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**KENMERK** CGM/110510-01  
**ONDERWERP** Advies 'Import and processing of genetically modified maize DAS-40728-9'

Geachte heer Atsma,

Naar aanleiding van een adviesvraag betreffende de import van de genetisch gemodificeerde maïslijn DAS-40728-9 van Dow AgroSciences Europe, deelt de COGEM u het volgende mee.

### **Samenvatting**


De COGEM is gevraagd om te adviseren over de mogelijke milieurisico's van import en verwerking van de genetisch gemodificeerde maïslijn DAS-40728-9. Deze maïslijn is met behulp van siliciumcarbide vezels getransformeerd, waarbij het van de bodembacterie *Sphingobium herbicidovorans* afkomstige *aad-1* gen is ingebracht in de conventionele maïslijn Hi-II. *Aad-1* codeert voor het enzym aryloxyalkanoaat dioxygenase. Als gevolg hiervan is de lijn tolerant voor verschillende synthetische auxineachtige plantenhormonen zoals het herbicide '2,4-dichlorophenoxyacetic acid' (2,4-D), en bepaalde 'aryloxyphenoxypropionate' (AOPP) bevattende herbiciden. De COGEM heeft niet eerder geadviseerd over deze maïslijn of over genetisch gemodificeerde gewassen die het *aad-1* gen tot expressie brengen.

De COGEM is van mening dat de moleculaire karakterisatie van maïslijn DAS-40728-9 adequaat is uitgevoerd.

Verwildering van maïsplanten is in Nederland nooit waargenomen. Daarnaast is opslag van maïsplanten in Nederland nagenoeg uitgesloten. Er zijn geen redenen om aan te nemen dat de ingebrachte eigenschap het verwilderingspotentieel van Maïs vergroten. Bovendien zijn er in Europa geen wilde verwanten van Maïs aanwezig waardoor uitkruising niet mogelijk is. Daarom acht de COGEM de kans dat incidenteel morsen tot verspreiding van deze maïslijn leidt verwaarloosbaar klein.

Concluderend heeft de COGEM op basis van de door haar uitgevoerde risicobeoordeling geen bezwaar tegen import en verwerking van maïslijn DAS-40728-9 en acht zij de risico's voor mens en milieu verwaarloosbaar klein.

Omdat andere instanties een voedselveiligheidsbeoordeling uitvoeren, heeft de COGEM bij deze vergunningaanvraag de risico's van incidentele consumptie niet beoordeeld.



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

Hoogachtend,



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# Import and processing of genetically modified maize DAS-40728-9

## COGEM advice CGM/110510-01

*The present application by Dow AgroSciences Europe (file EFSA/GMO/NL/2010/89) concerns import and processing for use in feed and food of the genetically modified maize line DAS-40728-9. Cultivation is not part of this application.*

*Maize line DAS-40728-9 was produced by the introduction of an *aad-1* expression cassette using whisker mediated transformation. As a result, the line tolerates a broad spectrum of selective herbicides. The plant is tolerant for several synthetic auxin acting herbicides like '2,4-dichlorophenoxyacetic acid' and certain 'aryloxyphenoxypropionate' containing herbicides. Maize line DAS-40728-9 has not been previously assessed by COGEM.*

*The applicant showed by Southern blot analyses that one copy of the insert was integrated at a single integration locus in the genome of DAS-40728-9 and that the backbone of the plasmid used for transformation was absent in DAS-40728-9. Bioinformatic analysis showed that no endogenous maize open reading frames (ORFs) were disrupted. Ten ORFs, encoding eight or more amino acids, spanning the 5' and 3' junctions of the insert and the genomic DNA were identified. The amino acid sequence was deduced and analyzed in silico for similarity to known toxins or allergens. No similarity was found. The molecular characterization of maize line DAS-40728-9 meets the criteria of COGEM.*

*During its long domestication process, maize has lost its ability to survive in the wild. In the Netherlands, the appearance of maize volunteers is rare and establishment of volunteers in the wild has never been reported. There are no reasons to assume that the introduced trait will increase the potential of maize to establish feral populations. The introduced gene cannot spread to closely related species since wild relatives of maize are not present in Europe.*

*In view of the above, COGEM is of the opinion that the risks for humans and the environment associated with import and processing of maize line DAS-40728-9 are negligible. A food/feed safety assessment is carried out by other organizations. Therefore, COGEM abstained from advice on the potential risks of incidental consumption.*

### **Introduction**

The present notification EFSA/GMO/NL/2010/89 by Dow AgroSciences Europe concerns import and processing of the genetically modified maize line DAS-40728-9. This maize line was produced by whiskers-mediated transformation and contains the constitutively expressed *aad-1* gene which encodes aryloxyalkanoate dioxygenase. As a result, DAS-40728-9 is tolerant for several synthetic auxin acting herbicides like 2,4-dichlorophenoxyacetic acid' (2,4-D). Additionally, the maize line is tolerant for certain 'aryloxyphenoxypropionate' (AOPP) containing herbicides

Maize line DAS-40728-9 has not been previously assessed by COGEM. COGEM has never advised on *aad-1* gene containing genetically modified crops before.

