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SCIENTIFIC OPINION

Scientific Opinion on application EFSA-GMO-NL-2011-93 for the placing on the market of the herbicide-tolerant genetically modified soybean MON 87708 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto[1]

EFSA Panel on Genetically Modified Organisms (GMO)[2,3]

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ABSTRACT

Soybean MON 87708 contains a single insert consisting of the *dmo* expression cassette. The DMO (dicamba mono-oxygenase) proteins confer tolerance to dicamba-based herbicides. Bioinformatic analyses of the inserted DNA and flanking regions do not raise safety issues. The levels of DMO proteins in soybean MON 87708 have been sufficiently analysed. The stability of the genetic modification has been demonstrated. No differences were identified in the compositional data of forage and seeds obtained from soybean MON 87708 or in its agronomic and phenotypic characteristics that would require further assessment with regard to safety by the GMO Panel. The safety assessment identified no concerns regarding the potential toxicity and allergenicity of the DMO proteins, or of soybean MON 87708. The compositional data indicating that soybean MON 87708 is as nutritious as non-GM soybean varieties were supported by the outcome of a chicken study. There are no indications of an increased likelihood of establishment and spread of feral GM soybean plants. Considering the scope of this application, potential interactions of soybean MON 87708 with the biotic and abiotic environment were not considered to be an issue. Environmental risks associated with an unlikely but theoretically possible horizontal gene transfer from soybean MON 87708 to bacteria have not been identified. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of soybean MON 87708. In conclusion, the GMO Panel considers that the information available for soybean MON 87708 addresses the scientific comments raised by the Member States, and that the soybean MON 87708, 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 or the environment, in the context of its intended uses.

[1] On request from the Competent Authority of the Netherlands for an application (EFSA-GMO-NL-2011-93) submitted by Monsanto, Question No EFSA-Q-2011-00122, adopted on 12 September 2013.

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[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Molecular Characterisation, Food and Feed Safety Assessment and Environmental Risk Assessment on GMO applications, including Christer Andersson, Annette Poëting, Sirpa Kärenlampi and Fabien Nogué for the preparatory work on this scientific opinion, and EFSA staff: Zoltán Divéki, Christina Ehlert, Antonio Fernández Dumont and Sylvie Mestdagh for the support provided to this scientific opinion.

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KEY WORDS

GMO, soybean MON 87708, Regulation (EC) No 1829/2003, DMO protein, food and feed safety, environment, import and processing

SUMMARY

Following the submission of an application (EFSA-GMO-NL-2011-93) under Regulation (EC) No 1829/2003[4] from Monsanto, 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 genetically modified (GM) herbicide-tolerant soybean MON 87708 (Unique Identifier MON-877Ø8-9) for food and feed uses, import and processing.

In delivering its Scientific Opinion the EFSA GMO Panel considered the application EFSA-GMO-NL-2011-93, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications. The scope of application EFSA-GMO-NL-2011-93 is for food and feed uses, import and processing of soybean MON 87708 within the European Union (EU), as for any other non-GM soybean, but excludes cultivation in the EU. The EFSA GMO Panel evaluated soybean MON 87708 with respect to the scope and the appropriate principles described in its Guidance Documents for the risk assessment of GM plants and derived food and feed, and on the post-market environmental monitoring of GM plants. The scientific evaluation included the molecular characterisation of the inserted DNA and the analysis of the expression of the corresponding proteins. An evaluation of the comparative analysis of compositional, phenotypic and agronomic characteristics was undertaken, and the safety of the newly expressed protein and the whole food/feed was evaluated with respect to potential toxicity, allergenicity and nutritional wholesomeness. Evaluation of the environmental impacts and of the post-market environmental monitoring plan was undertaken.

The molecular characterisation data establish that the genetically modified soybean MON 87708 contains one copy of an intact *dmo* expression cassette in a single locus. No other parts of the plasmid used for transformation are present in soybean MON 87708. The results of the bioinformatic analyses of the inserted DNA and the flanking regions do not raise safety issues. The levels of DMO proteins in soybean MON 87708 have been sufficiently analysed and the stability of the genetic modification has been demonstrated.

The EFSA GMO Panel compared the compositional, phenotypic and agronomic characteristics of soybean MON 87708, the conventional counterpart (A3525) and other non-GM soybean reference varieties, and assessed all statistically significant differences between soybean MON 87708 and A3525 for which equivalence with the non-GM reference varieties could not be established. It is concluded that no differences were identified in the compositional data of forage and seeds obtained from soybean MON 87708 or in its agronomic and phenotypic characteristics that would require further assessment with regard to safety by the EFSA GMO Panel.

The DMO proteins are degraded by proteolytic enzymes, and bioinformatics-supported studies demonstrated that these proteins show no similarity to known toxic and allergenic proteins. No toxicity of the DMO proteins was observed in 28-day and acute toxicity studies in mice. The result of a 90-day feeding study in rats with diets containing toasted defatted soybean meal from soybean MON 87708, its conventional counterpart or any of two non-GM soybean varieties did not raise safety concerns. There are no indications that the genetic modification might significantly change the overall allergenicity of soybean MON 87708 when compared with that of its conventional counterpart. The compositional data indicating that soybean MON 87708 is as nutritious as non-GM soybean varieties were supported by the outcome of a chicken study. In conclusion, the EFSA GMO Panel is of the opinion that soybean MON 87708 is as safe and as nutritious as its conventional counterpart and non-GM reference varieties, in the context of its intended uses.

The scope of application EFSA-GMO-NL-2011-93 is for food and feed uses, import and processing and does not include cultivation. Therefore, there is no requirement for scientific information on possible environmental effects associated with the cultivation of soybean MON 87708 in the EU.

[4] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. Official Journal of the European Communities, L268, 1–23.

There are no indications of an increased likelihood of establishment and spread of feral GM soybean plants in event of accidental release into the environment of viable soybean MON 87708 grains during transport and processing for food and feed uses. Considering its intended uses as food and feed, potential interactions of soybean MON 87708 with the biotic and abiotic environment were not considered to be an issue by the EFSA GMO Panel. The unlikely, but theoretically possible, transfer of the recombinant gene from soybean MON 87708 to environmental bacteria does not raise any safety concern because no selective advantage will be conferred on the recipients. The scope of the post-market environmental monitoring plan provided by the applicant is in line with the intended uses of soybean MON 87708. Furthermore, the EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the post-market environmental monitoring plan.

In conclusion, the EFSA GMO Panel considers that the information available for soybean MON 87708 addresses the scientific issues indicated in the relevant Guidance Documents of the EFSA GMO Panel and the scientific comments raised by the Member States, and that soybean MON 87708, 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 or the environment, in the context of its intended uses.

It should be noted that the assessment of potential consumer health risks resulting from dicamba residues and its metabolites in soybean MON 87708 is under the remit of the EFSA Pesticides Unit.

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BACKGROUND

On 9 February 2011, EFSA received from the Dutch Competent Authority an application (Reference EFSA-GMO-NL-2011-93) for authorisation of the herbicide tolerant genetically modified soybean MON 87708 (Unique Identifier MON-877Ø8-9) for food and feed uses, import and processing, submitted by Monsanto within the framework of Regulation (EC) No 1829/2003 on genetically modified food and feed.[5]

After receiving the application EFSA-GMO-NL-2011-93 and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed the Member States and the European Commission (EC) and made the summary of the dossier 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 Regulation (EC) No 1829/2003. On 29 March 2011, EFSA received additional information requested under completeness check (on 18 March 2011). On 13 May 2011, EFSA declared the application as valid in accordance with Article 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to the Member States and the EC and consulted nominated risk assessment bodies of the Member States, including the 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 State had three months after the date of receipt of the valid application (until 13 August 2011) to make their opinion known.

The Scientific Panel on Genetically Modified Organisms of EFSA (EFSA GMO Panel) carried out a scientific assessment of genetically modified soybean MON 87708 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 GM plants and derived food and feed (EFSA 2006a, 2011a) and on the post-market environmental monitoring (PMEM) of GM plants (EFSA, 2006b, 2011b). Furthermore, the EFSA GMO Panel also took into consideration the scientific comments of Member States, the additional information provided by the applicant and the relevant scientific publications.

