

Aan de minister van  
Volkshuisvesting, Ruimtelijke  
Ordening en Milieubeheer  
Mevrouw dr. J.M. Cramer  
Postbus 30945  
2500 GX Den Haag

**DATUM** 16 april 2008  
**KENMERK** CGM/080416-01  
**ONDERWERP** Advies import en verwerking van soja 305423 x 40-3-2 (EFSA/GMO/NL/2007/47)

Geachte mevrouw Cramer,

Naar aanleiding van de adviesvraag betreffende het dossier EFSA/GMO/NL/2007/47, getiteld "Application for the authorization of genetically modified 305423 x 40-3-2 soybean and derived food and feed in accordance with Regulation (EC) No 1829/2003" voor de import en verwerking van genetisch gemodificeerde soja door Pioneer Hi-Bred International Inc. adviseert de COGEM als volgt:

**Samenvatting:**

De COGEM is gevraagd te adviseren over de toelating van sojalijn 305423 x 40-3-2 voor import en verwerking. De lijn is tot stand gekomen door middel van een conventionele kruising van de genetisch gemodificeerde ouderlijnen 305423 en 40-3-2. Deze kruisingslijn bevat een *gm-fad2-1* genfragment en een *gm-hra* gen resulterend in respectievelijk een verhoogd vetzuurgehalte en tolerantie voor acetolactaatsynthase remmende herbiciden. Daarnaast is de sojalijn tolerant voor herbiciden met als werkzame stof glyfosaat door de aanwezigheid van het *cp4 epsps* gen. De COGEM heeft reeds positief geadviseerd over import en verwerking van de beide ouderlijnen. Bovendien heeft zij positief geadviseerd over teelt van ouderlijn 40-3-2.

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 voor verwildering en zijn er geen redenen om aan te nemen dat de geïntroduceerde genen het verwilderingspotentieel vergroten. Daarnaast zijn er in Europa geen wilde verwanten van soja aanwezig zodat uitkruising niet mogelijk is. De COGEM acht daarom de kans dat incidenteel morsen leidt tot verspreiding van 305423 x 40-3-2 binnen Nederland, verwaarloosbaar klein.

Concluderend acht de COGEM de risico's voor het milieu verwaarloosbaar klein en daarom heeft zij geen bezwaar tegen import en verwerking van de onderhavige sojalijn.

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, stylized loop followed by a horizontal line that ends in a small hook.

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 soybean line 305423 x 40-3-2

## COGEM advice CGM/080416-01

### Summary

*The present notification by Pioneer Hi-Bred International Inc. concerns the import and processing for use in feed and food of the hybrid soybean line 305423 x 40-3-2. Cultivation is not part of this application.*

*The soybean line was produced by cross-breeding two genetically modified lines. The hybrid line contains the *gm-fad2-1* gene fragment, resulting in a high oleic phenotype. Furthermore, the *gm-hra* gene is expressed which confers tolerance to acetolactate synthase-inhibiting herbicides. Finally, the line is also tolerant to glyphosate-containing herbicides due to the presence of the *cp4 epsps* gene.*

*Previously, COGEM advised positively on import and processing of both parental lines. COGEM has also given a positive advice on the cultivation of soybean line 40-3-2.*

*In Europe, wild relatives of soybean are not present and modern soybean cultivars do not possess any of the attributes commonly associated with problematic weeds. In addition, survival of soybean is not possible in the North-Western European climate. Establishment of feral soybean populations has never been observed in European countries. There is no reason to assume that the inserted genes would increase the potential of the soybean to establish feral populations. Therefore, COGEM is of the opinion that incidental spillage of the soybeans will not pose a risk to the environment.*

*In consideration of these aspects, COGEM is of the opinion that the import and processing of soybean line 305423 x 40-3-2 poses a negligible risk to the environment.*

### Introduction

The present application by Pioneer Hi-Bred International Inc., file EFSA/GMO/NL/2007/47, concerns the import and processing of soybean line 305423 x 40-3-2 for use in feed and food. This line contains the *gm-fad2-1* gene fragment, resulting in a high oleic phenotype. In addition, the *gm-hra* gene is expressed which confers tolerance to acetolactate synthase-inhibiting herbicides. Finally, the line is also tolerant to glyphosate-containing herbicides due to the presence of the *cp4 epsps* gene.

Since 1996, Soybean 40-3-2 is approved for processing and food and feed use in the European Union (1).

### **Previous COGEM advices**

In 1995, 2000 and 2007 respectively, COGEM advised positively on import and processing of both of the parental soybean lines 40-3-2 and 305423 (2,3,4,5). Furthermore, in 2006, COGEM has also given a positive advice on the cultivation of line 40-3-2 (3).

### **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. The optimum temperature for soybean growth is between 25°C and 30°C. In the Netherlands, 16.8°C was the average summer temperature from 1971 to 2006. The average temperature of the three warmest summers since 1901 was 18.6°C (6). In addition, soybean is susceptible to frost damage and 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 (6). Moreover, during the Dutch growth season the days are long, whereas soybean is a quantitative short-day plant that needs short days for fructification.

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 (8). Therefore, insect-borne exportation of pollen is limited (7). Hybridization with other species is not possible because there are no wild relatives of soybean in Europe.

