposure of the gastrointestinal tract and soil microorganisms to recombinant DNA and with accidental release into the environment of viable soybean FG72 seeds during transport and processing.

Soybean FG72 was developed to provide tolerance against isoxaflutole- and glyphosate-based herbicides by expressing the HPPD W336 and 2mEPSPS proteins, respectively.

As the scope of the present application excludes cultivation, environmental concerns in the EU related to the use of these herbicides on soybean FG72 do not apply.

[57] Dossier: Part I—Section/Section A5.
[58] Additional information: 13/6/2012.
[59] Dossier: Part I—Section D7.10.
[60] Dossier: Part I—Section D7.11.

3.4.1.1. Environmental risk assessment

Potential unintended effects on plant fitness due to the genetic modification[61]

Cultivated soybean species (*Glycine max* (L.) Merr.) belong to the sub-genus *Soja* of the genus *Glycine*. The species originated from eastern Asia and is a highly domesticated crop (Lu, 2005). The major worldwide soybean producers are the USA, Brazil, Argentina, China and India.[62] In the EU, soybean is mainly cultivated in Italy, Romania, France, Croatia, Austria and Hungary (Dorokhov et al. 2004; Krumphuber, 2008).[63] Cultivated soybean seeds rarely display any dormancy characteristics, and only under certain environmental conditions grow as volunteers in the year following cultivation. If volunteers occur, they do not compete well with the succeeding crop, and can easily be controlled mechanically or chemically (OECD, 2000). Although the introduction of isoxaflutole and glyphosate tolerance genes would reduce the chemical options available to control volunteer soybean plants, there will still be an ample choice of alternative herbicides in many crops. In the field, shattered soybean seeds usually do not overwinter owing to predation, rotting or fatal germination or as a result of management practices prior to planting the subsequent crop, resulting in death (Owen, 2005).

The herbicide tolerance traits can be regarded as providing only a potential agronomic and selective advantage for this GM soybean plant where and when isoxaflutole- and/or glyphosate-based herbicides are applied. Survival of soybean plants outside cultivation is mainly limited by a combination of low competitiveness, absence of a dormancy phase and susceptibility to plant pathogens and cold climatic conditions. As these general characteristics are unchanged in soybean FG72, and herbicide tolerance provides a selective advantage only where the target herbicides are applied, this is unlikely to increase their ability to survive over the seasons. Therefore, it is considered very unlikely that soybean FG72 will differ from conventional soybean varieties in its ability to survive until subsequent seasons or to establish feral populations under European environmental conditions.

As described in Section 3.2.1.1, the 2013 field trials fulfilled the EFSA guidance (EFSA GMO Panel, 2011a) and were considered to gather information on the agronomic and phenotypic characteristics of soybean FG72. The 2011 field trials[64] did not include GM plots exposed to the intended herbicides to which soybean FG72 is tolerant. However, these trials provided additional information on the agronomic and phenotypic characteristics of soybean FG72 in comparison with its conventional counterpart MST45.

The soybean plants tested in the 10 locations of the 2008 field trial (see Section 3.2.1.1) were assessed for seed phenotypic characteristics and germination capacity of soybean FG72 compared with the conventional counterpart, Jack, under controlled conditions.

Pollen morphology, viability and germination were evaluated, comparing characteristics of soybean FG72 with its conventional counterpart, Jack, under controlled conditions in 2009.[65]

Although some differences were observed (Section 3.2.1.2) under specific environmental conditions, they were not consistent and do not indicate a significant plant response associated with the event or any change in fitness.