On 10 August 2011, 17 August 2011, 10 February 2012, 5 July 2012, 7 November 2012, 11 February 2013, 19 February 2013 and 13 May 2013, the EFSA GMO Panel asked for additional data on soybean MON 87708. The applicant provided the requested information on 18 October 2011, 14 March 2012, 1 August 2012, 13 November 2012, 15 February 2013, 21 February 2013 and 6 June 2013. After evaluation of the full data package, the EFSA GMO Panel finalised its risk assessment of soybean MON 87708.

In giving its scientific opinion on GM soybean MON 87708 to the EC, the 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 6 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).

[5] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. Official Journal of the European Communities, L268, 1-23.

[6] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2011-00122

[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. Official Journal of the European Communities, L106, 1-38.

TERMS OF REFERENCE

The EFSA GMO Panel was requested, to carry out a scientific risk assessment of the soybean MON 87708 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.

ASSESSMENT

1. Introduction

The genetically modified (GM) soybean MON 87708 (Unique Identifier MON-877Ø8-9) was assessed with respect to its scope, taking account of the appropriate principles described in the Guidance Documents of the EFSA GMO Panel for the risk assessment of GM plants and derived food and feed (EFSA, 2006a, 2011a), and on the post-market environmental monitoring of GM plants (EFSA, 2006b, 2011b). The risk assessment presented here is based on the information provided in the application relating to soybean MON 87708 submitted in the EU, scientific comments raised by the Member States and relevant scientific publications.

The scope of application EFSA-GMO-NL-2011-93 is for food and feed use, import and processing of soybean MON 87708 within the EU. Thus, soybean MON 87708 will be imported into the EU mixed with other soybean varieties and will be used as food or feed, or for the production of a large number of derived products, as any commercial soybean variety. The main product for human use is soybean oil. Around 10 % of the heat-processed (toasted) defatted soybean meal goes to production of soybean products for human consumption, including flours, soybean protein concentrates and various textured products simulating meats, seafood and cheeses. The rest of the toasted defatted soybean meal goes into animal feed, in the EU mainly feed for poultry, pig and cattle (OECD, 2001). Whole soybeans are used to produce soy sprouts, baked soybeans and roasted soybeans. There is also a limited direct use of soybeans as animal feeds.[8]

Soybean MON 87708 was developed to confer tolerance to herbicides containing dicamba (3,6-dichloro-2-methoxybenzoic acid).[9] Dicamba tolerance is achieved by the expression of dicamba mono-oxygenase (DMO) proteins, which demethylate dicamba, producing 3,6-dichlorosalicylic acid and formaldehyde. It should be noted that the acceptable daily intake (ADI) proposed for the metabolite 3,6-dichlorosalicylic acid, which is produced in soybean MON 87708, is lower than the ADI proposed for dicamba.[10] The assessment of potential consumer health risks resulting from dicamba residues and its metabolites in soybean MON 87708 is outside the remit of the EFSA GMO Panel and is currently ongoing[10] in the EFSA Pesticides Unit.

The genetic modification is intended to improve agronomic performance only and is not intended to influence the nutritional aspects, the processing characteristics and the overall use of soybean as a crop.

2. Issues raised by Member States

Issues raised by Member States are addressed in Annex G of the overall opinion[11] and were taken into consideration during the evaluation of the risk assessment.

3. Molecular characterisation

3.1. Evaluation of relevant scientific data

3.1.1. Transformation process and vector constructs[12]

MON 87708 was developed by *Agrobacterium tumefaciens* (also know as *Rhizobium radiobacter*)-mediated transformation of meristematic tissue from the embryos of germinated soybean A3525 (*Glycine max* (L.) Merr.) seeds with plasmid vector PV-GMHT4355. The regeneration of the transformed tissue was achieved without a callus phase.

[8] Technical dossier/Section D7.5.
[9] Technical dossier/Section D1.
[10] EFSA-Q-2011-01268: Dicamba - Application to modify the existing MRL in soybean.
[11] http://registerofquestions.efsa.europa.eu/
[12] Technical dossier/Sections C and D1.

The plasmid PV-GMHT4355 contained two T-DNAs (T-DNA I and II):

- The *dmo* expression cassette (T-DNA I), which confers tolerance to dicamba herbicide contains the following genetic elements between the right and left borders: PC1SV promoter from *Peanut chlorotic streak caulimovirus*; *TEV*, 5′ non-translated region (leader sequence) from *Tobacco etch virus*, involved in the regulation of gene expression; *RbcS* encoding chloroplast transit (targeting) peptide and the first 24 amino acids of the mature ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO) small subunit protein from pea (*Pisum sativum*); *dmo*, a derivative of the coding sequence of DMO from *Stenotrophomonas maltophilia*; *E9*, 3′ non-translated region of pea RuBisCO small subunit, functioning as a polyadenylation signal.

- The CP4 *epsps* cassette (T-DNA II), which confers glyphosate tolerance carries the following genetic elements between the right and left borders: FMV promoter from *Figwort mosaic virus*; *DnaK* 5′ non-translated leader sequence from *Petunia hybrida Hsp70* gene, involved in the regulation of gene expression; sequence encoding CTP2, a chloroplast transit (targeting) peptide from the *shkG* gene of *Arabidopsis thaliana* (*shkG* encodes EPSPS, 5-enolpyruvylshikimate-3′-phosphate synthase); CP4 *epsps*, a codon-optimised coding sequence of *aroA* gene from *Agrobacterium* sp. strain CP4 coding for CP4 EPSPS; *E9*, 3′ non-translated region of pea RuBisCO small subunit, functioning as a polyadenylation signal.

The vector backbone contained elements necessary for the maintenance and selection of the plasmid in bacteria: *ori*V, the origin of replication from the broad-host-range plasmid RK2 for the maintenance of plasmid vector in *Agrobacterium*; *rop*, repressor of primer protein from ColE1 plasmid, playing a role in the maintenance of plasmid copy number in *Escherichia coli*; *ori*-pBR322, the origin of replication from plasmid pBR322 and required for the maintenance of PV-GMHT4355 in *E. coli*; bacterial promoter, coding and 3′ non-translated sequences of *aad*A from tansposon Tn*7*, an aminoglycoside-modifying enzyme conferring resistance to spectinomycin and streptomycin for selection of the plasmid in *E. coli* and *Agrobacterium*.

During transformation, both T-DNAs were inserted into the soybean genome. Glyphosate tolerance conferred by the CP4 EPSPS protein derived from T-DNA II served as a marker during the initial selection of transformants. The T-DNA II insert was segregated away from the soybean during the conventional breeding process.

3.1.2. Transgene constructs in the genetically modified plant[13]

Southern analysis was used to determine the number of copies and insertion sites, and to confirm the presence or absence of vector backbone sequences. The approach used was acceptable in terms of both coverage and sensitivity.

Southern analyses indicated that soybean event MON 87708 contains a single insert with one copy of the intact DMO expression cassette. No elements from T-DNA II or the vector backbone were detected. Southern analyses of genomic DNA from soybean MON 87708 were performed using appropriate combinations of restriction endonucleases and 10 overlapping probes covering the whole plasmid. The probes corresponding to the different elements of the two T-DNAs showed the expected hybridisation signals, whereas no signal was observed for any of the probes corresponding to the vector backbone.

The nucleotide sequence of the insert as well as both 5′ and 3′ flanking regions were determined. Comparisons with the conventional soybean genomic sequences indicated that, in soybean MON 87708 there was an 899 bp deletion, a 128 bp insertion adjacent to the 5′ of the insert, and a 35-bp insertion adjacent to the 3′ of the insert. Bioinformatic analyses of the genomic sequences flanking the insert and the deleted region were carried out to assess any potential interruption of known

[13] Technical dossier/Section D2.

soybean genes. BLASTN searches were performed against the GenBank EST (Expressed Sequence Tag) database and non-redundant nucleotide database and a BLASTX search against the non-redundant amino acid database. The results did not indicate the interruption of any gene in soybean MON 87708, and also confirmed that the insert is located in the nuclear genome.

In order to assess whether the open reading frames (ORFs) present within the insert and spanning the junction sites raise any safety issue, their putative translation products were compared to the GenBank protein database for all proteins and its subset for toxins, as well as to the FARRP database for allergens.[14] In addition, the presence of eight amino acid perfect matches between known allergens and the putative new ORFs was examined. No alignment met or exceeded the Codex Alimentarius (2003) and the EFSA (2010) threshold for potential allergenicity and no relevant similarities to known toxic proteins were found. These analyses support the conclusion that, even in the unlikely event that any of the new ORFs at the junctions or present within the insert were translated, this would not raise a safety issue.

