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Aan de minister van
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**DATUM** 13 oktober 2010
**KENMERK** CGM/101013-03
**ONDERWERP** Advies import en verwerking van sojalijn MON87705 met veranderde vetzuursamenstelling

Geachte mevrouw Huizinga,

Naar aanleiding van de adviesvraag betreffende het dossier EFSA/GMO/NL/2010/78 voor de import en verwerking van genetisch gemodificeerde soja MON87705, ingediend door Monsanto Europe S.A., adviseert de COGEM als volgt.

**Samenvatting**
De COGEM is gevraagd te adviseren over de toelating van sojalijn MON87705 voor import en verwerking. In deze lijn is de expressie van de *FAD2-1A* en *FATB1A* genen geremd wat resulteert in sojabonen met een veranderde vetzuursamenstelling. Het gehalte aan palmitinezuur, stearinezuur en linolzuur is verlaagd. Het gehalte aan oliezuur is verhoogd. Daarnaast brengt deze lijn het *cp4 epsps* gen tot expressie waardoor zij tolerant is voor glyfosaat bevattende herbiciden.
Teelt en overwintering van sojabonen is in Nederland niet mogelijk omdat soja een korte dagplant is, sterk koudegevoelig is en hoge temperaturen nodig heeft voor kieming en ontwikkeling. Verder beschikt soja niet over eigenschappen die nodig zijn voor verwildering en er zijn geen redenen om aan te nemen dat de geïntroduceerde eigenschappen het verwilderingspotentieel vergroten. Daarnaast zijn er in Europa geen wilde verwanten van soja aanwezig waardoor uitkruising niet mogelijk is. De COGEM acht daardoor de kans dat incidenteel morsen in Nederland tot verspreiding van MON87705 leidt verwaarloosbaar klein.
Hoewel de COGEM het door de aanvrager opgestelde ‘general surveillance’ plan onderschrijft, ziet zij een enkel punt voor verbetering.
Op basis van de overwegingen in dit advies acht de COGEM de risico’s van import en verwerking van sojalijn MON87705 verwaarloosbaar klein. De COGEM wijst erop dat een beoordeling van de voedselveiligheid, inclusief incidentele consumptie, door een andere instantie wordt uitgevoerd en geen onderdeel is van de risicoanalyse in dit advies.

De door de COGEM gehanteerde overwegingen en het hieruit voortvloeiende advies treft u hierbij aan als bijlage.

Hoogachtend,

Prof. dr. ir. Bastiaan C.J. Zoeteman
Voorzitter COGEM

c.c. Dr. I. van der Leij
Drs. H.P. de Wijs

# Import and processing of genetically modified glyphosate tolerant soybean MON87705 with an altered fatty acid profile

## COGEM advice CGM/101013-03

**Summary**

*The present application of Monsanto Company (file EFSA/GMO/NL/2010/78) concerns the import and processing for use in feed and food of soybean line MON87705. Cultivation is not part of this application.*

*Soybean line MON87705 was obtained by Agrobacterium-mediated transformation of conventional soybean. The line contains a FAD2-1A/ FATB1A suppression cassette resulting in an altered fatty-acid profile of the soybean seeds. Additionally, the line expresses the cp4 epsps gene derived from Agrobacterium sp., conferring tolerance to glyphosate containing herbicides.*

*In Europe, closely related species of soybean are not present and therefore, hybridization with other species is not possible. Soybean does not possess any of the attributes commonly associated with problematic weeds and establishment of feral soybean populations has never been observed in Europe. Due to the climatic and geographical conditions, survival of soybean is not possible in the Netherlands. Because there is no reason to assume that the inserted genes would introduce the potential for soybean to establish feral populations, COGEM is of the opinion that incidental spillage of soybean will not pose a risk to the environment.*

*In the opinion of COGEM, the molecular analysis of soybean line MON87705 has been sufficiently performed. Although the general surveillance (GS) plan could be improved by a guarantee that operators will monitor for unanticipated effects, COGEM considers the current GS plan sufficient for import and processing of soybean line MON87705.*

