COMMISSIE
COGEM
GENETISCHE
MODIFICATIE

BEZOEKADRES:
A. VAN LEEUWENHOEKLAAN 9
3721 MA BILTHOVEN

POSTADRES:
POSTBUS 578
3720 AN BILTHOVEN

TEL.: 030 274 2777
FAX: 030 274 4476
INFO@COGEM.NET
WWW.COGEM.NET

Aan de staatssecretaris
van Infrastructuur en Milieu
Mevrouw W.J. Mansveld
Postbus 30945
2500 GX Den Haag

DATUM 07 mei 2013
KENMERK CGM/130507-01
ONDERWERP Aanvullend advies over de teelt van gg-maïslijn 59122

Geachte mevrouw Mansveld,

Naar aanleiding van een adviesvraag betreffende de teelt van genetisch gemodificeerde maïs 59122 van Pioneer Hi-Bred International, deelt de COGEM u het volgende mee.

Samenvatting:
De COGEM is naar aanleiding van het verschijnen van de EFSA opinie over maïslijn 59122 opnieuw gevraagd om te adviseren over de teelt van deze genetisch gemodificeerde maïslijn. Maïslijn 59122 brengt de *cry34Ab1* en *cry35Ab1* genen, die een onderling versterkend effect hebben, tot expressie en is hierdoor resistent voor bepaalde keverachtigen, zoals de maïswortelkever (*Diabrotica* spp.). Daarnaast brengt deze maïslijn het *pat* gen tot expressie, waardoor zij tolerant is voor glufosinaat-ammonium bevattende herbiciden.
De COGEM kwam eerder tot de conclusie dat bij de teelt van maïslijn 59122 de risico's voor mens en milieu hoogstwaarschijnlijk verwaarloosbaar klein zijn. De aangeleverde informatie was echter niet voldoende om eventuele effecten van 59122 maïs op lieveheersbeestjes uit te kunnen sluiten. Bij een laboratoriumstudie waarbij lieveheersbeestjes aan een hoge hoeveelheid Cry eiwit werden blootgesteld, hadden de lieveheersbeestjeslarven een lager gewicht. Dit zou mogelijk een effect kunnen hebben op het aantal nakomelingen. De COGEM vond het daarom noodzakelijk dat bij het monitoren specifiek aandacht aan lieveheersbeestjes wordt besteed.
De aanvrager heeft nieuwe laboratoriumstudies uitgevoerd om effecten op Europese lieveheersbeestjes te onderzoeken. Bij deze studies werden de lieveheersbeestjes echter blootgesteld aan lage of onbekende hoeveelheden Cry eiwit. De resultaten van aanvullende veldproeven kunnen niet gebruikt worden om conclusies te trekken over een eventueel effect van 59122 maïs op lieveheersbeestjes, omdat in de veldproef slechts geringe aantallen lieveheersbeestjes werden aangetroffen. De aanvullende studies naar de mogelijke effecten op lieveheersbeestjes nemen daarom de eerdere bezorgdheid van de COGEM niet weg.
Gezien het bovenstaande vindt de COGEM het nog steeds van belang dat bij een toelating voor teelt van 59122 maïs specifiek op lieveheersbeestjes wordt gemonitord tenzij er door de aanvrager overtuigende aanvullende experimentele gegevens worden overlegd.

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. Drs. H.P. de Wijs, Hoofd Bureau GGO
Dr. I. van der Leij, Ministerie van IenM

Additional advisory report on cultivation of maize line 59122

COGEM Advisory report CGM/130507-01

This advisory report concerns the cultivation of the genetically modified maize line 59122. The maize line contains the cry34Ab1, cry35Ab1 *and* pat *genes conferring tolerance to glufosinate-ammonium-containing herbicides and resistance to certain coleopteran insects such as the corn rootworm (*Diabrotica *spp.).*

Previously, COGEM concluded that the cultivation of maize line 59122 most likely poses a negligible risk to human health and the environment. However, COGEM was of the opinion that case specific monitoring of ladybirds should be carried out.

Recently, EFSA published its opinion on cultivation of genetically modified maize 59122. The Dutch Ministry of Infrastructure and the Environment (IenM) asked COGEM whether the opinion of the EFSA GMO panel and the additional information that is provided by the applicant gives reason to reconsider the necessity for case specific monitoring of ladybirds (Coccinellidae).