Considering the scope of the application, special attention is given to those agronomic characteristics that may affect the survival, establishment and fitness of FG72 soybean seeds which could be accidentally released into the environment: early and final stand count, seedling vigour, plant height, days to flowering, yield, 100 seed weight, shattering and pollen germination. None of the observed differences (except plant health at growth stage R1 and days to maturity in the 2013 field trial) were indicative of a consistent plant response associated with the event. The observed differences are

[61] Dossier: Part I—Sections D3.4, E3.1.
[62] http://faostat.fao.org/ (based on 2013 data production, accessed April 2015).
[63] http://ec.europa.eu/eurostat/web/agriculture/data/database (based on 2014 data production, accessed April 2015).
[64] Additional information: 13/6/2012.
[65] Dossier: Part I—Section D7.4.

unlikely to be biologically significant in terms of increased weediness potential. This is further confirmed by the 2011 field trials, in which no biologically relevant differences were observed between soybean FG72 and its conventional counterpart.

From the data presented in the application, there is no indication of an increased weediness potential of soybean FG72 compared to conventional soybean and it can be considered that soybean FG72 has no altered survival, multiplication or dissemination characteristics compared with its conventional counterpart except in the presence of isoxaflutole- and glyphosate-based herbicides.

In addition to the data presented by the applicant, the EFSA GMO Panel is not aware of any scientific report of increased spread and establishment of existing GM soybeans or any change in survival capacity, including overwintering (Dorokhov et al., 2004; Owen, 2005; Bagavathiannan and Van Acker, 2008; Lee et al., 2009).

The EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects of the soybean FG72 in Europe will not be different from that of conventional soybean varieties.

Potential for gene transfer[66]

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 seed dispersal and cross-pollination.

(a) Plant to bacteria gene transfer

Genomic plant DNA is a component of several food and feed products derived from soybean. It is well documented that DNA present in food and feed becomes substantially degraded during processing and digestion in the human or animal gastrointestinal tract. However, a low level of exposure of fragments of ingested DNA, including the recombinant fraction of such DNA, to microorganisms in the digestive tract of humans, domesticated animals and other environments exposed to the GM plant or plant material is expected.

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

A successful HGT would require stable insertion of the transgene sequences into a bacterial genome and a selective advantage conferred on the transformed host. The only known mechanism that facilitates horizontal transfer of non-mobile, chromosomal DNA fragments into bacterial genomes is homologous recombination. This requires the presence of stretches of DNA sequences that are similar in the recombining DNA molecules and, in addition to substitutive gene replacement, facilitates the insertion of non-homologous DNA sequences if their flanking regions share sequence similarity with bacterial sequences in the recipient.

Soybean FG72 was developed through direct gene transfer methodology and contains two genetic elements sharing homology to those in bacteria, i.e. the coding sequence of the 4-hydroxyphenylpyruvate dioxygenase (*hppdPfW336*) of *P. fluorescens* and the contiguous 3' untranslated region of the nopaline synthase gene (*nos* terminator) from the T-DNA of *A. tumefaciens*. Both bacterial species, *P. fluorescens* and *A. tumefaciens*, typically occur in soil, water and plant rhizospheres and they are not considered to be prevalent in the main receiving environment, i.e. the gastrointestinal tract of humans or animals. However, occurrence of the recombinant genes outside their immediate receiving environment in the habitats cannot be ruled out (Hart et al., 2009) and is therefore also considered here.

[66] Dossier: Part I—Section A2.2.2, E3.1, E3.2; additional information:17/6/2013 and 20/4/2015.

On a theoretical basis (i.e. without any study providing experimental evidence for HGT in the case of GM food and feed derived from soybean FG72 or any other GM plant), it can be assumed that, as an extremely rare event, homologous recombination can occur between the recombinant *hppdPfw336* gene or the *nos* terminator from soybean FG72 and their natural variants, as they may occur in *P. fluorescens* (for *hppdPfw336*) and *A. tumefaciens* (for *nos* terminator) present in the environment. Such recombination events, however, would only replace natural variants (substitutive recombination) and are therefore unlikely to provide any new property connected to a selective advantage for the recipient organisms (EFSA, 2009b).