*In conclusion, COGEM is of the opinion that import and processing of soybean line MON87705 poses a negligible risk to the environment and has no objections against an authorization for import and processing of MON87705. COGEM points out that a food/feed safety assessment is carried out by other organizations. Therefore, COGEM abstains from advice on the potential risks of incidental consumption.*

**Introduction**

The present application (file EFSA/GMO/NL/2010/78) by Monsanto Europe S.A., concerns the import and processing of genetically modified soybean MON87705. The line has been developed to enhance the suitability of soybean oil for food and industrial use.

MON87705 contains a *FAD2-1A/ FATB1A* suppression cassette resulting in an altered fatty-acid profile of the soybean seeds. The level of the saturated fatty acids palmitic acid and stearic acid as well as the polyunsaturated fatty acid linoleic acid has been decreased. The level of mono-unsaturated fatty acid oleic acid has been increased. Additionally, the line expresses the *cp4 epsps* gene derived from *Agrobacterium* sp., conferring tolerance to glyphosate containing herbicides.

Soybean seeds with low saturated fatty acid levels improve the processing of soybean oil.[1] A decrease in the level of the polyunsaturated fatty acid linoleic acid enhances the oxidative stability of the soybean oil due to the reduction of the number of instable double bonds.

Monounsaturated fatty acid rich oil has been associated with decreased incidence of cardiovascular events.[2]

MON87705 was developed by *Agrobacterium*-mediated transformation of conventional soybean and has not been assessed by the EFSA before.

**Previous COGEM advice**

COGEM did not issue an advice on MON87705 soybean before. In 2007 and 2008 COGEM positively assessed two genetically modified soybean lines containing a *FAD2-1* suppression cassette.[3,4] In one line the cassette was combined with a *cp4 epsps* expression cassette.[4] Recently, COGEM issued a positive advice on another soybean line with an altered fatty acid profile based on the presence of a *Pj.D6D* and *Nc.Fad3* expression cassette.[5]

**Aspects of the crop**

Soybean (*Glycine max*) is a member of the genus *Glycine* and belongs to the *Fabaceae* (*Leguminosae*) family. Soybean is grown from equatorial to temperate zones. Due to the climatic and geographical conditions, cultivation of soybean is impossible in the Netherlands. The optimum temperature for soybean growth is between 25°C and 30°C. In the Netherlands, 16.6°C was the average summer temperature from 1971 to 2009. The average temperature of the three warmest summers since 1901 was 18.6°C.[6] In addition, soybean does not survive freezing.[7] In the Netherlands frost is common; during winter on average 38 days are measured with a minimum temperature below 0°C.[6] Moreover, during the Dutch growth season the days are long, whereas soybean is a quantitative short-day plant that needs short days for induction of flowering.[7]

Soybean is predominantly a self-pollinating species. The cross-pollination rate of soybean is less than 1%.[7] The dispersal of pollen is limited because the anthers mature in the bud and directly pollinate the stigma of the same flower.[7] In Europe, hybridization with other species is not possible because there are no closely related species of soybean.[7]

The soybean plant is not weedy in character.[7] Cultivated soybean rarely displays dormancy and seeds of cultivated soybean poorly survive in soil.[8] Soybean volunteers are rare and do not effectively compete with other cultivated plants or primary colonizers.[7] In addition, volunteers are easily controlled mechanically or chemically.[7] Establishment of feral soybean populations has never been observed in Europe.

Soybean is grown primarily for the production of beans, has a multitude of uses in the food and industrial sectors and represents one of the major sources of edible vegetable oil and proteins for livestock feed use.[7] Today, soybean is the most prevalently grown oilseed in the world, with approximately 222.1 million metric tons produced in 2007, which represented 56% of world oilseed production that year.[9] Soybean is grown as a commercial crop in over 35 countries. The major producers of soybean are the US, Brazil, Argentina and China.[10]

**Molecular characterization**

*Properties of the introduced gene cassettes*

The in the plant genome inserted *FAD2-1A/FATB1-A* suppression cassette contains endogenous *FATB1-A* and *FAD2-1A* sense and antisense gene segments generating an inverted repeat.

