



Aan de minister van  
Volkshuisvesting, Ruimtelijke  
Ordening en Milieubeheer  
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**DATUM** 14 april 2010  
**KENMERK** CGM/100414-01  
**ONDERWERP** Advies: import en verwerking van sojabonen met veranderde vetzuursamenstelling

Geachte mevrouw Huizinga,

Naar aanleiding van een adviesvraag betreffende het dossier EFSA/GMO/UK/2009/76 voor de import en verwerking van genetisch gemodificeerde soja MON87769 ingediend door Monsanto Europe S.A., adviseert de COGEM als volgt.

#### **Samenvatting**

De COGEM is gevraagd te adviseren over de toelating van sojalinj MON87769 voor import en verwerking. Deze lijn brengt de genen *Pj.D6D* en *Nc.Fad3* tot expressie wat resulteert in sojabonen met een veranderde vetzuursamenstelling.

Teelt en overwintering van sojabonen is in Nederland niet mogelijk, omdat soja een sterk koudegevoelige, korte dagplant is en hoge temperaturen nodig heeft voor kieming en ontwikkeling. Verwildering van soja is in Europa nog nooit waargenomen. Verder zijn er geen redenen om aan te nemen dat de geïntroduceerde eigenschap het verwilderingpotentieel vergroot. Daarnaast zijn er in Europa geen wilde verwanten van soja aanwezig zodat uitkruising niet mogelijk is. De COGEM acht derhalve de kans dat incidenteel morsen binnen Nederland tot verspreiding van MON87769 leidt verwaarloosbaar klein. Daarnaast is de COGEM van mening dat de moleculaire karakterisering adequaat is uitgevoerd en dat er geen redenen zijn om aan te nemen dat het verhoogde vetzuurgehalte in de sojaboon een risico vormt voor het milieu.

Op basis van de overwegingen in dit advies acht de COGEM de risico's van import en verwerking van sojalinj MON87769 verwaarloosbaar klein. De COGEM wijst erop dat een beoordeling van de voedselveiligheid, inclusief incidentele consumptie, door een andere instantie wordt uitgevoerd en daarom 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,

A handwritten signature in black ink, consisting of a large loop on the left and a long horizontal stroke extending to the right.

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

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

# **Import and processing of genetically modified soybean MON87769 expressing two desaturase genes**

## **COGEM advice CGM/100414-01**

### **Summary**

*The present application of Monsanto Company (file EFSA/GMO/UK/2009/76) concerns the import and processing for use in feed and food of soybean line MON87769. Cultivation is not part of this application.*

*Soybean line MON87769 was obtained by Agrobacterium tumefaciens mediated transformation. The soybean expresses the Pj.D6D gene derived from Primula juliae and Nc.Fad3 derived from Neurospora crassa resulting in the production of stearidonic acid (SDA), an omega-3 fatty acid.*

*The applicant showed by Southern blot analyses that one copy of the insert was integrated at a single integration locus in the genome of MON87769 and that the backbone of the plasmid used for transformation was absent in MON87769. Sequence analyses spanning the 5' and 3' junctions of the insertion site and the genomic DNA indicated a 9 bp deletion of genomic DNA and two small insertions of 17 and 8 bp. No endogenous soybean open reading frames (ORFs) were disrupted. Sequence analyses also identified several sequences of eight amino acids or greater in length. No sequence similarities between any known toxins or allergens were demonstrated. In the opinion of COGEM, the molecular analysis of MON87769 has been adequately performed.*

*In Europe, closely related species of soybean are not present and soybean does not possess any of the attributes commonly associated with problematic weeds. Besides, establishment of feral soybean populations has never been observed in Europe. Hybridization with other species is not possible because there are no closely related species of soybean present. 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 or increase 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 conclusion, COGEM is of the opinion that import and processing of soybean line MON87769 poses a negligible risk to the environment and has no objections against an authorization for import and processing of MON87769. 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/UK/2009/76) by Monsanto Europe S.A., concerns the import and processing of genetically modified soybean MON87769. Soybean MON87769 was produced by Agrobacterium tumefaciens mediated transformation of conventional soybean and expresses the Pj.D6D gene derived from Primula juliae and Nc.Fad3 derived from Neurospora

*crassa*. The introduction of these genes results in the seed specific production of stearidonic acid (SDA), an omega-3 fatty acid.