Soybean FG72 also includes the coding sequence of the *2mepsps* gene which originates from maize (*Zea mays* L.). Because this recombinant gene is not of bacterial but of plant origin, HGT is expected to be in the same range as other plant genes and, thus, the likelihood can be expected to be negligible.

In addition to homology-based recombination processes, illegitimate recombination that does not require DNA similarity between the recombining DNA molecules is theoretically possible. However, the transformation rates for illegitimate recombination are considered to be 10^{10}-fold lower than for homologous recombination (Hülter and Wackernagel, 2008; EFSA, 2009b). Illegitimate recombination events have not been detected in studies that have exposed bacteria to high concentrations of GM plant DNA (EFSA, 2009b). Thus, this process, in comparison with homologous recombination, is not considered to contribute significantly to HGT events. In comparison with the above-described homology-facilitated recombination processes, the contribution of illegitimate recombination is extremely low.

The EFSA GMO Panel concludes that the *hppdPfw336* gene from soybean FG72 may, on a theoretical basis, replace similar genes by homologous recombination with environmentally present *P. fluorescens* or *A. tumefaciens* or, theoretically, other bacterial species too. The exposure of DNA from soybean FG72 to *P. fluorescens* and *A. tumefaciens* is expected to be very low, considering that both are not common members of the gut microbiota. Owing to the natural occurrence of the *hppd* gene or gene variants with high similarity in bacteria in the environment, a low-level gene transfer or gene replacement in *P. fluorescens* or other bacteria, caused by soybean FG72, is not regarded as conferring a new trait or selective advantage. Considering the scope and the above assessment, the EFSA GMO Panel has therefore not identified any concern associated with HGT from soybean FG72 to bacteria.

(b) Plant to plant gene transfer

Considering the scope of application EFSA-GMO-BE-2011-98 and the physical characteristics of soybean seeds, a possible pathway of gene dispersal is from grain spillage and pollen of occasional feral GM soybean plants originating from accidental seed spillage during transport and/or processing.

The genus *Glycine* is divided into two distinct sub-genera: *Glycine* and *Soja*. Soybean is in the subgenus *Soja*. The sub-genus *Glycine* contains 16 perennial wild species, while the cultivated soybean, *Glycine max*, and its wild and semi-wild annual relatives, *Glycine soja* and *Glycine gracilis*, are classified in the sub-genus *Soja* (OECD, 2000). Owing to the low level of genomic similarity among species of the genus *Glycine*, *Glycine max* can cross only with other members of *Glycine* subgenus *Soja* (Hymowitz et al., 1998; Lu, 2005). Hence, the three species of the sub-genus *Soja* are capable of cross-pollination and the hybrid seed that is produced can germinate normally and produce plants with fertile pollen and seed (Abe et al., 1999; Nakayama and Yamaguchi, 2002). However, since *G. soja* and *G. gracilis* are indigenous to Australia, China, Japan, Korea, the Philippines, the far eastern region of Russia, the South Pacific and Taiwan, and as they have not been reported in other parts of the world where the cultivated soybean is grown (Dorokhov et al., 2004; Lu, 2005), the plant-to-plant gene transfer from soybean is restricted to cultivated areas and the occasional soybean plants resulting from seed spillage in the EU.

Soybean (*Glycine max*) is an annual almost completely self-pollinating crop in the field, which has a percentage of cross-pollination usually lower than 1 % (Weber and Hanson, 1961; Caviness, 1966;

Ray et al., 2003; Lu, 2005; Yoshimura et al., 2006; Abud et al., 2007). Soybean pollen dispersal is limited because the anthers mature in the bud and directly pollinate the stigma of the same flower (OECD, 2000). However, cross-pollination rates as high as 6.3 % have been reported for closely spaced plants (Ray et al., 2003), suggesting the potential for some within-crop gene flow in soybean. These results indicate that natural cross-pollination rates can fluctuate significantly among different soybean varieties under particular environmental conditions such as a favourable climate for pollination and an abundance of pollinators (Gumisiriza and Rubaihayo, 1978; Ahrent and Caviness, 1994; Kikuchi et al., 1998; Ray et al., 2003; Lu, 2005).