FATB1-A and FAD2-1A are thioesterase and desaturase enzymes respectively, and both involved in the fatty acid metabolism. Due to the presence of the inverted repeat, transcription of the *FAD2-1A/FATB1-A* cassette generates double stranded RNA (dsRNA). Via the RNA interference (RNAi) pathway, the dsRNA mediates suppression of the endogenous *FATB* and *FAD2* RNA levels in soybean seeds resulting in a decrease in the level of palmitic, stearic, and linoleic acid, as well as an increase in the level of oleic acid. According to the applicant, the assembled gene transcript does not encode a functional protein.

EPSPS is a naturally endogenous plant enzyme involved in the biosynthesis of aromatic amino acids. Glyphosate inhibits EPSPS, resulting in a lack of amino acids essential for growth and development of plants. The *cp4 esps* gene is derived from *Agrobacterium* sp. strain CP4 and encodes the CP4 EPSPS protein which has a much reduced affinity for glyphosate. The integration of the *cp4 esps* gene cassette results in plants tolerant to glyphosate containing herbicides.[11]

*Overview of the construction and inserted genetic elements of MON87705*

Soybean MON87705 was produced by *A. tumefaciens* mediated transformation of meristematic soybean tissue using the binary vector PV-GMPQ/HT4404. PV-GMPQ/HT4404 contains two transfer DNAs (T-DNAs). T-DNA I contains the *cp4 epsps* expression cassette and a partial *FAD2-1A/FATB1-A* suppression cassette. A *FMV/Tsf1* chimeric promoter drives constitutive expression of the CP4 EPSPS protein. The partial *FAD2-1A/FATB1-A* suppression cassette contains the endogenous soybean sense gene segments of the first *FAD2-1A* intron and the *FATB1-A* 5' untranslated region (UTR) and *FATB1-A* plastid targeting sequence. The gene segments are under regulation of the endogenous soybean seed 7Sα' promoter. T-DNA II contains a partial *FAD2-1A/FATB1-A* suppression cassette which consists of the antisense gene segments of the first *FAD2-1A* intron and the *FATB1-A* 5'UTR, and is flanked by the H6 3'UTR of *Gossypium barbadense* (Pima cotton). During plant transformation, the two T-DNAs co-integrated adjacent to each other at one locus in the soybean genome, creating a DNA insert that contains the *cp4 epsps* expression cassette and a complete *FAD2-1A/FATB1-A* suppression cassette. An overview of the insert introduced in MON87705 and its flanking regions is given below:

- Unique 5' flanking sequence of the insert. Soybean genomic DNA.
- Sequence flanking the 5' end of the insert. 2374 bp of soybean genomic DNA, duplicated from the sequence flanking the 3' end of the insert.
- B-Left Border. 259 bp sequence from the B-Left Border region remaining after integration.
- Intervening sequence. Sequence used in DNA cloning.
- P-*FMV/Tsf1*. Chimeric promoter consisting of enhancer sequences from the promoter of the *Figwort mosaic virus* 35S RNA combined with the promoter from the *Tsf1* gene of *A. thaliana* that encodes elongation factor EF-1α.
- L-*Tsf1*. 5' Untranslated leader sequence (exon 1) from the *Tsf1* gene of *A. thaliana* that encodes elongation factor EF-1α.
- I-*Tsf1*. Intron with flanking exon sequence from the *Tsf1* gene of *A. thaliana* that encodes elongation factor EF-1α.