The soybean plant is not weedy in character (8). Cultivated soybean rarely displays dormancy (8) and seeds of cultivated soybean survive poorly in soil (9). Soybean volunteers are rare and do not effectively compete with other cultivated plants or primary colonizers (8). In addition, volunteers are easily controlled mechanically or chemically (8). Establishment of feral soybean populations has never been observed in European countries.

More information on aspects of soybean can be found in previous COGEM advices on the single parental soybean lines (3,4).

### **Molecular characterization**

The hybrid soybean line 305423 x 40-3-2 was produced by cross-breeding the parental soybean lines 305423 and 40-3-2. The molecular characterization of these parental lines will be briefly discussed. A more detailed description of the inserted genes can be found in previous COGEM advices (2,3,4,5).

### *Properties of the introduced genes resulting in a high oleic phenotype*

Soybean line 305423 was genetically modified with a *gm fad2-1* gene fragment. The gene fragment is part of the coding region of the soybean endogenous omega-6 desaturase gene 1 (*FAD2-1*) and does not code for a functional protein. Transcription of the *gm-fad2-1* gene fragment in seeds acts to suppress transcription of omega-6-desaturase, resulting in a high oleic phenotype.

### *Properties of the introduced genes conferring herbicide tolerance*

Besides fragment *gm-fad2-1*, a *gm-hra* gene is introduced in soybean line 305423. Due to two mutations, the *gm-hra* gene is an optimized form of the endogenous acetolactate synthase (*als*) gene. As a consequence, confers tolerance to ALS-inhibiting herbicides.

The ALS enzyme catalyzes the first step in the biosynthesis of the essential amino acids valine, leucine and isoleucine. The mode of action of ALS-inhibiting herbicides is based on the interruption of this enzyme. Interruption will result in disruption of the formation of the amino acids causing the plant to die. The *gm-hra* gene has mutations in the *als* gene, which have resulted in an increased tolerance for these herbicides because of a decrease in the binding affinity between enzyme and herbicide.

In addition, a functional *cp4 epsps* gene is present in soybean line 40-3-2. This gene encodes for a CP4 EPSPS protein possessing a high tolerance to glyphosate. EPSPS is a naturally occurring enzyme involved in the biosynthesis of aromatic amino acids. In non-transgenic soybean lines, glyphosate acts by binding to and inhibiting the function of naturally occurring EPSPS. Consequently, aromatic amino acids are no longer formed, leading to plant death. In contrast, CP4 EPSPS is not affected by glyphosate because of a reduced binding affinity to this substance. Because 40-3-2 expresses *cp4 epsps*, it has acquired a high tolerance to glyphosate (10).

EPSPS proteins are active in the chloroplasts of a plant cell. The sequence encoding the chloroplast transit peptide is fused to the *epsps* gene, resulting in the transport of the transgenic CP4 EPSPS protein to the chloroplast (11).

### *Molecular analysis*

In the past, COGEM assessed the molecular analysis of the parental soybean lines 40-3-2 and 305423. Concerning line 40-3-2, COGEM concluded that the molecular analysis of this line was adequate (3).

In the opinion of COGEM the molecular analysis of parental soybean line 305423 was incomplete. It could not be excluded that new open reading frames would be created due to the insertion. These new open reading frames might have potential toxic or allergenic adverse effects (4). Recently, COGEM abstains from advises on the potential risks of

incidental consumption in case a food/feed assessment is already carried out by other organizations. Therefore, considerations of the COGEM relative to previous objections regarding the molecular analysis in view of incidental consumption are not included. The outcome of the assessment of consumption by other organizations was not known at the moment of completion of this advice.

With regard to the considerations above, COGEM is of the opinion that the molecular analysis of hybrid line 305423 x 40-3-2 does not indicate that import and processing of this line would pose a risk to the environment.

### **General surveillance plan**

A general surveillance plan is supplied by the applicant. COGEM is of the opinion that this plan is sufficient. In addition, a general surveillance plan is of less importance because soybean cannot survive in the North-Western European climate.

General surveillance has been introduced to be able to observe unexpected effects of the cultivation of genetically modified crops (12). The setting or population in which these effects could occur is either not or hardly predictable (12). In the present application, observations for 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 305423 x 40-3-2 soybean. Monitoring will occur, amongst others, at loading docks. COGEM points out that general surveillance could also focus on the route of transportation.

### **Advice**

COGEM has been asked to advice on import and processing for use in food and feed of hybrid soybean line 305423 x 40-3-2. Previously, COGEM advised positively on import and processing of both parental lines. COGEM has also given a positive advice on the cultivation of soybean line 40-3-2 (2,3,4,5).

Because cultivation is not part of the present application, the risk assessment focuses on the accidental spillage of soybean.

COGEM is of the opinion that the Dutch 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 increase 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 is very unlikely to lead to the spread of soybean within

the European Union. In addition, wild relatives of soybean are not present in Europe and therefore introgression of the inserted genes into wild relatives cannot occur.

The molecular analysis of hybrid line 305423 x 40-3-2 does not indicate that import and processing of this line would pose a risk to the environment.

In consideration of the aspects above, COGEM is of the opinion that import and processing of soybean 305423 x 40-3-2 poses negligible risks to the environment.

## References

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