Plant-to-plant gene transfer could therefore occur under the following scenario: imports of soybean FG72 seeds; processing outside of importing ports; transport in regions of soybean production in Europe; spillage of GM seeds during transport; germination and development of spilled seeds within soybean fields or in the very close vicinity of cultivated soybean fields; and overlap of flowering periods and environmental conditions favouring cross-pollination. The overall likelihood of cross-pollination between GM soybean plants and cultivated soybean is therefore extremely low. Apart from seed production areas, GM plants and plants derived from out-crossing with this GM soybean will not persist overtime. Dispersal of soybean seeds by animals is not expected owing to the characteristics of the seed, but accidental release into the environment of seeds may occur during transport and processing for food, feed and industrial uses. However, even in soybean fields, seeds usually do not survive during the winter owing to predation, rotting, germination or due to management practices prior to planting the subsequent crop (Owen, 2005). It is expected that the survival rate of soybean seeds accidentally spilled outside cultivated fields would be even lower than in cultivated fields.

The EFSA GMO Panel takes into account the fact that this application does not include cultivation of the soybean within the EU, so that likelihood of cross-pollination between cultivated soybean and occasional soybean plants resulting from seed spillage is considered to be extremely low, considering also the low rate of natural cross-pollination in soybean, usually well below 6 % under field conditions (Ray et al., 2003). However, in countries cultivating this GM soybean and producing seed for export, there is a potential for admixture in seed production and thus the introduction of GM seeds through this route.

In conclusion, as soybean FG72 has very unlikely altered survival, multiplication or dissemination characteristics, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes from this GM soybean in Europe is extremely unlikely to differ from that of conventional soybean varieties.

Interactions of the GM plant with target organisms[67]

Interactions of soybean FG72 with target organisms are not considered an issue by the EFSA GMO Panel, as there are no target organisms.

Interactions of the GM plant with non-target organisms[68]

Considering the scope of application EFSA-GMO-BE-2011-98, which exclude cultivation and due to the low level of exposure to the environment, potential interactions of the GM plant with non-target organisms were not considered a relevant issue by the EFSA GMO Panel.

Interactions with the abiotic environment and biochemical cycles[69]

Considering the scope of application EFSA-GMO-BE-2011-98, which exclude cultivation and due to the low level of exposure to the environment, potential interactions with the abiotic environment and biogeochemical cycles were not considered a relevant issue by the EFSA GMO Panel.

[67] Dossier: Part I—Section E3.3.
[68] Dossier: Part I—Section E3.4.
[69] Dossier: Part I—Section E3.6.

3.4.1.2. Post-market environmental monitoring[70]

The objectives of a post-market environmental monitoring (PMEM) plan, according to Annex VII of Directive 2001/18/EC, are (1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the environmental risk assessment 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 environmental risk assessment.

Monitoring is related to risk management, and thus a final adoption of the PMEM plan falls outside the mandate of EFSA. However, the EFSA GMO Panel gives its opinion on the scientific content of the PMEM provided by the applicant (EFSA, 2006b; EFSA GMO Panel, 2011b). The potential exposure to the environment of soybean FG72 would be through faecal material from animals fed the GM soybean or through accidental release into the environment of GM soybean seeds (e.g. during transport and/or processing). The EFSA GMO Panel is aware that, owing to the physical characteristics of soybean seeds and methods of transport, accidental spillage cannot be excluded. Hence, it is important that appropriate management systems are in place to restrict seeds of soybean FG72 entering cultivation, as this would require specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

The PMEM plan proposed by the applicant includes (1) the description of an approach involving operators (federations involved in soybean import and processing) reporting to the applicant via a centralised system any observed adverse effect(s) of GMOs on human health and the environment; (2) a coordinating system established by EuropaBio for the collection of the information recorded by the various operators; and (3) the use of networks of existing surveillance systems (Lecoq et al., 2007; Windels et al., 2008). The applicant proposes to submit a PMEM report on an annual basis.