3.2. Information on the expression of the insert[15]

The DMO precursor protein undergoes alternative processing, resulting in two monomeric forms (DMO and DMO+27). The active DMO enzyme is a trimer. Total DMO proteins levels were analysed by an enzyme-linked immunosorbent assay (ELISA) using leaf, root, forage and mature seed materials, from replicated field trials across eight soybean-growing regions in the USA in 2009. The plants were treated with dicamba. Considering the scope of the application, the DMO proteins levels in seed and forage are considered most relevant. The mean DMO level was 40 µg/g dry weight (dw) (range 21–65 µg/g dw) in mature seeds. In forage, the mean DMO level was 32 µg/g dw (range 15–54 µg/g dw).

3.2.1. Inheritance and stability of inserted DNA[16]

Genetic stability of the inserted DNA was studied over five generations by Southern analysis. The restriction enzyme/probe combinations used were sufficient to conclude that all of the generations tested retained the single copy insert together with its flanking regions. The insert is therefore stably inherited.

Supporting evidence for the stability was obtained by a quantitative, structure-specific endonuclease-based assay over three generations in approximately 3200 plants. This analysis also provided information on the zygosity of the plants which was consistent with a single genetic locus segregating according to Mendelian principles.

3.3. Conclusion

Molecular characterisation data establish that soybean MON 87708 contains a single insert. Bioinformatic analyses did not reveal disruption of known soybean genes or the formation of ORFs that would raise a hazard. Levels of the newly expressed DMO proteins have been sufficiently analysed, and the stability of the inserted DNA was confirmed over several generations. The EFSA GMO Panel considers that all of the molecular data sets are sufficient for the molecular characterisation.

[14] Technical dossier/Section D3 (c).
[15] Technical dossier/Sections D3 (a), (b) and (d).
[16] Technical dossier/Section D5.

4. Comparative analysis

4.1. Evaluation of relevant scientific data

4.1.1 Production of material for the comparative assessment[17]

The application EFSA-GMO-NL-2011-93 for food and feed use, import and processing of soybean MON 87708 within the EU presented compositional data on seed and forage material collected in a field trial performed in the USA in 2009. Some aspects of these studies have been published (Harrison et al., 2011). The field trials compared the composition of soybean MON 87708 with a suitable conventional counterpart. The conventional counterpart was the non-transgenic Asgrow variety A3525, which was the soybean variety originally transformed to establish transformation event MON 87708.

The field trial was performed at eight sites within soybean cultivation areas in the USA. At each site the following test materials were grown in a randomized complete block design with four replicates: soybean MON 87708 (treated and untreated with dicamba), the conventional counterpart (soybean A3525) and three non-GM soybean reference varieties. All test materials were treated with conventional herbicides. Overall the field trials included 14 non-GM soybean reference varieties.[18] The test materials were characterised by event-specific polymerase chain reaction (PCR) for the presence or absence of the MON 87708 event. One replication of the conventional counterpart at one site (Indiana) was excluded from the analysis because of the adventitious presence of a different GM soybean event coming from a GM soybean grown at the same site. Forage and seed material were missing from one of the four replicates of the conventional counterpart at one site (Kansas), and from one of the four replicates of soybean MON 87708 at one site (Iowa).

4.1.2 Compositional analysis[19]

On request of the EFSA GMO Panel, the applicant analysed the compositional data using the most recent statistical methodology recommended by the EFSA GMO Panel (EFSA, 2010, 2011a). This recommends a test of difference to determine whether the GM plant is different from its conventional counterpart, and a test of equivalence to determine whether the GM plant falls within the range of natural variation estimated from the non-GM soybean reference varieties. As described in EFSA 2011a, the result of the equivalence test is categorised into four possible outcomes to facilitate drawing appropriate 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.

The key constituents included in the compositional analysis of soybean seeds and forage were selected according to OECD recommendations (OECD, 2001). Soybean seeds were harvested and analysed for proximates (protein, fat, ash, and moisture, and carbohydrate by calculation), fibre fractions (acid detergent fibre (ADF) and neutral detergent fibre (NDF)), amino acids, fatty acids, vitamin E, anti-nutrients (i.e. phytic acid, trypsin inhibitor, lectins, stachyose and raffinose) and other secondary metabolites (isoflavones). Forage was analysed for proximates and for fibre fractions (ADF, NDF). In total, 56 parameters were analysed in seed, seven of which were also analysed in forage. Fourteen

[17] Technical dossier/Sections C and D7.1–7.3 and additional information received October 2011, March 2012 and August 2012.

[18] The non-GM reference materials were FS3591; Crows C3908; NK S38-T8; Croplan HT3596STS; Midland 363; Stewart SB3454; Quality Plus 365C; Channel Bio 3461; Pioneer 93M52; NK 32Z3; Garst 3585N; Channel Bio 37002; Crows C37003N; Wilken 3316.

[19] Technical dossier/Section D7.1 and additional information received October 2011, March 2012 and August 2012.

parameters having more than 50 % of the observations below the limit of quantification were excluded from the analysis.[20]

Samples sprayed with conventional herbicides showed statistically significant differences between soybean MON 87708 and its conventional counterpart for 20 parameters in seeds (protein and carbohydrates; the amino acids arginine, aspartic acid, glutamic acid, histidine, isoleucine, leucine, lysine, phenylalanine, proline and valine; and the fatty acids C16:0 palmitic acid, C18:1 oleic acid, C18:3 linolenic acid, C20:1 eicosenoic acid and C22:0 behenic acid; and the anti-nutrients phytic acid, trypsin inhibitors and stachyose), and for two parameters in forage (protein and carbohydrate). The test of equivalence indicated that the level of 18 of these parameters in soybean MON 87708 fell within the equivalence limits established from the non-GM soybean reference varieties. Equivalence could not be established for four parameters in seeds (equivalence category III - EFSA, 2011). These were total fat (MON 87708 16.45% dw, A3525 16.48% dw, reference varieties 18.41% dw), carbohydrates (MON 87708 38.56% dw, A3525 37.47% dw, reference varieties 36.59% dw), 20:1 eicosenoic acid (MON 87708 0.11% total fatty acids (FA), A3525 0.12% total FA, reference varieties 0.15% total FA), and 22:0 behenic acid (MON 87708 0.27% total FA, A3525 0.28% total FA, reference varieties 0.32% total FA). The test of equivalence could not be performed on trypsin inhibitor because of the lack of variation among the non-GM soybean reference varieties for this compound.

The EFSA GMO Panel evaluated the parameters for which equivalence could not be demonstrated and concluded that no further assessment was needed as their biochemical role is well known and the magnitude of the reported levels lack relevance from a food and feed safety and nutritional point of view.

Samples of soybean MON 87708 sprayed with dicamba in addition to required conventional herbicides and the conventional counterpart sprayed only with required conventional herbicide showed statistically significant differences for 21 parameters in seeds (protein, moisture and carbohydrates, the amino acids arginine, aspartic acid, glutamic acid, histidine, leucine, phenylalanine and proline, the fatty acids 16:0 palmitic acid, 18:1 oleic acid, 18:2 linoleic acid, 18:3 linolenic acid, 20:1 eicosenoic acid, and 22:0 behenic acid, and vitamin E, phytic acid, stachyose, genistein and daidzein) and for one parameter in forage (total fat). The test of equivalence indicated that the level of 16 of these parameters in soybean MON 87708 fell within the equivalence limits established from the non-GM soybean reference varieties, whereas equivalence could not be established for five parameters (equivalence category III - EFSA, 2011) and therefore required further evaluation. These were total fat (MON 87708 16.33% dw, A3525 16.48% dw, reference varieties 18.41% dw), carbohydrates (MON 87708 38.50% dw, A3525 37.47% dw, reference varieties 36.59% dw), 20:1 eicosenoic acid (MON 87708 0.11% total FA, A3525 0.12% total FA, reference varieties 0.15% total FA), and 22:0 behenic acid (MON 87708 0.27% total FA, A3525 0.28% total FA, reference varieties 0.32% total FA) in seeds, and protein (MON 87708 24.03% dw, A3525 23.81% dw, reference varieties 21.64% dw) in forage. The test of equivalence could not be performed on trypsin inhibitor because of the lack of variation among the non-GM soybean reference varieties, however the test of difference was not significant.

