

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

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KENMERK CGM/080207-02
ONDERWERP Advice cultivation of maize variety 59122

Geachte mevrouw Cramer,

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 gevraagd opnieuw te adviseren over de vergunningaanvraag voor de teelt van de genetisch gemodificeerde maïslijn 59122 naar aanleiding van de beantwoording van een aantal vragen die de COGEM in het voorgaande advies stelde.

Door introductie van het *cry34Ab1* gen en het *cry35Ab1* gen afkomstig uit *Bacillus thuringiensis* is de maïs resistent voor bepaalde insecten uit de orde van de *Coleoptera*, waaronder de maïswortelkever (*Diabrotica* spp.). De genen *cry34Ab1* en *cry35Ab1* hebben een onderling versterkend effect. Daarnaast is maïslijn 59122 door de introductie van het *pat* gen tolerant voor herbiciden met als werkzame stof glufosinaat-ammonium.

De COGEM kwam eerder tot de conclusie dat de gegevens betreffende de effecten op niet-doelwitorganismen onvoldoende waren. Naar aanleiding hiervan zijn een aantal vragen opgesteld en is de aanvrager verzocht om aanvullende gegevens te overleggen. De vragen hadden betrekking op de statistische betekenis van een aantal laboratoriumtesten, de relevantie van de geteste niet-doelwitorganismen en de methodologie van de veldproeven. De aanvrager heeft de resultaten van twee nieuwe veldstudies aangeleverd en een aantal bestaande gegevens opnieuw geanalyseerd.

De COGEM is op basis van deze nieuwe gegevens van mening dat de risico's voor mens en milieu bij de teelt van maïslijn 59122 verwaarloosbaar klein zijn, onder voorbehoud dat bij de monitoring extra aandacht wordt besteed aan Europese lieveheersbeestjes en verwante kevers .

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. Dr. ir. D.C.M. Glandorf
Dr. I. van der Leij

Cultivation of genetically modified maize line 59122

COGEM advice CGM/080207-02

This notification concerns the cultivation of the genetically modified maize line 59122. The maize line harbors the genes cry34Ab1, cry35Ab1 and a pat gene conferring tolerance to glufosinate-ammonium-containing herbicides and resistance to certain Coleopteran insects such as the corn rootworm (Diabrotica spp.).

In Europe, no wild relatives of maize are present and establishment of maize plants in the wild has never been observed. There are no reasons to assume that the inserted traits will increase the potential of the maize line to establish feral populations. In addition, the appearance of volunteers is very rare under Northwest European agronomical conditions.

Previously, COGEM advised negatively on the cultivation of maize line 59122. Based on the studies on non-target organisms provided, COGEM had some serious reservations on the conclusion of the applicant that maize line 59122 exerts no negative effects on non-target organisms. The applicant was asked to provide more detailed and confirmatory data to underpin his conclusion.

The applicant provided additional information regarding the statistical relevance of existing laboratory studies, representativeness of the selected NTO test species for the European situation and the methodology used for NTO testing with maize 59122. Furthermore, data from two additional field tests were provided.

Based on the additional information provided by the applicant and the considerations put forward in this advice, COGEM is of the opinion that the cultivation of maize line 59122 poses a negligible risk to human health and the environment, under the condition that specific monitoring for Ladybird beetles is incorporated.

Introduction

The scope of the present notification by Pioneer Hi-Bred International concerns the cultivation of maize line 59122. The maize line expresses the genes *cry34Ab1*, *cry35Ab1* conferring resistance to certain coleopteran insects such as the corn rootworm (*Diabrotica spp.*). The genes *cry34Ab1* and *cry35Ab1* exert a synergistic effect. The *cry34Ab1* gene confers an average resistance for 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.

Previous COGEM advices

Previously, COGEM advised positively on the import of maize line 59122 (5). In Europe, no wild relatives of maize are present and establishment of maize plants in the wild has never been observed. There are no reasons to assume that the traits inserted will increase the potential of the maize line to establish feral populations. In addition, the appearance of volunteers is very rare under Northwest European agronomical conditions. Furthermore, it was concluded that the molecular characterization was adequate.

However, in 2007 COGEM advised negatively on the cultivation of maize line 59122 due to insufficient information regarding the effects on non-target organisms. COGEM was of the opinion that the studies on the effects on non-target organisms (NTOs) provided contained some serious shortcomings.