### **Previous COGEM advice**

To date, COGEM has not advised on soybean MON87769 or the specific genes introduced in this soybean. However, in 2006 COGEM advised positively on the import and processing of soybean 305423 expressing a *gm-fad2-1* gene fragment resulting in a high oleic phenotype.<sup>1</sup> Also in 2008 COGEM advised positively on the import and processing of hybrid soybean line 305423 x 40-3-2. In addition to a high oleic phenotype, this hybrid line is tolerant to glyphosate-containing herbicides due to the presence of the *cp4 epsps* gene.<sup>2</sup>

### **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.<sup>3</sup> In addition, soybean does not survive freezing. In the Netherlands frost is common; during winter on average 38 days are measured with a minimum temperature below 0 °C.<sup>3</sup> 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.

Soybean is predominantly a self-pollinating species. The cross-pollination rate of soybean is less than 1%.<sup>4</sup> Cross-pollination occurs by insects. The dispersal of pollen is limited because the anthers mature in the bud and directly pollinate the stigma of the same flower. Therefore, insect-born exportation of pollen is limited.<sup>4</sup> In Europe, hybridization with other species is not possible because there are no closely related species of soybean.

The soybean plant is not weedy in character.<sup>5</sup> Cultivated soybean rarely displays dormancy<sup>5</sup> and seeds of cultivated soybean survive poorly in soil.<sup>6</sup> Soybean volunteers are rare and do not effectively compete with other cultivated plants or primary colonizers.<sup>5</sup> In addition, volunteers are easily controlled mechanically or chemically.<sup>5</sup> Establishment of feral soybean populations has never been observed in Europe.

### **Molecular characterisation**

Soy line MON87769 was produced by *A. tumefaciens* mediated transformation of meristematic soybean tissue. This soy line expresses the *Pj.D6D* gene derived from *P. juliae* and *Nc.Fad3* derived from *N. crassa* resulting in the production of stearidonic acid (SDA), an omega-3 fatty acid.

### ***Overview of the construction and inserted genetic elements of parental soybean line MON87769***

The binary transformation plasmid PV-GMPQ1972 was used to produce the genetically modified soybean line MON87769. PV-GMPQ1972 contains two separate transfer-DNA's (T-DNA's). T-DNA I contains the desaturase genes *Pj.D6D* and *Nc.Fad3* and T-DNA II harbours a selectable marker (*cp4 epsps*). Following selection of the transformants, the inserted T-DNA II was segregated from progeny through subsequent traditional breeding and genetic selection processes. The inserted T-DNA I containing the desaturase genes was maintained. The result is a soybean containing only the *Pj.D6D* and *NcFAD3* expression cassettes. An overview of the introduced T-DNA I sequences is given below:

- *Sequence flanking 5' end of the insert. G. max* genomic DNA.
- *B-Right border*. 42 bp of the DNA region from *A. tumefaciens* containing the right border sequence used for transfer of the T-DNA.
- *P-7Sa'*. Seed-specific promoter and leader sequence from the *Sphas 1* gene of *G. max*.
- *CS-Pj.D6D*. Coding sequence for the fatty acid delta-6 desaturase from *P. juliae*.
- *T-tml*. 3' non-translated region of the *tml* gene from *A. tumefaciens* octopine-type Ti plasmid.
- *P-7Sa*. Promotor and leader sequence from the *Sphas2* gene from *G. max*.
- *CS-Nc.Fad3*. Codon optimised coding sequence for the gene from *N. crassa* encoding delta-15 desaturase.
- *T-E9*. 3' non-translated region of the *Pisum sativum rbcS2* gene which functions to direct polyadenylation of the mRNA.
- *B-Left Border*. 273bp of the DNA region from *A. tumefaciens* containing the left border sequence used for transfer of the T-DNA.
- *Sequence flanking 5' end of the insert. G. max* genomic DNA.