The EFSA GMO Panel is of the opinion that the PMEM plan proposed by the applicant is in line with the scope of application EFSA-GMO-BE-2011-98, as the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. No case-specific monitoring is necessary. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

3.4.2. Conclusion

The scope of application EFSA-GMO-BE-2011-98 is for food and feed uses, import and processing and does not include cultivation. Considering the scope of soybean FG72, the environmental risk assessment is concerned with indirect exposure mainly through manure and faeces from animals fed with seeds or feed produced by soybean FG72 and with the accidental release into the environment of viable seeds produced by soybean FG72 during transport and processing.

In the case of accidental release into the environment of viable seeds of soybean FG72 during transport and processing, there are no indications of an increased likelihood of establishment and spread of feral soybean FG72 plants, except in the presence of isoxaflutole- and/or glyphosate-based herbicides. In addition, the low levels of environmental exposure of these GM soybean plants and the newly expressed proteins through other routes indicate that the risk to non-target organisms is extremely low. The EFSA GMO Panel considers that it is unlikely that the recombinant DNA in soybean FG72 transfers to bacteria. A risk caused by a rare but theoretically possible transfer of the recombinant gene from soybean FG72 to bacteria in the environment has not been identified by the GMO Panel, because expression of these genes would not provide any selective advantage in the context of its intended use.

The PMEM plan provided by the applicant and the reporting intervals are in line with the scope of application EFSA-GMO-BE-2011-98 and the requirements of the EFSA GMO Panel for post-market

[70] Dossier: Part I—Section E4.

environmental monitoring of GM plants. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

4. Conclusions

The EFSA GMO Panel was asked to carry out a scientific assessment of soybean FG72 for import, processing, and food and feed uses in accordance with Regulation (EC) No 1829/2003.

The molecular characterisation data provided for soybean FG72 did not give rise to safety issues.

The differences in agronomic and phenotypic characteristics observed in plant health at growth stage R1 and days to maturity were not consistent and did not indicate a significant plant response associated with the event or any change in fitness. None of the differences identified in the composition of grain and forage between soybean FG72 and the conventional counterpart necessitated further assessment regarding food and feed safety.

The safety assessment identified no concerns regarding the potential toxicity or allergenicity of the newly expressed 2mEPSPS and HPPD W336 protein in soybean FG72, and found no evidence that the genetic modification might significantly change the overall allergenicity of soybean FG72. Based on the comparative analysis, the nutritional characteristics of food and feed derived from soybean FG72 is not expected to differ from that of food and feed derived from non-GM soybean varieties.

The EFSA GMO Panel concludes that soybean FG72, assessed in this application, is as safe and nutritious as its conventional counterpart and the non-GM soybean reference varieties tested. In addition, the EFSA GMO Panel found no indication that the introduction of the event FG72 into other soybean varieties would affect its safety with respect to potential effects on human and animal health.

Considering the scope of soybean FG72, which excludes cultivation, there is no requirement for scientific assessment of possible environmental effects associated with the cultivation of this GM soybean. In the event of accidental release into the environment of viable seeds of soybean FG72 (e.g. during transport and processing), there are no indications of an increased likelihood of establishment and spread of feral soybean plants, except in the presence of isoxaflutole- and/or glyphosate-based herbicides. The low levels of environmental exposure of these GM soybean plants indicate that the risk to non-target organisms is extremely low. The unlikely but theoretically possible transfer of the recombinant gene from soybean FG72 to environmental bacteria does not give rise to concern owing to the lack of a selective advantage in the context of its scope. The PMEM plan provided by the applicant and the reporting intervals are in line with the scope of application EFSA-GMO-BE-2011-98 and requirements of the EFSA GMO Panel for post-market environmental monitoring of GM plants. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