The EFSA GMO Panel further evaluated these parameters for which equivalence could not be demonstrated and concluded that no further assessment was needed as their biochemical role is well known and the magnitude of the reported levels lack relevance from a food and feed safety and nutritional point of view.

[20] These were C8:0 caprylic acid, C10:0 capric acid, C12:0 lauric acid, C14:0 myristic acid, C14:1 myristoleic acid, C15:0 pentadecanoic acid, C15:1 pentadecenoic acid, C16:1 palmitoleic acid, C17:0 heptadecanoic acid, C17:1 heptadecenoic acid, C18:3 gamma-linolenic acid, C20:2 eicosadienoic acid, C20:3 eicosatrienoic acid and C20:4 arachidonic acid.

4.1.3 Agronomic and phenotypic characteristics[21]

Based on data collected at the eight sites in the USA field trial in 2009 (the same field trial used to collect seeds and forage for compositional studies: see Section 4.1.1 and 4.1.2), the applicant performed a comparative assessment of the phenotypic and agronomic characteristics of soybean MON 87708 and its conventional counterpart (soybean A3525). The phenotypic and agronomic characteristics evaluated were early stand count, seedling vigour, days to 50 % flowering, flower colour, plant height, lodging, pod shattering, final stand count, seed moisture, 100 seed weight, yield, and plant growth stages. In addition, specific studies were carried out to investigate pollen viability, pollen diameter, and dormancy/germination.

On request of the EFSA GMO Panel, the applicant analysed the data on phenotypic and agronomic characteristics using the most recent statistical methodology recommended by the EFSA GMO Panel (EFSA, 2010, 2011a). The test of difference of phenotypic and agronomic characteristics identified statistically significant differences between soybean MON 87708 and its conventional counterpart for only one endpoint (100 seed weight). Soybean MON 87708 both sprayed and not sprayed with dicamba had a lower 100 seed weight than its conventional counterpart (in both cases 14.6 g vs. 15.6 g). The equivalence test indicated that all the analysed characteristics fell within the equivalence limits established from the non-GM soybean reference varieties, except for 100 seed weight (100 seed weight: 15.0-17.7 g). Equivalence could not be established for 100 seed weight (equivalence category III - EFSA, 2011); therefore, this parameter required further evaluation.

The difference in 100 seed weight might be either incidental or indicative of unintended effects due to the genetic modification. The applicant was unable to explain why non-equivalence between the GM soybean and the non-GM soybean reference varieties had occurred. However, considering the magnitude of the difference in 100 seeds weight, its inherent variability and lack of impact on other parameters investigated, including yield, the EFSA GMO Panel concludes that this difference does not pose safety concerns in the context of the scope of this application.

A specific study was performed at one of the field trial sites to investigate pollen morphology and viability. There was no difference in percent viable pollen, pollen diameter and pollen morphology between soybean MON 87708 and the conventional counterpart, soybean A3525.

A study focusing on seed germination and dormancy characteristics was performed in germination chambers using seeds collected from three field trial sites. No difference in percent viable hard seed or percent viable firm-swollen seed was observed between seeds of soybean MON 87708 and the conventional counterpart tested at six different temperature regimes. The percent germinated seed of soybean MON 87708 was lower than that of the conventional counterpart at 10 ^{o}C (98.9 % vs. 99.7 %) and 10/30 ^{o}C (98.6 % vs. 99.7 %), whereas no difference was observed at 20 ^{o}C, 30 ^{o}C, 10/20 ^{o}C and 20/30 ^{o}C. A higher percent dead seed was observed for soybean MON 87708 than for the conventional counterpart at 10^{o}C (0.8 % vs. 0.2 %) and 10/30 ^{o}C (1.4 % vs. 0.3 %). As these differences were small in magnitude and fell within the range estimated from the non-GM soybean reference varieties, the EFSA GMO Panel did not find these observations indicative of relevant alterations in germination characteristics.

Comparable responses to abiotic stressors such as cold, compaction, drought, flood, frost, hail, nutrient deficiency and wind were observed. These data on the environmental interaction of soybean MON 87708 as compared with the conventional counterpart were obtained in materials that had received equivalent maintenance herbicide treatments, i.e. they were not treated with dicamba. No differences between soybean MON 87708 and the conventional counterpart for any of the diseases on this legume crop were observed.

[21] Technical dossier/Section D4 and additional information October 2011, March 2012 and August 2012.

Overall, the EFSA GMO Panel found these differences small in magnitude and unlikely to be biologically meaningful in relation to an adverse environmental impact of soybean MON 87708 compared with the conventional counterpart.

4.2. Conclusion

Based on the information available, it is concluded that no differences were identified in the compositional data of forage and seeds obtained from soybean MON 87708 or in its agronomic and phenotypic characteristics that would require further assessment with regard to safety by the EFSA GMO Panel.

5. Food/feed safety assessment

5.1. Evaluation of relevant scientific data

5.1.1. Effects of processing[22]

Soybean MON 87708 will be used for production and manufacturing of food and feed products in the same way as 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 DMO proteins in soybean MON 87708, the EFSA GMO Panel has no reason to assume that the characteristics of soybean MON 87708 and derived processed products would be different from those products derived from conventional soybean varieties, except for the presence of the DMO proteins. The three major processed fractions produced from whole soybean are oil, protein-rich meal and lecithin. The processing to produce these fractions on a pilot scale, which included heat treatment, resulted in severe loss of the DMO activity.[23]

Heat stability of the DMO proteins

On request of the EFSA GMO Panel, the applicant provided information on the thermal stability of the DMO enzymes.[23] The stability to heat of aqueous solutions of the DMO proteins was assessed by measuring DMO activity and its intactness studied by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). As expected, the DMO enzymes were denatured at elevated temperatures. Complete loss of activity was found after incubation at 55^{o}C and above. SDS-PAGE demonstrated that incubation of DMO at 55 ^{o}C had no effect on protein size. Incubation for 30 minutes at 75 ^{o}C or at higher temperatures (95 ^{o}C) resulted in a visible reduction in DMO protein band intensity.

5.1.2. Toxicology[24]

5.1.2.1. Protein used for safety assessment

As described previously, soybean MON 87708 expresses two versions of the monomer dicamba mono-oxygenase (DMO) protein, DMO and DMO+27 (see Section 3.1.3). The DMO proteins used in the resistance to degradation studies, the study on acute oral toxicity and some of the substrate specificity studies were purified directly from seeds of soybean MON 87708. On the other hand, the 28-day repeated-dose toxicity study and some of the substrate specificity studies were performed with DMO proteins produced in *E. coli*. The characterisation of the DMO and DMO+27 protein variants, with regard to physico-chemical characteristics and functional activity, was performed with the individual proteins and/or with a protein mixture. The molecular weights were determined by SDS-PAGE, the identity and immunoreactivity with immunoblot analysis; partial amino acid sequencing was achieved by N-terminal sequence analysis and proteolytic peptide mapping by matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry. These analyses were

[22] Technical dossier/Section D7.6.
[23] Additional information October 2011.
[24] Technical dossier/Section D7.8 and additional information October 2011, March 2012 and August 2012.

complemented with the determination of both glycosylation status and functional activity. Together the studies confirmed the structural and functional equivalence of the two DMO proteins expressed in soybean MON 87708 with the corresponding proteins expressed in *E. coli*.

5.1.2.2. Toxicological assessment of expressed novel proteins in soybean MON 87708[25]

The DMO proteins in soybean MON 87708 are derived from the bacterium *Stenotrophomonas maltophilia*, which is ubiquitous in the environment. Occasionally, it has been found in clinical isolates (Denton, 1998).

Crystallographic studies demonstrated the role of the carboxylic group of dicamba in binding to the active site of DMO, and the chloride atoms in providing the correct orientation. The applicant identified a number of naturally occurring benzoic, phenolic and phenylpropanoic acids which showed elements of structural similarities with dicamba and tested these in an assay positive for dicamba demethylation. No evidence of catabolism was seen with any other potential substrate tested, indicating a high specificity of the DMO for dicamba.[26]

(a) Bioinformatics studies

Bioinformatic analyses[27] of the amino acid sequence of the DMO protein expressed in soybean MON 87708 revealed no significant similarities to known toxic proteins.