Aspects of the crop

Maize (*Zea mays* L.) is a member of the grass family *Poaceae*. Maize is being cultivated as an agricultural crop, originating from Central America. Although insect pollination can not be completely excluded, maize is predominantly wind pollinated (1;2). According to literature, pollen viability varies between 30 minutes and 9 days (2;3;4). In Europe, no wild relatives of maize are present and, therefore, hybridization with other species can not occur.

The appearance of volunteers is very rare under Dutch conditions. Grains exhibit no germination dormancy, resulting in a short persistence. In addition, only few seeds remain on the field after harvesting of fodder maize (1). Establishment of maize plants in the wild has never been observed in the Netherlands.

Molecular characterization

The molecular characterization of maize 59122 was previously evaluated by COGEM in an application concerning import and processing for the use in feed and food of maize line 59122. It was concluded that the molecular characterization was adequate and that the risk of formation of toxic or allergenic products with the modification of maize 59122 is negligible.

Environmental risk assessment

In its previous advice, COGEM formulated a series of questions to the applicant concerning the statistical power of certain laboratory studies provided, representativeness of the selected NTO test species for the European situation and the methodology used for NTO testing with maize 59122. Furthermore, COGEM asked for more confirmatory data from field tests.

Recently, the applicant has provided additional information to answer these questions. These points will be discussed below, starting with remarks on the laboratory studies which are followed by a discussion on the quality of the additional field studies the applicant provided.

Laboratory studies

COGEM previously placed some remarks related to the statistical relevance of amongst others annex 21 (toxicity testing with ladybird beetle *Coleomegilla maculata*) and annex 24 (toxicity testing with parasitic Hymenopteran *Nasonia vitripennis*). In response, the applicant carried out *post hoc* statistical power calculations for the ladybird beetle study and the study with the parasitic Hymenopteran *Nasonia vitripennis*.

In general, the applicant states that the methodological design of NTO laboratory toxicity studies with the Cry34/35Ab1 proteins was in line with international guidelines on ecotoxicity testing. Furthermore, the applicant emphasizes that detecting a magnitude of effect size that ranges from 30% to 50% or less with a statistical power of 80% or more is generally considered as adequate to confirm the absence of biologically significant effects. On this point, COGEM agrees with the applicant.

Also, the applicant underlines that the *post hoc* power calculations demonstrate that these two studies allow to detect a magnitude of effect size that is considerably lower than what is generally considered as safe from risk.

Statistical significance was calculated for toxicity testing with *N. vitripennis*. Previously it was unclear whether the number of wasps in the control group who drowned were accounted for in the mortality calculation. The applicant now confirms that these wasps were omitted from the mortality calculation. Furthermore, *post hoc* statistical power calculations for the parasitic Hymenopteran study demonstrate that statistical power for this toxicity testing is sufficient. COGEM agrees on this point with the applicant.

The applicant concludes that the statistical power on the ladybird beetle toxicity study was sufficient and that the Cry34/35Ab1 proteins at concentrations that represent exposure under field conditions do not cause developmental or mortality effects on ladybird beetle larvae.

Although COGEM is of the opinion that the newly calculated statistical power of these studies is indeed sufficient, she places a remark regarding the conclusion of the applicant that no negative effects are found in the laboratory study on the effect of maize 59122 on ladybird beetles (*C. maculata*). In the opinion of COGEM the conclusion of the applicant is less clear cut than stated in the additional information.

Results from the tier-1 experiment concerning *C. maculata* larvae fed directly with a Cry34Ab1 and Cry35Ab1 containing diet (at a level 10 times the expected environmental concentration), indicate no significant difference in mortality. However, sub-lethal effects such as a reduction in larval weight resulting in a slower development appear to be significant in this study. A reduction in larval weight might have a pronounced effect on the life expectation and reproduction ability of insects and thus on population dynamics. The tier-2 study with a diet containing Cry34Ab1 and Cry35Ab1 pollen at a, according to the applicant, realistically high end exposure rate (at a level 1.5 times the expected environmental concentration), caused no developmental or mortality effects on *C. maculata* larvae. The applicant concludes from the tier-1 and tier-2 experiments, that no harmful effects are caused by the Cry34/35Ab1 proteins on *C. maculata*.

COGEM is of the opinion that, notwithstanding the overall results, the measured effect on larval growth in the tier-1 experiment cannot be neglected. The results of the statistical calculations are just within range of non-significance with a one-sided P-value. With a two-sided P-value, the observed effect would have been significant.