In its previous advice, COGEM was of the opinion that monitoring of ladybirds is necessary because in laboratory experiments exposure of ladybird larvae to high levels of Cry34Ab1/Cry35Ab1 proteins resulted in a reduced larval weight, which might affect the rate of reproduction.

The applicant performed additional studies to assess possible adverse effects on Coccinella septempunctata*, a ladybird common in Europe. However, in these studies, ladybirds were exposed to low or unknown levels of Cry34Ab1/Cry35Ab1 proteins, making it impossible to draw conclusions on the absence or presence of an effect.*

Additional field trials with 59122 maize were performed in Hungary in which visual observations were carried out to obtain more information on the occurrence of coccinellid species in maize fields. In all plots of the field trial (planted with 59122 maize or the non-transgenic control) ladybirds were present in low numbers. Due to the low abundance of ladybirds the results cannot be used to draw conclusions about possible effects of 59122 maize on ladybirds.

In summary, the additional information does not remove COGEM's previous concerns. Therefore, in case 59122 maize is authorised for cultivation in COGEM's view case specific monitoring of ladybirds, in particular of spider mite consuming ladybirds (i.e. ladybirds belonging to the Stethorini tribe), remains a necessity unless conclusive additional experimental data is provided showing unequivocally the absence of an effect.

Introduction

Maize line 59122 expresses the *cry34Ab1* and *cry35Ab1* genes, conferring resistance to certain coleopteran insects such as the corn rootworm (*Diabrotica* spp.). The *cry34Ab1* and *cry35Ab1* genes exert a combinatory effect. The *cry34Ab1* gene confers an average resistance to the corn rootworm, while *cry35Ab1* alone confers no resistance at all. Expression of both genes in the

same plant however, results in a maximum resistance against the corn rootworm. Maize line 59122 also contains a copy of the *pat* gene, conferring tolerance to glufosinate-ammonium containing herbicides.

COGEM has previously advised on the environmental risks of cultivation of maize line 59122.[1,2] In its latest advice, COGEM concluded that the cultivation of maize line 59122 most likely poses a negligible risk to human health and the environment, but was of the opinion that case specific monitoring of ladybirds should be carried out. COGEM considered case specific monitoring of ladybirds necessary because the results from a laboratory study with *Coleomegilla maculata* raised concerns (see below).[2]

Recently, EFSA published its opinion on cultivation of genetically modified maize line 59122. EFSA considers it unlikely that 59122 will have adverse effects on the environment, except for the possible development of Cry34Ab1/Cry35Ab1 resistant coleopteran target pests.[3]

The Dutch Ministry of Infrastructure and the Environment (IenM) asked COGEM whether the opinion of the EFSA GMO panel and the additional information that is provided by the applicant gives reason to reconsider the necessity for case specific monitoring of ladybirds (Coccinellidae).

Information assessed previously

The applicant previously performed laboratory studies to assess possible effects of Cry34Ab1/Cry35Ab1 proteins on *Coleomegilla maculata* (Annex 21). A significant reduction in larval weight was observed when larvae were exposed to artificial diet containing Cry34Ab1 and Cry35Ab1 proteins at concentrations of 900 ppm and 1 ppm respectively. According to the applicant, these concentrations correspond to ten times the expected environmental concentration. No significant differences on mortality or development were observed when larvae were exposed to inbred 59122 pollen. According to the applicant, the expression level in inbred 59122 pollen (117 µg/g for Cry34Ab1; Cry35Ab1 not determined) corresponds to 1.5 times the expected environmental concentration. The expected environmental concentrations are based on the average Cry34Ab1/Cry35Ab1 expression levels in hybrid pollen, which according to the applicant is typical for field exposure.

C. maculata larvae were also exposed to other Cry34Ab1/Cry35Ab1 expressing inbred maize lines. COGEM noted that the statistical analysis of mortality when exposed to pollen of inbred maize line 2.14 (175 µg/g Cry34Ab1; 75,5 ng/g Cry35Ab1) was just within range of non-significance with a one-sided P-value.