(b) Resistance to degradation by proteolytic enzymes[28]

The resistance to degradation by pepsin of DMO proteins (DMO and DMO+27) produced and purified from soybean seeds of MON 87708 (purity 81%) was measured in solutions containing pepsin and the test protein at pH 1.2. The integrity of the test protein was analysed by gel electrophoresis followed by protein staining and Western analysis. No DMO proteins were detected within 30 seconds of incubation. A 21-kDa fragment present in the DMO enzyme preparation was stable during the digestion with pepsin and appeared as weakly stained bands on the colloidal blue gel staining gels. However, this fragment was shown, by N-terminal sequencing, to be a stable impurity in the DMO preparation from soybean MON 87708 and not to derive from the DMO enzyme.

The resistance of the DMO proteins to degradation by pancreatin was also assessed in solutions containing pancreatin and the test protein at pH 7.5. The integrity of the test protein was analysed by gel electrophoresis followed by Western analysis. No DMO proteins were detected within five minutes of incubation. The EFSA GMO Panel notes that resistance to degradation by pancreatin is not required by either the EFSA Guidance Document (EFSA, 2006a) or Codex Alimentarius (CAC, 2009).

(c) Acute toxicity testing

The DMO proteins (DMO and DMO+27) extracted from seeds of soybean MON 87708 induced no adverse effects in an acute oral toxicity study in CD-1 mice administered a single dose of 140 mg/kg bw.

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

[25] Technical dossier/Section D7.8.1.
[26] Technical dossier/Section D7.8.1 and additional information July 2012, November 2012.
[27] Technical dossier/Section D7.8.1 and additional information February 2013.
[28] Technical dossier/Section D7.8.1.

(d) Repeated-dose feeding toxicity study[29]

On request of the EFSA GMO Panel, the applicant supplied a 28-day feeding toxicity study in mice with a mixture of the DMO and DMO+27 proteins supplied in the diet in a ratio approximately equal to the one at which they occur in soybean MON 87708 (i.e. 2:3). The study was conducted in accordance with the OECD Guideline 407 on individually housed Crl:CD-1 mice (14 mice per sex per group) fed diets targeted to administer 0, 17, 51 or 171 mg DMO proteins/kg bw per day (actual doses: 0, 17.5, 52.4 and 174 mg/kg bw per day in males; 0, 15.5, 53 and 179.7 mg/kg bw per day in females). The animals were observed regularly for clinical signs, and feed consumption and body weights were recorded. At the end of the treatment period, haematological and serum chemistry analyses were performed. All animals were sacrificed and underwent a detailed necropsy examination with selected organs weighed. Tissues from all animals in the high-dose and control groups were subjected to a comprehensive histological examination. Data for the test groups were compared with those of the control group and, when relevant, also with historical control data.

Administration of DMO proteins did not induce any deaths or clinically relevant findings at any of the dose levels. There were no relevant differences in mean body weight, body weight gain or food consumption. The only statistically significant difference in the clinical pathology parameters assessed was an incidental higher mean absolute neutrophil count in males of the high-dose group, which was mainly driven by an unusually high value in one animal showing incidental inflammation of the skin. A slightly significantly higher mean spleen weight (relative to body weight) was seen in a male group given the high dose than in the control group; this was not associated with histopathological changes and considered the expression of biological variability. No macroscopic or microscopic findings in the examined organs and tissues attributable to the test material were reported. The highest dose administered in this study, i.e. 174 mg/kg bw per day in males and 179.7 mg/kg bw per day in females, is considered the no observed adverse effect level (NOAEL).

Assuming an intake of 200 g of soybean per 70 kg adult per day in the EU and that all soybean consumed is derived from soybean MON 87708, the daily intake of DMO proteins would be in the region of 110 μg/kg bw. The highest estimated intake of DMO proteins in adults is about 1000-fold lower than the NOAEL from the 28-day feeding study.

5.1.2.3. Animal feeding studies[30]

(a) Subchronic toxicity study in rats[31]

A 90-day rat feeding study was performed using a protocol adapted from OECD Guideline 408. Individually housed Crl:CD[SD] rats were assigned to eight groups (12 rats per sex per group) and offered *ad libitum* diets containing 15% or 30% toasted and defatted soybean meal respectively from soybean MON 87708 (test groups fed diets containing soybean MON 87708 treated with the intended herbicide dicamba),[32] from the conventional counterpart (control groups), or from two commercial non-GM varieties (reference groups). Analysis of soybean meals showed their nutritional equivalence. Diets were adjusted to contain approximately equal levels of calories, protein, and other nutrients (analytically confirmed) and to be balanced for rats. Data from the groups fed diets containing soybean MON 87708 were compared with their corresponding control groups using a one-way parametric analysis of variance for all quantitative endpoints.[33]

All animals appeared healthy throughout the study. Body weight gain was transiently lower (up to 11 %; statistically significant) in females given the diet containing 15 % soybean MON 87708 (weeks 0-6) and in males given the diet containing 30 % soybean MON 87708 (weeks 0-3); partial recovery

[29] Additional information March 2012.
[30] Technical dossier/Section D7.8.4.
[31] Technical dossier/Section D7.8.4 and additional information June 2013.
[32] Additional information June 2013.
[33] Additional information June 2013.

was seen during the course of the study, resulting in a minimally lower cumulative body weight gain in rats fed diets containing soybean MON 87708 than in the corresponding controls (4.4 % in females, 3.8 % in males respectively). These changes are not considered relevant as they were minimal, were not accompanied by significant differences in body weight and were within, or close to, the ranges of groups fed reference varieties (the mean cumulative body weight gain in grams for females given 15 % diet was: control 136, test diet 130 and reference varieties 127-145; in the case of males fed 30 % diets it was: controls 338, test diet 325 and reference varieties 330-350). No biologically relevant differences in feed intake were seen among groups.

Statistically significant differences in clinical pathology and urinalysis parameters between rats fed diets containing soybean MON 87708 and control animals (i.e. lower mean absolute monocytes counts in females fed the 15 % MON 87708 diet; higher mean percent eosinophils, higher alanine aminotransferase activity and serum chloride levels in male rats fed 30 % MON 87708; changes in urinary specific gravity, pH and volume in females fed 15 % test diet; and lower spleen weight in female rats given diets containing 15 % soybean MON 87708) were considered incidental and not relevant because the differences were minimal, were not associated with changes in related parameters or in histopathology, and were within the range of reference dietary groups and/or historical control data. At macroscopic or microscopic examination (histopathology on rats given 30 % inclusion rate diets) no MON 87708-diet related findings were observed, and all the detected changes were consistent with the background pathology of rats of this strain and age.

(b) Chicken feeding study

The applicant provided a 42-day broiler chicken feeding study,[34] in which the growth of birds given diets containing soybean meal produced from soybean MON 87708 (treated with dicamba)[35] was compared with that of birds given meal prepared from the conventional counterpart (soybean A3525) and another six commercial non-GM soybean varieties. A total of 960 one-day-old broilers (Cobb × Cobb 500) were assigned to one of the eight treatments, each treatment initially consisting of 10 replicate pens of 12 birds, reduced to 10 birds per pen (five male and five females replicates) on day 7, under a randomized complete block structure.

Each diet consisted predominantly of one of the eight soybean meals, maize grain and maize gluten. The main dietary constituents were analysed for protein, moisture, and amino acids prior to diet preparation to formulate isocaloric diets with a similar amount of soybean meal. The soybean content varied with the age of the birds starting with 32.5 % w/w for the first 21 days of feeding, and reducing to 29.5-30.5 % during the final stages of growth. The diets, formulated based on nutrient requirements recommended by the National Research Council (NRC, 1994) were analysed for crude nutrients, minerals, amino acids, fatty acids and antinutrients. Diets were shown to be essentially free of pesticide residues[36] and mycotoxins.

Body weight, weight gain, feed intake and feed to gain ratio were measured or calculated at the end of the trial. Birds were then killed, processed and analysed for carcase yield. Data were subject first to a two-factor (diet and sex) analysis of variance. When no interaction was detected, the test group was evaluated against the control and reference groups together. This was done using a mixed linear model analysis.