Representativeness of the NTO's tested

In the opinion of COGEM, the parasitic Hymenopteran *Nasonia vitripennis* is not representative for the parasitic *Hymenoptera* in maize ecosystem. *N. vitripennis* is a parasitic hymenopteran which parasitizes fly pupae in bird's nests. The applicant explains that although *N. vitripennis* is not normally present in maize, it was selected as an indicator species because it is known to be a sensitive species that can be reared and handled under laboratory conditions and is used commonly as a surrogate for parasitic Hymenoptera in tier-1-ecotoxicity studies.

COGEM notes the justification of the applicant for his choice for *N. vitripennis* as an indicator species for parasitic *Hymenoptera* in maize. Based on these results alone however, COGEM cannot conclude maize 59122 has no adverse effects on parasitic *Hymenoptera* in maize ecosystems. In addition to these laboratory studies, field study results will have to confirm that no harmful effects occur in parasitic *Hymenoptera* in maize ecosystems.

Field study

In its previous advice, COGEM was of the opinion that the quality of the field study from Spain (2005) was not sufficient to be able to conclude that no effects on NTOs are to be expected with the cultivation of event 59122. This is especially the case since maize 59122 is a newly developed variety which has entered the market only recently. Results of earlier experiments with this maize event are not available. Because the maize in the

Spain field study (2005) was planted too late in the season, no relevant conclusions could be drawn regarding the effects of maize 59122 on NTOs.

The applicant provided two additional field studies performed in Spain (2006) and Hungary (2006). Regarding the planting dates for the NTO field trials, the applicant states that these were within the range that is considered acceptable for normal planting. The Spain field study of 2005 was planted at the end of June, whilst these new field studies were planted earlier. Additionally, late planting of maize is not unusual in certain regions of Europe, as it is sometimes grown as a follow-on crop to beans in certain regions of Spain. COGEM consents that the additional field studies were performed within range of the normal planting season.

Regarding the additional field studies, COGEM notes that minimal information is provided regarding weed control and present weed populations. Although the Hungary field study provided information on the treatment of non-gm control maize, they do not present the results of this treatment. COGEM remarks that since weed control information is relevant for the development of NTOs, it would be preferable to add this information to the dossier.

Overall the field studies provided show no significant harmful effects on the NTOs present in maize 59122 compared to the non-gm maize control. The field studies confirm the findings of the laboratory toxicity studies on *N. vitripennis* that maize event 59122 has no adverse effect on parasitic Hymenopteran species present in maize ecosystems.

Regarding the effects on *C. maculata* COGEM notes that although laboratory toxicity testing demonstrated a possible adverse effect on the growth of *C. maculata* larvae; in the field no such effect was observed on ladybird beetles.

Post-market monitoring plan / general surveillance

Laboratory studies as well as field studies have been taken into consideration regarding a possible adverse effect on *C. maculata* larval growth. COGEM has some reservations concerning the conclusion of the applicant that no negative effects are found in the laboratory study on the effect of maize 59122 on larvae of ladybird beetles (*C. maculata*) since the results were just within range of non-significance with the statistical methodology used. However, no adverse effect on *C. maculata* in the additional field studies was observed. COGEM also takes into account that ladybird beetles do not feed directly on the plant, but on other insects such as aphids, which are usually not present in the maize ecosystem. COGEM is of the opinion that adverse effects on ladybird beetles are unlikely, however the findings of the laboratory studies remain a point of concern.

Therefore, COGEM is of the opinion that the applicant should pay extra attention to the monitoring of *C. maculata* in maize 59122.

Advice

The present application concerns the cultivation of maize line 59122. Maize line 59122 expresses the *pat* gene and the genes *cry34Ab1* and *cry35Ab1* providing the plant with a herbicide tolerance trait as well as resistance to certain coleopteran insects. In the past, COGEM advised positively on the import of this particular maize line.

There are no wild relatives of maize in the Netherlands and the appearance of volunteers under Dutch conditions is very rare. Furthermore, there are no reasons to assume that the inserted traits will increase the now absent potential of the maize line to run wild. COGEM is of the opinion that the molecular characterization is adequate.

In relation to this notification, COGEM previously had some serious questions regarding the data presented concerning the testing of potential effects on NTOs. In reaction to these questions, the applicant provided additional information regarding the statistical relevance of existing laboratory studies, representativeness of the NTO test species selected for the European situation and the methodology used for NTO testing with maize 59122. Furthermore, data from two additional field tests were provided.

Based on the additional information provided by the applicant and the considerations put forward in this advice, COGEM is of the opinion that the cultivation of maize line 59122 poses a negligible risk to human health and the environment, under the condition that specific monitoring for ladybird beetles is incorporated.

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