### ***Properties of the genes introduced in MON87769***

The genetically modified soybean line MON87769 contains the *P. juliae Pj.D6D* gene and the *N. crassa Nc.Fad3* gene. The introduction of these genes results in the seed-specific production of the Pj $\Delta$ 6 and Nc $\Delta$ 15 desaturase proteins.

$\Delta$ 6 desaturase is responsible for the conversion of  $\alpha$ -linolenic acid (ALA) into stearidonic acid (SDA). SDA is an omega-3 fatty acid which is a normal metabolic precursor to the longchain, poly-unsaturated omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in humans and animals. Longchain poly-unsaturated omega-3 fatty acids have been linked to reductions in cardiovascular disease, cancer, inflammation and neurological disorders.<sup>7</sup>  $\Delta$ 6 desaturase may also convert linoleic acid (LA) into  $\gamma$ -linolenic acid (GLA). The addition of  $\Delta$ 15 desaturase increases ALA levels, allowing greater flux to SDA accumulation in MON87769. It also lowers the LA levels (by conversion of LA into ALA) and hence lowers the substrate pool for GLA production.

### ***Molecular analysis MON87769***

The applicant showed by Southern blot analyses that one intact copy of the T-DNA I containing the *Pj.D6D* and *NcFAD3* expression cassettes was integrated at a single integration locus in the genome of MON87769. Furthermore, the applicant demonstrated by Southern blot analysis that the backbone of plasmid PV-GMPQ1972 and the T-DNA II, harbouring the *cp4 epsps* expression cassette, was absent in MON87769. Results obtained by PCR amplification and DNA sequence analyses confirmed that an intact insert was integrated. Sequence analyses spanning the 5' and 3' junctions of the insertion site and the genomic DNA indicated a 9 bp deletion of genomic DNA and two small insertions of 17 and 8 bp. Bioinformatic analysis by BLASTn and BLASTx searches (GenBank databases, June 2009) indicate that it is unlikely that endogenous soybean open reading frames were disrupted by the T-DNA I insertion. Furthermore, DNA sequences spanning the 5' and 3' junctions of the MON87769 insertion site and the genomic DNA were analyzed from stop codon to stop codon. Five sequences of eight amino acids or greater in length spanning the 5' junction and six sequences of eight amino acids or greater in length spanning the 3' junction were identified. Bioinformatic analyses were performed by alignment searches in allergen, toxin and protein databases (AD\_2009, TOX\_2009 and PRT\_2009) respectively. The results of these analyses demonstrated no sequence similarities between any known toxins or allergens and the eleven putative polypeptides.

COGEM is of the opinion that the molecular characterization of MON87769 has been adequately performed and meets the criteria laid down by COGEM.<sup>8</sup> These criteria match the EFSA criteria.<sup>9,10</sup>

### **Environmental risk assessment**

The current application of soybean line MON87769 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 as 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. The introduced traits do not increase the ability of soybean seed to survive in the Dutch climatic conditions. In view of the above, there are no reasons to assume that the expression of the *Pj.D6D* and *Nc.Fad3* genes increases the potential of MON87769 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.<sup>11</sup> This application is submitted under Regulation (EC) 1829/2003, therefore a food/feed assessment is carried out by EFSA. Other organizations who advice 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 completion of this advice.

### **General surveillance plan**

General surveillance (GS) has been introduced to be able to observe unexpected adverse effects of GM 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 MON87769 soybean. Recently, COGEM formulated criteria on which GS plans concerning Dutch applications for import and cultivation of GM crops have to comply.<sup>12</sup> Although the 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 MON87769 soybean.

### **Advice**

COGEM has been asked to advise on import and processing for use in food and feed of soybean line MON87769. COGEM is of the opinion that incidental spillage of soybean leading to the spread of soybean within the Netherlands is negligible. Establishment of feral soybean populations in European countries has never been observed and there is no reason to assume that the presence and expression of the introduced genes increases the potential of soybean to establish feral populations. In addition, closely related species of soybean are not present in Europe and therefore introgression of the inserted genes into closely related species cannot occur.

The molecular analysis of MON87769 is adequate. Based on the aspects discussed, COGEM is of the opinion that import and processing of soybean MON87769 poses negligible risks to the environment.

### **References**

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