- Intervening sequence. Sequence used in DNA cloning.
- TS-*CTP2*. Targeting sequence from the *ShkG* gene encoding the transit peptide region of *A. thaliana* EPSPS that directs transport of the CP4 EPSPS protein to the chloroplast.
- CS-*cp4 epsps*. Codon modified coding sequence of the *aroA* gene from the *Agrobacterium* sp. strain CP4 encoding the CP4 EPSPS protein.
- Intervening sequence. Sequence used in DNA cloning.
- T-*E9*. 3' Untranslated region of the pea *RbcS2* gene which functions to direct polyadenylation of the mRNA.
- Intervening sequence. Sequence used in DNA cloning.
- P-*7Sα'*. Non-coding promoter and leader sequence from the *Sphas1* gene of *G. max* encoding β-conglycinin storage protein (α'-βcsp) that directs transcription in seed.
- Intervening sequence. Sequence used in DNA cloning.
- *FAD2-1A*. Partial sequence from the first intron of the *G. max FAD2-1A* gene.
- *FATB1-A*. Partial sequence from the 5' untranslated region and the plastid targeting sequence from *G. max FATB1-A* gene.
- Intervening sequence. Sequence used in DNA cloning.
- B-Right Border. 20 bp sequence from the B-Right Border region remaining after integration.
- B-Left Border. 38 bp sequence from the B-Left Border region remaining after integration.
- *FATB1-A*. Partial sequence from the 5' untranslated region and the plastid targeting sequence from *G. max FATB1-A* gene.
- *FAD2-1A*. Partial sequence from the first intron of the *G. max FAD2-1A* gene.
- Intervening sequence. Sequence used in DNA cloning.
- T-*H6*. 3' UTR of the H6 gene from *G. barbadense* encoding a fiber protein involved in secondary cell wall assembly.
- Intervening sequence. Sequence used in DNA cloning.
- B-Left Border. 275 bp sequence from the B-Left Border region remaining after integration.
- Sequence flanking 3' end of the insert. Soybean genomic DNA including the 2374 bases duplicated at the 5' end of the flanking sequence of the insert.
- Unique 3' flanking sequence of the insert. Soybean genomic DNA.

*Molecular analysis*

The applicant confirmed by Southern blot analyses that a single copy of the T-DNA I and T-DNA II are integrated at a single integration locus in the genome of MON87705. The integration of the T-DNAs was stable over several generations. Additionally, Southern blot analyses showed that the backbone of plasmid PV-GMPQ/HT4404 is absent.

DNA sequence analysis of overlapping PCR products across the insert confirmed that T-DNA I and T-DNA II have been integrated adjacent to each other. The *FAD2-1A* and *FATB1-A* gene suppression elements, located at the junction of T-DNA I and T-DNA II, are arranged as an inverted repeat. COGEM points out that at this junction, the T-DNA I and T-DNA II border sequences have been rearranged. In the schematic representation depicted in the application, the overlap between the PCR products appears to be minimal. The extent of the overlap cannot be determined as primer sequences are not provided. Due to the inverted repeat structure it is possible that performance of the PCR reaction was problematic. Therefore, COGEM cannot

exclude that the sequence data contain flaws. However, in case the data are incorrect, it does not lead to the expression of putative ORFs. Due to the inverted repeat, putative ORFs will be processed into small interfering (si) RNAs via the RNAi pathway and, as a consequence, translation is blocked. In COGEM's opinion, sequence data have to be correct and unquestionable. Therefore, COGEM suggests confirming the sequence at the T-DNA I and T-DNA II junction by using alternative primer sets based on the sequence data obtained. COGEM proposes designing primers at the unexpected Left Border sequence, present at the junction, and primers at either side of this Left Border sequence.

Sequence analysis of approximately 3000 bp on either side of the integration site, demonstrated that the flanking regions consist of soybean DNA with a 36 bp deletion as well as a 2374 bp insertion at the 5' end of the insert. The 2374 bp insertion was homologous with 2374 bp present in the flanking region at the 3' end of the insertion site, apart from one single nucleotide difference. According to the applicant, this 2374 bp duplication occurred during integration of the T-DNA sequences. Additional sequence analysis revealed that, both the *cp4 epsps* expression cassette and the *FAD2-1A/FATB1-A* suppression cassette in MON87705 match the cassettes in PV-GMPQ/HT4404, apart from a 30 bp truncation at the 3' end of the *FATB1-A* antisense sequence. According to the applicant, the 30 bp truncation did not have an impact on the function of the suppression cassette.