No significant differences in performance, carcase yield or the analysis of breast and thigh meat was observed when birds fed MON 87708 meal were compared with birds fed meal from either the control or the non-GM soybean varieties. Since body weight showed a significant diet by sex interaction, data were analysed separately for each sex. Final body weight, daily weight gain and feed intake were significantly lower in female birds fed with MON 87708, but not in male birds. The differences

[34] Technical dossier/MSL0022551 (2010) & RAR-10-030 (2010).
[35] Additional information June 2013.
[36] Tested pesticides are organophosphates, organonitrogens, organochlorinated pesticides, N-methylcarbamate pesticides.

observed for females, although significant, were small (for example, body weight differing only by 80 g in a 2.3-kg bird). Furthermore, the feed to gain ratio was not significantly different between sexes.

Diets containing the various soybean meals were designed to deliver the same nutrition and consequently, the growth of birds given diets containing soybean meal produced from soybean MON 87708 was not expected to differ significantly from that of birds given meal prepared from the conventional counterpart soybean A3525. This held true for male birds, confirming the expectation that no unintended effects of a magnitude sufficient to affect growth had been introduced following the genetic modification. The differences observed for female birds were not considered to be of biological significance because the feed to gain ratio was not significantly different between sexes or between treatment groups.

5.1.3. Allergenicity[37]

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

5.1.3.1. Assessment of allergenicity of the newly expressed proteins[38]

A weight-of-evidence approach is followed, taking into account all the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (CAC, 2009; EFSA, 2006a, 2011a).

The *dmo* gene originates from *Stenotrophomonas maltophilia*, an aerobic, non-fermentative, Gram-negative bacterium present in the environment as well as in humans. The bacterium has not been reported to give rise to allergenicity. The total amount of DMO in seed, the most important food material from soybean, reaches a level of 40 μg/g dry weight (i.e. around 0.01 % of total soybean protein) in both dicamba-treated and non-treated soybeans.

Bioinformatic analyses[39] of the amino acid sequence of the DMO+27 protein (which contains the DMO amino acid sequence), using the criterion of 35 % identity in a 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 the DMO+27 protein and known allergens, and confirmed the outcome of the previous bioinformatic analyses.

The studies on resistance to proteolytic enzymes of the newly expressed proteins have been described in Section 5.1.2.2(b).

Based on all available information, the EFSA GMO Panel considers that there are no indications that the newly expressed DMO proteins in soybean MON 87708 may be allergenic in the intended conditions of use.

5.1.3.2. Assessment of allergenicity of the whole GM plant or crop[40]

Allergenicity of the whole GM plant could be increased (as an unintended effect) as a result of the genetic modification, for example through qualitative or quantitative modifications of the pattern of expression of endogenous allergenic proteins.

[37] Technical dossier/Section D7.9.
[38] Technical dossier/Section D7.9.1. and additional information February 2013.
[39] Additional information February 2013.
[40] Technical dossier/Section 7.9.2.

According to the EFSA GMO Panel Guidance Documents (EFSA, 2006a, 2011a), when the plant receiving the introduced gene is known to be allergenic, the applicant should test any potential change in the allergenicity of the whole GM plant by comparing the allergen repertoire with that of its appropriate comparator(s). In this context, soybean is also considered a common allergenic food.[41]

The applicant performed *in vitro* allergenicity studies with extracts of soybeans MON 87708, its conventional counterpart (soybean A3525), and 17 non-GM reference varieties. The IgE binding of soybean proteins to sera from 13 individuals clinically documented to be allergic to soybean, and from five non-allergic individuals, was quantified using an ELISA method to investigate whether the allergenicity potential of soybean MON 87708 is altered in comparison with that of its conventional counterpart and the non-GM reference varieties. The sera from allergic individuals had similar reactivity to proteins in extracts from soybean MON 87708 and the conventional counterpart.

In addition and to further address the potential for changes in endogenous allergen repertoire of soybean MON 87708, the applicant supplied the results of two-dimensional (2D) SDS-PAGE analysis of extracts of soybean MON 87708 and its conventional counterpart followed by Western blotting with individual sera from eight individuals allergic to soybean. These studies demonstrated no meaningful differences in the IgE binding patterns between the extracts of proteins derived from soybean MON 87708 and its conventional counterpart.

In the context of the present application, and based on all the available information, the EFSA GMO Panel concludes that there are no indications that the genetic modification might significantly change the overall allergenicity of soybean MON 87708 when compared with that of its conventional counterpart.

5.1.4. Nutritional assessment of GM food/feed[42]

The intended trait of soybean MON 87708 is herbicide tolerance, with no intention to alter the nutritional parameters. The evaluation of compositional data, indicating that soybean MON 87708 is as nutritious as non-GM soybean varieties, was supported by the outcome of a chicken study (see Section 5.1.2.3(b)). The EFSA GMO Panel concludes that the data provided support the view that diets formulated with defatted soybean meal derived from soybean MON 87708 are as nutritious as those formulated with defatted soybean meal derived from commercial non-GM soybean varieties.

5.1.5. Post-market monitoring of GM food/feed[43]

No data indicating that soybean MON 87708 is any less safe than its conventional counterpart have emerged. In addition, soybean MON 87708 is as nutritious as non-GM soybean varieties. Therefore, and in line with the Guidance Documents (EFSA, 2006a, 2011a), the EFSA GMO Panel is of the opinion that post-market monitoring of the GM food/feed is not necessary.

5.2. Conclusion

The DMO proteins are degraded by proteolytic enzymes, and bioinformatics-supported studies demonstrated that these proteins show no similarity to known toxic and allergenic proteins. No toxicity of the DMO protein was observed in 28-day and acute toxicity studies in mice. The result of a 90-day feeding study in rats did not raise safety concerns. There are no indications that the genetic modification might significantly change the overall allergenicity of soybean MON 87708 when compared with that of its conventional counterpart. The compositional data indicating nutritional equivalence were corroborated by the chicken study. In conclusion, the EFSA GMO Panel is of the opinion that soybean MON 87708 is as safe and as nutritious as its conventional counterpart and non-GM reference varieties, in the context of its intended use.

[41] 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, L310, 11-14.
[42] Technical dossier/Section D7.10.
[43] Technical dossier/Section D7.11.

6. Environmental risk assessment and monitoring plan

6.1. Evaluation of relevant scientific data

6.1.1. Environmental risk assessment

The scope of application EFSA-GMO-NL-2011-93 is for food and feed uses, import and processing and does not include cultivation. Considering this scope, the environmental risk assessment is concerned with the indirect exposure mainly through manure and faeces from animals fed grain produced by soybean MON 87708 and with the accidental release into the environment of viable grains produced by soybean MON 87708 during transport and processing.

As the scope of the present application excludes cultivation, environmental concerns in the EU related to the use of dicamba-based herbicides on soybean MON 87708 do not apply.

6.1.1.1. Unintended effects on plant fitness due to the genetic modification[44]

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 (Lu, 2005). The major worldwide soybean producers are Argentina, Brazil, China, North Korea, South Korea and the USA. In the EU, soybean is mainly cultivated in Italy, France, Romania, Croatia, Hungary and Austria (Dorokhov et al. 2004).[45] Cultivated soybean seeds rarely display any dormancy characteristics, and only under certain environmental conditions do they 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). In soybean fields, seeds usually do not survive during the winter owing to predation, rotting, germination resulting in death, or management practices prior to planting the subsequent crop (Owen, 2005). The herbicide tolerance trait can be regarded as providing only a potential agronomic and selective advantage to this GM soybean plant where and when dicamba-based herbicides are applied. However, survival of soybean plants outside cultivation where dicamba-based herbicides are applied is limited mainly 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 MON 87708, herbicide tolerance is not likely to provide a selective advantage outside cultivation. Even if dicamba-based herbicides are applied to these plants, this will not change their ability to survive over seasons. Therefore, it is considered very unlikely that soybean MON 87708 will differ from conventional soybean varieties in its ability to survive until subsequent seasons or to establish feral populations under European environmental conditions.

Laboratory tests and field studies have been carried out to assess the phenotypic and agronomic characteristics as well as ecological interactions of GM soybean as described in Section 4.1.3.