Although COGEM concluded that the cultivation of maize line 59122 most likely poses a negligible risk to human health and the environment, the results of the laboratory study on *C. maculata* was considered a point of concern. A reduction in larval weight might have an effect on life expectation and reproduction ability. If the reproduction rate would be affected this would influence population dynamics. Because COGEM could not exclude the possible occurrence of effects on reproduction due to the observed lower larval weight, COGEM deemed case specific monitoring of ladybirds necessary.

Exposure of Coccinellidae

Ladybirds may be exposed to Cry proteins directly (when consuming maize pollen) or indirectly (when prey contains Cry proteins). Most of the Coccinellidae are predaceous and prey on Sternorrhyncha (homopteran insects like aphids and scale insects). [4] Aphids feed on the phloem sap of maize plants, which has been shown to contain no or very small amounts of Cry protein for maize expressing another Cry protein, i.e. Cry1Ab.[5] Aphids collected from fields planted with other maize events that expressed either Cry1Ab or Cry3Bb1 proteins contained no or negligible amounts of Cry proteins.[7,6]

In contrast to most Coccinellidae, members of the Stethorini tribe prey on spider mites (Acari: Tetranychidae).[4] *Stethorus punctillum*, for instance, prey exclusively on spider mites.[7]. Spider mites (*Tetranychus urticae*) are known to contain high levels of Cry proteins. Laboratory experiments with another maize event expressing the Cry3Bb1 protein indicated that the level of Cry3Bb1 protein in spider mites was in the range of the concentration present in maize leaves.[6,8] Field-collected spider mites were found to contain levels up to 4.6 times higher than the level in maize leaves from another maize event expressing Cry1Ab.[7,9] Cry1Ab and Cry3Bb1 proteins have been shown to remain in its active form in spider mites.[10,11]

Many Coccinellidae consume some pollen, but pollen is an adequate larval diet for only a few select genera. The applicant performed some preliminary studies to investigate whether Cry34Ab1/Cry35Ab1 proteins could accumulate in prey species (corn leaf aphids and spider mites). The study that assessed Cry34Ab1/Cry35Ab1 protein accumulation in corn leaf aphids contains several flaws (such as a dilution error and contamination of control aphids with Cry34Ab1 protein). The study on spider mites exposed the spider mites to 59122 maize for only two days and not all study details (such as the number of replicates) are provided.

COGEM is of the opinion that the quality of the preliminary studies is insufficient to draw conclusions about the Cry protein accumulation in prey species under field conditions. The available scientific literature, however, suggests that aphidophagous ladybirds are mainly exposed to Cry proteins via plant material such as pollen. In addition, the available scientific literature indicates that Cry proteins can accumulate in spider mites and spider mite consuming ladybirds such as *S. punctillum* may thus be exposed to Cry protein levels exceeding those found in maize leaves.

Additional information

Coccinella septempunctata

The applicant performed additional studies to assess possible effects of 59122 maize on *C. septempunctata,* a ladybird species common in Europe.[12]

C. septempunctata larvae were exposed to a mixed diet of pollen and ground *Ephestia kuehniella* eggs in a 1:3 ratio (by weight). No statistically significant differences were observed on mortality or development. COGEM notes that the level of Cry34Ab1 protein in the 59122 maize pollen was 2.8 µg/g (dry weight), whereas the level of Cry34Ab1 protein in 59122 maize pollen collected from European field trials in 2003 and 2004 ranged between 45.4 and 146 µg/g

(dry weight). The applicant states that the level of Cry35Ab1 protein was not determined, because historically this level was found to be below the level of detection in pollen. Because of the low Cry34Ab1 expression level in the pollen used in the diet and because the Cry protein exposure was further reduced by the *E. kuehniella* eggs in the mixed diet, COGEM is of the opinion that this study is of limited value for the assessment of possible effects of 59122 maize on *C. septempunctata* and other ladybirds.

In another study, *C. septempunctata* L2 larvae* were placed in aphid isolators containing bird cherry-oat aphids (*Rhopalosiphum padi*) and naturally occurring pollen densities. No significant difference in the numbers of L2 larva that molted to L3 was observed between 59122 maize and the isogenic maize line. In addition, twenty to thirty *C. septempunctata* larvae (L1 stage) were placed on aphid infested maize plants. No statistically significant differences were observed in adult weight of *C. septempunctata.* COGEM notes that the expression level of Cry34Ab1/Cry35Ab1 in the pollen was not determined and points out that this severely hampers the interpretation of the study results.