The applicant performed Northern blot analysis on RNA isolated from MON87705 and immature conventional soybean seeds in order to examine suppression of the RNA levels of the endogenous *FAD2-1A* and *FATB1-A* genes. The analysis showed that the *FAD2-1A* and *FATB1-A* RNA levels were greatly reduced in MON 87705 compared to the control.

Bioinformatic analysis by BLASTn and BLASTx searches (GenBank databases, January 2010) showed that no endogenous soybean open reading frames or regulatory elements were disrupted by the T-DNA insertion. The DNA sequences spanning the 5' and 3' junctions of the MON87705 insertion site and the genomic DNA were bioinformatically analyzed from stop codon to stop codon for theoretical new fusion proteins (AD, TOX, and PRT databases, 2010). Results of these analyses demonstrated no structurally-relevant similarities between any known toxins or allergens and the putative polypeptides.

In conclusion, COGEM is of the opinion that the molecular characterization of MON87705 meets the criteria laid down by COGEM.[12]

**Environmental risk assessment**

The current application of soybean line MON87705 concerns import and processing. In case of spillage soybean seed may be released into the environment. Due to the climatic and geographical conditions cultivation of soybean is impossible in the Netherlands. Soybean is a quantitative short-day plant that needs short days for induction of flowering, the optimum temperature for growth is between 25°C and 30°C, and soybean does not survive freezing. In view of the above, there are no reasons to assume that the introduced traits increase the ability of soybean seed to survive in the Dutch climatic conditions and increase the potential of MON87705 to establish feral populations in case of incidental spillage.

Since 2008 COGEM abstains from giving advice on the potential risks of incidental consumption in case a food/feed assessment is already carried out by other organizations.[13] This application is submitted under Regulation (EC) 1829/2003, therefore a food/feed assessment is carried out by EFSA. Other organizations who advise the competent authorities can perform an additional assessment on food safety although this is not obligatory. In the Netherlands a food and/or feed assessment for Regulation (EC) 1829/2003 applications is carried out by RIKILT. Regarding the risks for food and feed, the outcome of the assessment by other organizations (EFSA, RIKILT) was not known at the moment of the completion of this advice.

**General surveillance plan**

General surveillance (GS) has been introduced to be able to observe unexpected adverse effects of genetically modified crops on the environment. The setting or population in which these effects might occur is either not, or hardly predictable.

The GS plan states that unanticipated adverse effects will be monitored by existing monitoring systems which include the authorization holder and operators involved in the handling and use of viable MON87705 soybean. Recently, COGEM formulated criteria which GS plans concerning Dutch applications for import and cultivation of GM crops have to comply with.[14] Although the general surveillance plan could be improved by a guarantee that operators will monitor for unanticipated effects, COGEM considers the general surveillance plan sufficient for import and processing of MON87705 soybean.

**Advice**

COGEM has been asked to advise on import and processing for use in food and feed of soybean line MON87705. In COGEM's point of view, the molecular analysis gives no reason to assume that MON87705 will pose a risk to the environment.

The North-Western European climate prohibits survival and establishment of soybean. Furthermore, modern soybean cultivars do not possess any of the characteristics commonly associated with problematic weeds and there is no reason to assume that presence and expression of the introduced genes increases the potential of soybean to establish feral populations. In addition, establishment of feral soybean populations in European countries has never been observed. COGEM is of the opinion that incidental spillage of soybean leading to the spread of soybean within the Netherlands is negligible. Closely related species of soybean are not present in Europe and therefore introgression of the inserted genes into closely related species can not occur.

Based on the aspects discussed, COGEM is of the opinion that import and processing of soybean MON87705 poses a negligible risk to the environment. A food/feed safety assessment is carried out by other organizations. Therefore, COGEM abstains from advice on the potential risks of incidental consumption.

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