Phenotypic and agronomic traits were evaluated in a field trial across eight locations in the US in 2009 (for further details, see Section 4.1.3). In addition, ecological interactions, such as soybean MON 87708 responses to abiotic and biotic stressors, were evaluated in the same trials. The statistical analysis indicated that dicamba-treated and non-treated soybean MON 87708 had lower 100 seed weight than its conventional counterpart and the non-GM reference varieties planted in these field trials. This parameter therefore required further evaluation (for further details, see Section 4.1.3).

Germination and dormancy of seeds from soybean MON 87708, its control and non-GM reference varieties, produced under different environmental conditions, were evaluated in growth chamber experiments following international protocols. Pollen characteristics were also assessed. Although some differences were observed under specific environmental conditions, these were not consistent and did not indicate a consistent plant response associated with the trait or any change in fitness.

[44] Technical dossier/Sections D4, D9.1 and D9.2 and additional information, November 2011.
[45] http://epp.eurostat.ec.europa.eu/portal/page/portal/agriculture/data/database.

Considering the intended uses of soybean MON 87708, special attention is paid to those agronomic characteristics which may affect the survival, establishment and fitness of soybean MON87708 grains which could be accidentally released into the environment: yield, plant height, shattering, germination, dormancy. The observed difference in 100 seed weight might result from a differentiated development of the crop or might be an indication of unintended effects due to the genetic modification. Regardless, this difference is unlikely to be biologically relevant in terms of increased persistence and invasiveness potential.

Therefore, from the data presented in the application, there is no indication of an increased persistence and invasiveness potential of soybean MON 87708 compared with conventional soybean, and it can be considered that soybean MON 87708 has no altered survival, multiplication or dissemination characteristics compared with its conventional counterpart, except under application of dicamba-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 and any change in survival capacity, including overwintering (Dorokhov et al., 2004; Owen, 2005; Bagavathiannan and Van Acker, 2008; Lee et al., 2009).

Therefore, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects of soybean MON87708 in Europe will not be different to that of conventional soybean varieties.

6.1.1.2. Potential for gene transfer[46]

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through horizontal gene transfer of DNA or through vertical gene flow via seed dispersal and cross-pollination.

(a) Plant to bacteria gene transfer

Genomic DNA is a component of many food and feed products derived from soybean. It is well documented that DNA present in food and feed becomes substantially degraded during 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 animals feeding on soybean MON87708 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 (2009) for further details).

A successful horizontal transfer 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 MON87708 contains genetic elements with identity or high similarity to those of bacteria. The coding sequence of DMO is highly similar to corresponding genes from DMO-producing *Stenotrophomonas maltophilia*, and the flanking regions of the recombinant gene insert contain approximately 40- and 250-bp-long sequences of the truncated right and left border, respectively, of

[46] Technical dossier/Section D6

the Ti plasmid of *Agrobacterium tumefaciens*. Neither *A. tumefaciens* nor *S. maltophilia* is considered to be prevalent in the main receiving environment, i.e. the gastrointestinal tract of humans or animals (Denton et al., 1998). Both occur in soil, and *S. maltophilia* has been isolated from the rhizosphere of plants (Berg et al., 1996). However, occurrence of the recombinant genes outside their immediate receiving environments in the habitats of both bacterial species cannot be ruled out (Hart et al., 2009) and is therefore also considered here.

On a theoretical basis (i.e. without any study providing experimental evidence for horizontal gene transfer in the case of GM food and feed derived from soybean MON 87708 or any other GM plant), it can be assumed that, as an extremely rare event, homologous recombination can occur between the recombinant *dmo* gene and the *dmo* gene of S. *maltophilia* or related bacteria present in the environment. Such recombination events would only replace natural variants (i.e. substitutive recombination) and are therefore unlikely to provide any new property resulting in a selective advantage for the recipient organisms (EFSA, 2009). Homologous recombination of the flanking regions with those on Ti plasmids of *A. tumefaciens* would result in gene replacement, whereby a *dmo* gene would be substituted for genes for crown gall formation (with loss of auxin-, cytokinin- and opine-synthesising genes). The current literature suggests that the minimum length of homologous DNA necessary to facilitate recombination is 150-bp (De Vries and Wackernagel, 2002; see also Annex 1 of EFSA, 2009). Therefore, the flanking regions of the Ti plasmid in the genome of MON87708 have a limited potential to facilitate homologous recombination of the recombinant *dmo* gene with naturally occurring *Agrobacterium* strains carrying Ti plasmids.

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, 2009). Illegitimate recombination events have not been detected in studies that have exposed bacteria to high concentrations of GM plant DNA (EFSA 2009). Thus, this process, in comparison with homologous recombination, is not considered to significantly contribute to horizontal gene transfer events. In comparison with the above described homology-facilitated recombination processes, the contribution of illegitimate recombination is extremely low.

The *dmo* gene of soybean MON87708 is regulated by a promoter of the peanut chlorotic streak caulimovirus. The expression of such a promoter–gene construct in bacteria is unknown, but generally the expression level of eukaryotic promoters in bacteria is inefficient (Warren et al., 2008).

In a worst-case scenario, considering the possibility of expression, an *A. tumefaciens* recipient would become capable of producing a DMO protein, but simultaneously it would lose its capacity for crown gall formation. The exposure of bacterial communities to the recombinant gene in soybean MON87708 must be seen in the context of the natural occurrence and level of exposure to alternative sources of similar genes to which bacterial communities are continually exposed. Owing to its specific life-style as a soil bacterium and plant pathogen, the EFSA GMO panel considers it unlikely that *A. tumefaciens* would gain selective advantage by acquisition and expression of the *dmo* gene from soybean MON 87708 by homologous recombination.

The EFSA GMO Panel concludes that the *dmo* gene from soybean MON87708 may, on a theorical basis, be transferred by homologous recombination to *A. tumefaciens* or to *S. maltophilia*. However, since neither *A. tumefaciens* nor *S. maltophilia* is considered to be a member of the gut microbiota, exposure to recombinant DNA of soybean MON 87708 is considered to be very low.

Owing to the occurrence of natural variants of the *dmo* gene in the environment, a low level of gene transfer to *A. tumefaciens* or *S. maltophilia* is not regarded to confer a novel selective advantage on environmental bacteria as potential recipients. Considering its intended uses as food and feed and the above assessment, the EFSA GMO Panel has therefore not identified a concern associated with horizontal gene transfer from soybean MON 87708 to bacteria.

(b) Plant-to-plant gene transfer

Considering the intended uses of soybean MON87708 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 grain spillage during transport and/or processing.

The genus *Glycine* is divided into two distinct subgenera: *Glycine* and *Soja*. Soybean is in the subgenus *Soja*. The subgenus *Glycine* contains 16 perennial wild species, whilst the cultivated soybean, *Glycine max*, and its wild and semi-wild annual relatives, *Glycine soja* and *Glycine gracilis*, are classified in the subgenus *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 subgenus *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 since they have not been reported in other parts of the world where the cultivated soybean is grown (Dorokhov et al. 2004; Lu 2005), plant-to-plant gene transfer from soybean in the EU is restricted to cultivated soybean.

Soybean (*Glycine max*) is an annual almost completely self-pollinating crop; the percentage that is cross-pollinated is 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 of 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 favourable climate for pollination and abundance of pollinators (Gumisiriza and Rubaihayo, 1978; Kikuchi et al., 1993; Ahrent and Caviness, 1994; Ray et al., 2003; Lu, 2005).

Plant-to-plant gene transfer could therefore occur under the following scenario: imports of soybean MON87708 grains (although most MON87708 grains will be processed in the country of production), processing outside of importing ports, transport in regions of soybean production in Europe, spillage of GM grains during transport, germination and development of spilled grains within soybean fields or in the very close vicinity to cultivated soybean fields, 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 outcrossing with this GM soybean will not persist overtime. Dispersal of soybean seeds by animals is not expected because of the characteristics of the seed, but accidental release into the environment of grains may occur during transport and processing for food, feed and industrial uses. However, cultivated soybean seeds rarely display any dormancy characteristics and only under certain environmental conditions will they 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). Even in soybean fields, seeds usually do not survive during the winter owing to predation, rotting, germination resulting in death or management practices prior to planting the subsequent crop (Owen, 2005).

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. 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, since soybean MON 87708 has no 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 will not differ from that of conventional soybean varieties.

6.1.1.3. Interactions of the GM plant with target organisms[47]

Considering the intended uses of soybean MON 87708, excluding cultivation, and the absence of target organisms, potential interactions of the GM plant with target organisms were not considered an issue by the EFSA GMO Panel.

