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DATUM 16 juli 2012
KENMERK CGM/120716-01
ONDERWERP Additional advice on cultivation of glyphosate tolerant soybean 40-3-2

Dear Sir,

Recently, EFSA published its opinion regarding the cultivation of genetically modified soybean 40-3-2.¹ Soybean 40-3-2 expresses the *cp4 epsps* gene and as a result it is tolerant to glyphosate containing herbicides. Soybean 40-3-2 has been cultivated since 1996 in countries such as the USA and Argentina. Prior to the accession of Romania to the EU, soybean 40-3-2 was also cultivated in Romania from 1999 to 2006.

In 2006, COGEM advised on the market application for cultivation of glyphosate tolerant soybean 40-3-2 (EFSA/GMO/NL/2005/24).² The Netherlands' Ministry of Infrastructure and the Environment (IenM) asked COGEM whether the recently published opinion of the EFSA GMO panel, including additional information from the applicant, sufficiently addresses previous comments of COGEM on this application.

In its previous advisory report, COGEM concluded that cultivation of soybean 4-3-2 poses negligible risks to the environment. Although COGEM considered the supplied general surveillance plan acceptable, COGEM pointed out that the general surveillance plan could be improved. Furthermore, COGEM commented on the studies on non-target organisms that were part of the application.

In the current advisory report, COGEM will discuss three aspects of the application in greater detail.

Molecular characterisation

Soybean 40-3-2 was obtained by particle bombardment and contains two inserts. One of the inserts consists of the *cp4 epsps* gene cassette lacking the first 354 bp of the 35S promoter. The region flanking the 35S promoter sequence was shown to be soybean DNA.³ Immediately adjacent to the 3' nos terminator of the *cp4 epsps* gene a 254 bp sequence of the *cp4 epsps* coding region is present.³ Next to this 254 bp sequence, soybean genomic DNA was found.⁴

¹ EFSA (2012). Scientific opinion on an application (EFSA-GMO-NL-2005-24) for the placing on the market of the herbicide tolerant GM soybean 40-3-2 for cultivation under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal 10(6): 2753

² COGEM (2006). Cultivation of glyphosate tolerant soybean 40-3-2. Advisory report CGM/0611128-01

³ Windels *et al.* (2001). Characterisation of the Roundup Ready soybean insert. Eur Food Res Technol 213: 107-112



This flanking soybean DNA might be rearranged genomic DNA or the result of a deletion event which may have happened during the integration of the gene cassette, because no PCR product could be obtained using primers for the insert-flanking plant sequences using DNA from untransformed soybean.³

The second insert present in soybean 40-3-2 is 72 bp long and derived from the *cp4 epsps* coding sequence. In the additional information that was provided in December 2008, the applicant reported that the 5' flanking regions of this second insert aligns with a repetitive stretch of A and T containing sequences. The origin of this sequence remains unclear. According to the applicant, sequence analysis has indicated that the inserts' 3' flanking sequence is derived from chloroplast DNA (84 bp). The sequence adjacent to this chloroplast DNA did not show similarities with the available data on soybean genomic DNA, thus its origin remains unclear.

The applicant updated the bioinformatic analysis of the open reading frames (ORFs) located at the junctions of the primary and secondary inserts and their flanking DNA. These results did not indicate a similarity with known allergens or toxins. COGEM noted that the analysis of the primary insert did not include an analysis of the ORFs present at the junctions of the 254 bp *cp4 epsps* fragment and its flanking DNA.

Although the molecular characterisation of soybean 40-3-2 contains some uncertainties, they are unlikely to affect the ecological characteristics of soybean 40-3-2. 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 organisations.⁵ COGEM notes that the current application only concerns the cultivation of soybean 40-3-2.

Non-target organisms

In its previous advisory report COGEM pointed out that the experiments that were carried out to study effects on non-target organisms display some shortcomings. As mentioned in several of its previous opinions, in COGEM's view experiments on non-target organisms are only necessary when there is a reason to expect that the introduced trait could adversely affect non-target organisms.⁶ As there is no indication that the transgenic CP4 EPSPSP protein could adversely affect non-target organisms, COGEM is of the opinion that the shortcomings in the studies on non-target organisms are of less importance.

General surveillance

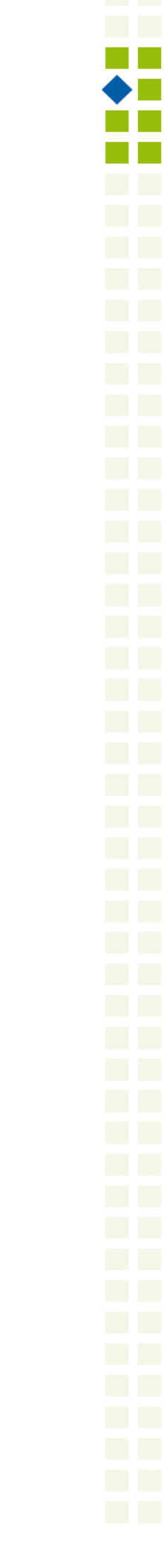
Since COGEM's previous advisory report on the cultivation of soybean 40-3-2, the general surveillance plan has been updated. The previous distinction between the detection of direct and indirect effects has been removed. The applicant states that adverse reports will be discussed in the mandatory general surveillance report, which will be prepared annually. In 2010, COGEM published a report on the principles that should be followed for general surveillance.⁷ In addition, in its opinion on cultivation of soybean 40-3-2, EFSA proposes

⁴ Goley *et al.* (2002). DNA sequences flanking the 3' end of the functional insert of Roundup Ready soybean event 40-3-2- are identical to DNA sequences from the wild-type soybean lines A5403 and 3244. Monsanto report MSL-17561

⁵ COGEM (2008). Toelichting advies GA21. Brief CGM/080117-02 (in Dutch)

⁶ COGEM (2011). Comments on the 'Guidance on the environmental risk assessment of GM plants' and on the 'Scientific opinion on the assessment of potential impacts of GM plants on NTOs'. Advisory report CGM/110214-02

⁷ COGEM (2010). General Surveillance. Topic report CGM/100226-01



several modifications to improve the general surveillance plan of soybean 40-3-2. COGEM is awaiting the revised harmonised general surveillance plan that is currently being developed by industry representatives. COGEM urges those involved to incorporate the principles previously published by COGEM in the revised harmonised general surveillance plan and to finalise the discussions on the content of this plan.

In conclusion, COGEM remains of the opinion that cultivation of soybean 40-3-2 poses a negligible risk to the environment.

Sincerely yours,



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Chair COGEM

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