Abundance of ladybirds in maize fields

The applicant also performed additional field trials with 59122 maize in Hungary (2007 and 2008). In the 2007 Hungarian field trial no statistical differences related to 59122 maize were observed in the community level analysis. To obtain more information on the occurrence of coccinellid species in the 2008 Hungarian field trial, the applicant made additional visual observations when pollen shed and the abundance of aphids and spider mites were at its peak. No statistical differences were observed in the community level analysis, except for a difference at the second sampling data which appeared to be driven by the abundance of aphids.

In both Hungarian field trials due to the low number of ladybirds the requirements for further statistical analysis of differences between 59122 and the non-transgenic control were not met for individual coccinellid taxa. The applicant concluded that the results from the field trials indicate that the ladybirds are present in low abundance even when abundant food sources are present.

Due to the low number of Coccinellidae observed in field trials, the results from the field trials cannot be used to draw any conclusions about possible effects of 59122 on ladybirds.

In the scientific literature, results are available from German and Spanish field trials planted with other maize events. In these field trials Coccinellidae were present in low numbers, but according to the authors they were one of the most abundant predatory groups.[13,14]

* The larval stages of a ladybird are called L1, L2, L3 and L4. The smallest larval stage is the L1 stage which is the larval stage that hatches from an egg. The L4 stage is the last larval stage before pupation and adulthood.

Environmental risk assessment

Ladybirds are potentially exposed to Cry34Ab1/Cry35Ab1 proteins via pollen and prey. The available information indicates that aphids contain no or negligible levels of Cry toxins. Aphidophagous ladybirds are therefore primarily exposed to Cry proteins when feeding on pollen. Coccinellid species belonging to the Stethorini tribe, such as *Stethorus punctillum*, prey on spider mites. The available information suggests that Cry proteins are able to accumulate to some extent in spider mites and remain in their active form. Spider mite consuming ladybirds may be exposed to Cry proteins at levels exceeding the levels in maize leaves.

A significant reduction in larval weight was observed in a laboratory study exposing *C. maculata* to Cry34Ab1/Cry35Ab1 proteins at a level exceeding ten times the expected environmental concentration (based on the expression level in hybrid pollen). No significant differences were observed on mortality or development when larvae were exposed to inbred 59122 maize pollen, but the effect of pollen from another Cry34Ab1/Cry35Ab1 protein expressing inbred maize line on *C. maculata* mortality was just within the range of non-significance with a one-sided P value.

A reduction in larval weight might have an effect on life expectation and reproduction ability. If the rate of reproduction would be affected this would influence population dynamics. The applicant did not investigate whether a reduced larval weight influences reproduction or population growth.

The applicant did perform additional studies with *C. septempunctata* larvae but in these studies ladybirds were exposed to low or unknown Cry34Ab1/Cry35Ab1 expression levels.

In European field trials carried out in 2003 and 2004, the Cry protein levels in pollen and maize leaves were highly variable. The Cry protein levels in pollen ranged from 45.4-146 µg/g Cry34Ab1 (dry weight) (Cry35Ab1 below limit of detection), and the Cry protein level in leaves ranged from the limit of detection to 667 µg/g Cry34Ab1 or 307 µg/g Cry35Ab1(dry weight).

Because the laboratory studies were carried out with low or unknown Cry34Ab1/Cry35Ab1 protein levels and the expression level of these Cry proteins in the field is variable, COGEM is of the opinion that the additional studies with *C. septempunctata* are of limited value. Due to the low abundance of ladybirds in the field trials, the field trial results cannot be used to draw conclusions about possible effects of 59122 maize on ladybirds.

According to literature, Coccinellidae are an abundant predator group in maize.[13,14] Although the number of ladybirds in maize fields is small, the number is similar to the number of ladybirds in other cultivated or semi-natural habitats.[15] Therefore, it cannot be concluded that maize is insignificant to ladybird populations.

Conclusion

COGEM is of the opinion that cultivation of 59122 maize most likely poses a negligible risk to ladybirds. However, concerns remain on the possible effect of 59122 maize on the rate of reproduction of ladybirds. The additional information that was provided by the applicant is insufficient to draw conclusions on this subject. Therefore, COGEM is of the opinion that in case 59122 is authorised for cultivation, case specific monitoring of ladybirds, in particular of spider mite consuming ladybirds (i.e. ladybirds belonging to the Stethorini tribe), remains a necessity unless conclusive additional experimental data is provided.