### **Aspects of the crop**

Maize (*Zea mays* L.) is a member of the grass family *Poaceae*. Maize is a highly domesticated crop, originating from Central America. Although insect pollination cannot be completely excluded, maize is predominantly wind pollinated.<sup>1,2</sup> According to literature,

pollen viability varies between 30 minutes and 9 days.<sup>2,3,4</sup> In Europe, no wild relatives of maize are present and therefore hybridization with other species cannot occur.

Throughout the world the appearance of volunteers is very rare. Seed kernels are the only survival structures of maize.<sup>5</sup> Due to the structure of the corn cob (ear on a stiff central cob enclosed in husks) natural dissemination of the kernels rarely occurs. Maize needs human intervention to disseminate its seed.<sup>5</sup> In addition, kernels exhibit poor dormancy resulting in a short persistence. Besides, maize can only survive within a narrow range of climatic conditions and, as maize is originally a subtropical crop, it is frost-sensitive.<sup>6</sup> Maize is very sensitive to weed competition.<sup>7</sup> During the long process of domestication, maize has lost the ability to survive in the wild.<sup>5</sup> Establishment of maize plants in the wild has never been observed in the Netherlands and COGEM is not aware of any reports of wild maize plants elsewhere in Europe.

### **Molecular characterization**

DAS-40728-9 maize was obtained by transformation of conventional maize line Hi-II with a linear *aad-1* containing expression cassette using silicon carbide 'whisker' fibers. Whiskers enable DNA entry into cells. The *aad-1* gene was derived from the gram negative soil bacterium *Sphingobium herbicidovorans*.

#### *The aad-1 expression cassette*

The *aad-1* gene was optimized for plant expression by adding an alanine residue at the second position. The *aad-1* containing expression cassette was cloned into plasmid pDAS1740. A 6236 base pair (bp) fragment containing the expression cassette was used for transformation. The cassette contained the following elements:

- Intervening sequence used for DNA cloning
- RB7 MAR v3; Matrix attachment region (MAR) from *Nicotiana tabacum*
- Intervening sequence used for DNA cloning
- Maize ZmUbi1 promoter; Ubiquitin promoter from *Z. mays*
- Intervening sequence used for DNA cloning
- *Aad-1* gene; synthetic, plant-optimized version of the aryloxyalkanoate dioxygenase gene from *S. herbicidovorans*
- Intervening sequence used for DNA cloning
- Maize ZmPer5 3' UTR; 3' untranslated region from *Z. mays* peroxidase gene
- Intervening sequence used for DNA cloning
- RB7 MAR v4; Matrix attachment region (MAR)
- Intervening sequence used for DNA cloning

#### *Properties of the introduced genetic elements*

The *aad-1* gene encodes aryloxyalkanoate dioxygenase. This enzyme is capable of cleaving several synthetic auxins, like the herbicide 2,4-D, and certain AOPP herbicides. As a result, the plant tolerates a broad spectrum of herbicides.

Synthetic auxins mimic the plant hormone auxin resulting in a disturbance of the hormone metabolism in broadleaf plants.<sup>8</sup> AOPPs inhibit the lipid biosynthesis in grasses by specifically inhibiting their monomeric acetyl-CoA carboxylases.<sup>8</sup> After cleavage by aryloxyalkanoate dioxygenase, 2,4-D is converted into the herbicidally inactive detoxified

2,4-dichlorophenol DCP. The AOPP herbicides are converted into their corresponding herbicidally inactive phenols.

Expression of the *aad-1* gene is controlled by the ZmUbiInt promoter and ZmPer5 termination sequences. The promoter provides constitutive expression in monocots. Matrix attachment regions (MARs) from *N. tabacum* were included to potentially increase the expression of the *aad-1* gene and to reduce the incidence of gene silencing.

#### *Molecular analysis*

The applicant showed by Southern blot analyses that one copy of the *aad-1* expression cassette was integrated at a single integration locus in the genome of DAS-40728-9 maize. For transformation an *aad-1* containing linear fragment of pDAS1740 was used. No backbone of plasmid was present which was confirmed by hybridization analysis.