6.1.1.4. Interactions of the GM plant with non-target organisms[48]

Owing to the intended uses of soybean MON 87708, which exclude cultivation and because of the low level of exposure to the environment, potential interactions of the GM plant with non-target organisms were not considered an issue by the EFSA GMO Panel.

6.1.1.5. Interactions with the abiotic environment and biogeochemical cycles[49]

Owing to the intended uses of soybean MON 87708, which exclude cultivation and because of the low level of exposure to the environment, potential interactions with the abiotic environment and biogeochemical cycles were not considered an issue by the EFSA GMO Panel.

6.1.2. Post-market environmental monitoring[50]

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 assumptions 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 which 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 plan provided by the applicant (EFSA, 2011). The potential exposure to the environment of soybean MON 87708 would be through manure and faeces from animals fed soybean MON 87708 or through accidental release into the environment of GM soybean grains (e.g. during transport and processing). The scope of the PMEM plan provided by the applicant is in line with the intended uses. As the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects, no case-specific monitoring is necessary.

The general surveillance 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 and a final report at the end of the consent.

The EFSA GMO Panel is of the opinion that the scope of the PMEM plan proposed by the applicant is in line with the intended uses of soybean MON 87708 as the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. In addition, the EFSA GMO Panel acknowledges the approach proposed by the applicant to put in place appropriate management systems to restrict environmental exposure in the case of accidental release of viable grains of soybean MON 87708. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the PMEM plan.

[47] Technical dossier/Sections D8 and D9.4.
[48] Technical dossier/Section D9.5.
[49] Technical dossier/Sections D9.8 and D10.
[50] Technical dossier/Section D11.

6.2. Conclusion

The scope of application EFSA-GMO-NL-2011-93 is for food and feed uses, import and processing and does not include cultivation. Considering the intended uses of soybean MON87708, the environmental risk assessment is concerned with the indirect exposure mainly through manure and faeces from animals fed grain produced by soybean MON 87708 and with the accidental release into the environment of viable grains produced by soybean MON 87708 during transport and processing.

In the event of accidental release into the environment of viable grains of soybean MON 87708 during transport and processing, there are no indications of an increased likelihood of establishment and spread of feral GM soybean plants, except under application of dicamba-based herbicides. Considering its intended uses as food and feed, potential interactions of soybean MON 87708 with the biotic and abiotic environment were not considered to be an issue by the EFSA GMO Panel. Furthermore, the EFSA GMO Panel is of the opinion that the unlikely but theoretically possible transfer of the recombinant gene from soybean MON 87708 to environmental bacteria does not raise concern owing to the lack of a selective advantage in the context of its intended uses.

The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of soybean MON 87708 and the Guidance Document of the EFSA GMO Panel on PMEM of GM plants (EFSA, 2011). In addition the EFSA GMO Panel acknowledges the approach proposed by the applicant to put in place appropriate management systems to restrict environmental exposure in cases of accidental release of viable grains of soybean MON 87708. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the PMEM plan.

CONCLUSIONS AND RECOMMENDATIONS

The EFSA GMO Panel was requested to carry out a scientific risk assessment of soybean MON 87708 for food and feed uses, import and processing in accordance with Regulation (EC) No 1829/2003.

The EFSA GMO Panel is of the opinion that the molecular characterisation provided for soybean MON 87708 is sufficient. The result of the bioinformatic analyses of the inserted DNA and the flanking regions do not raise any safety concerns. The levels of DMO proteins in soybean MON 87708 have been sufficiently analysed and the stability of the genetic modification has been demonstrated.

Based on the information available, it is concluded that no differences were identified in the compositional data of forage and seeds obtained from soybean MON 87708 or in its agronomic and phenotypic characteristics that would require further assessment with regard to safety by the EFSA GMO Panel.

The DMO proteins are degraded by proteolytic enzymes, and bioinformatics-supported studies demonstrated that these proteins show no similarity to known toxic and allergenic proteins. No toxicity of the DMO protein was observed in 28-day and acute toxicity studies in mice. The result of a 90-day feeding study in rats with diets containing toasted defatted soybean meal from soybean MON 87708, its conventional counterpart or any of two non-GM soybean varieties did not raise safety concerns. There are no indications that the genetic modification might significantly change the overall allergenicity of soybean MON 87708 when compared with that of its conventional counterpart. The compositional data indicating that soybean MON 87708 is as nutritious as non-GM soybean varieties were supported by the outcome of a chicken study. The EFSA GMO Panel is of the opinion that soybean MON 87708 is as safe and as nutritious as its conventional counterpart and non-GM reference varieties, in the context of its intended uses.

Considering the scope of this application, which excludes cultivation, there is no requirement for scientific assessment on possible environmental effects associated with the cultivation of this GM soybean. In the case of accidental release into the environment of viable grains of soybean MON 87708 during transport and processing, there are no indications of an increased likelihood of

establishment and spread of feral GM soybean plants, except under application of dicamba-based herbicides. Considering its intended uses as food and feed, potential interactions of soybean MON 87708 with the biotic and abiotic environment were not considered to be an issue by the EFSA GMO Panel. Furthermore, the EFSA GMO Panel is of the opinion that the unlikely but theoretically possible transfer of the recombinant gene from soybean MON 87708 to environmental bacteria does not raise concern owing to the lack of a selective advantage in the context of its intended uses.

The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of soybean MON 87708 and the Guidance Documents of the EFSA GMO Panel on PMEM of GM plants. In addition, the EFSA GMO Panel acknowledges the approach proposed by the applicant to put in place appropriate management systems to restrict environmental exposure in the event of accidental release of viable grains of soybean MON 87708. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the PMEM plan.

In conclusion, the EFSA GMO Panel considers that the information available for soybean MON 87708 addresses the scientific issues indicated in the relevant Guidance Documents of the EFSA GMO Panel and the scientific comments raised by the Member States, and that soybean MON 87708, 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 or the environment, in the context of its intended uses.

It should be noted that the assessment of potential consumer health risks resulting from dicamba residues and its metabolites in soybean MON 87708 is under the remit of the EFSA Pesticides Unit.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of the Netherlands received on 9 February 2011 concerning a request for placing on the market of soybean MON 87708 in accordance with Regulation (EC) No 1829/2003.

2. Acknowledgement letter dated 2 March 2011 from EFSA to the Competent Authority of the Netherlands.

3. Letter from EFSA to applicant, dated 18 March 2011, requesting additional information under completeness check.

4. Letter from applicant to EFSA, received 29 March 2011, providing additional information under completeness check.

5. Letter from EFSA to applicant, dated 13 May 2011, delivering the 'Statement of Validity' for application EFSA-GMO-NL-2011-93, soybean MON 87708 submitted by Monsanto under Regulation (EC) No 1829/2003.

6. Letter from EFSA to applicant dated 10 August 2011 requesting additional information and stopping the clock.

7. Letter from EFSA to applicant dated 17 August 2011 requesting additional information and maintaining the clock stopped.

8. Letter from applicant to EFSA received 18 October 2011 providing additional information.

9. Letter from EFSA to applicant dated 10 February 2012 requesting additional information and maintaining the clock stopped.

10. Letter from applicant to EFSA received 14 March 2012 providing additional information.

11. Letter from EFSA to applicant dated 24 May 2012 re-starting the clock.

12. Letter from EFSA to applicant dated 5 July 2012 requesting additional information and stopping the clock.

13. Letter from applicant to EFSA received 1 August 2012 providing additional information.

14. Letter from EFSA to applicant dated 7 November 2012 requesting additional information and maintaining the clock stopped.

15. Letter from applicant to EFSA received 13 November 2012 providing additional information.

16. Letter from EFSA to applicant dated 11 February 2013 requesting additional information and maintaining the clock stopped.

17. Letter from applicant to EFSA received 15 February 2013 providing additional information.

18. Letter from EFSA to applicant dated 19 February 2013 requesting additional information and maintaining the clock stopped.

19. Letter from applicant to EFSA received 21 February 2013 providing additional information.

20. Letter from EFSA to applicant dated 13 May 2013 requesting additional information and maintaining the clock stopped.

21. Letter from applicant to EFSA received 6 June 2013 providing additional information.

22. Letter from EFSA to applicant dated 5 September 2013 re-starting the clock.

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