The experimental data should provide information on the maximum level of bioaccumulation of Cry proteins in spider mites over a prolonged time period and should assess the effect of Cry proteins on spider mite consuming ladybirds at levels corresponding to a worst case bioaccumulation scenario in spider mites.

In summary, the additional information provided does not remove COGEM's previous concerns regarding the reduced larval weight of *C. maculata* ladybirds that was observed in a laboratory study. Therefore, in case 59122 maize is authorised for cultivation, in COGEM's view case specific monitoring of ladybirds, in particular of spider mite consuming ladybirds (i.e. ladybirds belonging to the Stethorini tribe), remains a necessity unless conclusive additional experimental data is provided showing unequivocally the absence of an effect.

References

1. COGEM (2007). Cultivation of GM maize line 59122. Advice CGM/070612-07
2. COGEM (2008). Cultivation of GM maize line 59122. Advice CGM/080207-02
3. EFSA (2013). Scientific Opinion on an application from Pioneer Hi-Bred International and Dow AgroSciences LLC (EFSA-GMO-NL-2005-23) for placing on the market of GM maize 59122 for food and feed uses, import, processing and cultivation under Regulation (EC) No 1829/2003. EFSA Journal 11(3): 3135
4. Giorgi JA, Vandenberg NJ, McHugh JV *et al.* (2009). The evolution of food preferences in Coccinellidae. Biological Control 51: 215-231
5. Raps A, Kehr J, Gugerli P *et al.* (2001). Immunological analysis of phloem sap of *Bacillus thuringiensis* corn and of the nontarget herbivore *Rhopalosiphum padi* (Homoptera Aphididae) for the presence of Cry1Ab. Molecular Ecology 10: 535-533
6. Meissle M & Romeis J (2009). The web-building spider *Theridion impressum* (Araneae: Theridiidae) is not adversely affected by Bt maize resistant to corn rootworms. Plant Biotechnology Journal. 7: 1-12
7. Obrist LB, Dutton A, Albajes R, Bigler F (2006). Exposure of arthropod predators to Cry1Ab toxin in Bt maize fields. Ecological Entomology 31: 143-154
8. Li Y & Romeis J (2010). *Bt* maize expressing Cry3Bb1 does not harm the spider mite, *Tetranychus urticae*, or its ladybird beetle predator, *Stethorus punctillum*. Biological control 53: 337-344

9. Álvarez-Alfageme F, Ferry N, Castañera P *et al.* (2008). Prey mediated effects of Bt maize on fitness and digestive physiology of the red spider mite predator *Stethorus punctillum* Weise (Coleoptera: Coccinellidae). Transgenic Res. 17: 943-954
10. Obrist LB, Dutton A, Romeis J & Bigler F (2006). Biological activity of Cry1Ab toxin expressed by Bt maize following ingestion by herbivorous arthropods and exposure of the predator *Chrysoperla carnea*. Biocontrol 51: 31-43
11. Meissle, M & Romeis J (2009). Insecticidal activity of Cry3Bb1 expressed in Bt maize on larvae of the Colorado potato beetle, *Leptinotarsa decemlineata*. Entomol. Exp. Appl. 131, 308–319
12. Meissle M, Álvarez-Alfageme F, Malone LA &Romeis J (2012). Establishing a database of bio-ecological information on non-target arthropod species to support the environmental risk assessment of genetically modified crops in the EU. Supporting publications 2012: EN-334
13. Rauschen S, Schaarschmidt F & Gathmann A (2009). Occurrence and field densities of Coleoptera in the maize herb layer: implications for Environmental Risk Assessment of genetically modified *Bt*-maize. Transgenic Research doi 10.1007/s11428-009-9351-3
14. De La Poza M, Pons X, Farinós GP *et al.* (2005). Impact of farm-scale Bt maize on abundance of arthropods in Spain. Crop Protection 677-684
15. Duelli P & Obrist MK (1998). In search of the best correlates for local organismal biodiversity in cultivated areas. Biodiversity and Conservation 7: 297-309