Results obtained by PCR amplification and DNA sequence analyses showed that the integrated *aad-1* expression cassette was intact whereas both flanking MAR-elements were truncated. The DNA sequence of the *aad-1* cassette matched the transgene sequence of pDAS1740 except for a single bp change (T to C) in the 3'UTR sequence.

Sequence analysis of the insertion locus revealed a 21 bp insertion as well as a two bp deletion of native maize genome at the 5' integration junction. Additionally, an insert of one bp was observed at the 3' integration junction. Furthermore, the sequence analysis confirmed that one copy of the expression cassette was inserted at the integration locus.

Bioinformatic analyses of the flanking borders of the integration site and the parental maize genomic locus showed that the insert is located in a maize Grande retrotransposon like sequence and that no endogenous maize ORFs were disrupted by the insertion.

DNA sequences spanning the 5' and 3' junctions of the DAS-40728-9 insertion site and the maize genomic DNA were analyzed for the presence of potential encoded fusion proteins. Sequences were analysed from stop to stop codon. Ten open reading frames were identified and screened for any matches with known toxins and allergens using bioinformatic tools. Fragments from at least 8 up to 80 contiguous amino acids were used as query sequences to detect possible homologies. Query sequences longer than 80 amino acids were split into overlapping sequences of 80 amino acid long fragments. No similarities with toxic proteins or allergens were detected.

In conclusion, COGEM is of the opinion that the molecular characterization of DAS-40728-9 has been adequately performed and meets the criteria laid down by COGEM.<sup>9</sup>

#### **Environmental risk assessment**

During the long process of domestication, maize has lost the ability to survive in the wild.<sup>5</sup> Maize needs human intervention to disseminate its seed.<sup>5</sup> Maize kernels exhibit poor dormancy resulting in a short persistence. Maize is very sensitive to weed competition and cannot persist as a weed.<sup>7</sup> Furthermore, maize is naturally frost sensitive and can only survive within a narrow range of climatic conditions. In the Netherlands, volunteers are rarely found and establishment of maize plants in the wild has never been observed. In Europe, no wild relatives of maize are present and therefore hybridization with other species cannot occur.<sup>5</sup>

Maize DAS-40728-9 expresses the *aad-1* gene. As a result, the maize line is tolerant for 2,4-D and AOPP herbicides. The current application concerns import and processing. In case of spillage maize kernels may be released into the environment. Maize kernels can only survive within a narrow range of climatic conditions. The introduced traits do not increase the

ability of maize kernels to survive in the environment. In addition, the applicant carried out an agronomic assessment for DAS-40728-9. This assessment does not give any indication to assume that DAS-40728-9 has an increased survivability compared to conventional maize lines. In view of the above, there are no reasons to assume that maize DAS-40728-9 has an increased potential for the establishment of feral populations in case of incidental spillage.

Since 2008, COGEM abstains from advices on the potential risks of incidental consumption in case a food/feed safety assessment is already carried out by other organizations. This application is submitted under Regulation (EC) 1829/2003, therefore a food/feed safety 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 safety 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**

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 DAS-40728-9 maize. Recently, COGEM formulated criteria on which GS plans concerning Dutch applications for import and cultivation of GM crops have to comply.<sup>10</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 DAS-40728-9 maize.

### **Advice**

COGEM has been asked to advice on import and processing of maize line DAS-40728-9. This genetically modified maize line expresses the *aad-1* gene from *S. herbicidovorans* and is tolerant for 2,4-D and certain AOPP containing herbicides. DAS-40728-9 was produced by whisker mediated transformation of conventional maize line Hi-II.

Putative polypeptides encoded at the junction sites of the insert and the maize genomic DNA of DAS-40728-9 did not possess any similarity to known toxins or allergens. The molecular characterization of maize line DAS-40728-9 meets the criteria of COGEM.

Maize has lost the ability to survive in the wild. In addition, maize needs human intervention to disseminate its seed. In the Netherlands, volunteers are rare and establishment of maize plants in the wild has never been observed. There is no reason to assume that expression of the *aad-1* gene increases the potential of maize to establish feral populations in case of incidental spillage. In addition, an agronomic assessment of DAS-40728-9 did not give any indication to assume that DAS-40728-9 has an increased survivability compared to conventional maize lines. Introgression of the introduced genes into closely related species cannot occur, as wild relatives of maize are not present in Europe.

In view of the above, COGEM is of the opinion that the risks for humans and the environment associated with import and processing of maize line DAS-40728-9 are

negligible. A food/feed safety assessment is carried out by other organizations. Therefore, COGEM abstained from advice on the potential risks of incidental consumption.

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