In conclusion, the EFSA GMO Panel considers that the information available for soybean FG72 addresses the scientific comments raised by Member States and that soybean FG72, as described in this application, is as safe as its conventional counterpart and non-GM soybean reference varieties with respect to potential effects on human and animal health and the environment in the context of the scope of this application.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of Belgium, received on 24 June 2011 concerning a request for authorisation for placing on the market of genetically modified soybean FG72 submitted under Regulation (EC) No 1829/2003 by Bayer CropScience and M.S. Technologies, LLC (application reference EFSA-GMO-BE-2011-98).

2. Acknowledgement letter dated 19 July 2011 from EFSA to the Competent Authority of Belgium.

3. Letter from EFSA to applicant dated 27 July 2011 requesting additional information under completeness check.

4. Letter from applicant to EFSA received on 2 September 2011 providing additional information under completeness check.

5. Letter from EFSA to applicant dated 22 September 2011 requesting additional information under completeness check.

6. Letter from applicant to EFSA received on 5 October 2011 providing additional information under completeness check.

7. Letter from EFSA to applicant dated 24 October 2011 delivering the 'Statement of Validity' of the application for the placing on the market of genetically modified soybean FG72 (EFSA-GMO-BE-2011-98) submitted in accordance with Regulation (EC) No 1829/2003 by Bayer CropScience and M.S. Technologies, LLC.

8. Letter from EFSA to applicant dated 8 February 2012 requesting additional information and stopping the clock.

9. Letter from EFSA to applicant dated 4 April 2012 requesting additional information and maintaining the clock stopped.

10. Letter from applicant to EFSA received on 8 May 2012 providing additional information.

11. Letter from applicant to EFSA received on 13 June 2012 providing additional information.

12. Letter from EFSA to applicant dated 6 December 2012 requesting additional information and maintaining the clock stopped.

13. Letter from applicant to EFSA received on 2 April 2013 providing additional information.

14. Letter from applicant to EFSA received on 28 August 2013 requesting clarifications on the progress of the application.

15. Letter from EFSA to applicant dated 29 August 2013 requesting additional information and maintaining the clock stopped.

16. Letter from EFSA to applicant dated 2 September 2013 providing clarifications on the progress of the application.

17. Letter from applicant to EFSA received on 23 September 2013 providing a timeline for submission of responses and requesting further clarifications on the progress of the application.

18. Letter from EFSA to applicant dated 25 October 2013 providing clarifications on the progress of the application.

19. Letter from applicant to EFSA received on 29 April 2014 providing additional information and spontaneous supplementary information.

20. Letter from EFSA to applicant dated 27 May 2014 requesting additional information and maintaining the clock stopped.

21. Letter from applicant to EFSA received on 17 July 2014 providing additional information.

22. Letter from EFSA to applicant dated 4 September 2014 requesting additional information and maintaining the clock stopped.

23. Letter from applicant to EFSA received on 27 October 2014 providing additional information.

24. Letter from applicant to EFSA received on 9 January 2015 requesting clarifications on the scientific review and clock status of the application.

25. Letter from EFSA to applicant dated 22 January 2015 re-starting the clock.

26. Letter from EFSA to applicant dated 10 February 2015 providing clarifications on the scientific review and clock status of the application.

27. Letter from EFSA to applicant dated 19 February 2015 requesting additional information and stopping the clock.

28. Letter from EFSA to applicant dated 3 March 2015 requesting additional information and maintaining the clock stopped.

29. Letter from applicant to EFSA received on 23 March 2015 providing additional information.

30. Letter from applicant to EFSA received on 1 April 2015 providing additional information.

31. Letter from EFSA to applicant dated 22 May 2015 requesting additional information and maintaining the clock stopped.

32. Letter from applicant to EFSA received on 3 June 2015 providing additional information.

33. Letter from EFSA to applicant dated 23 June 2015 re-starting the clock.

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