COMMISSIE
COGEM
GENETISCHE
MODIFICATIE

Aan de minister van
Volkshuisvesting, Ruimtelijke
Ordening en Milieubeheer
Mevrouw dr. J.M. Cramer
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DATUM 12 juni 2007
KENMERK CGM/070612-07
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 te adviseren over de vergunningaanvraag voor de teelt van de genetisch gemodificeerde maïslijn 59122. 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*.). Het *cry34Ab1* gen alleen geeft enige mate van tolerantie tegen de maïswortelkever, het *cry35Ab1* gen alleen geeft geen tolerantie. Expressie van beide genen leidt tot optimale resistentie tegen de maïswortelkever. Daarnaast is maïslijn 59122 door de introductie van het *pat* gen tolerant voor herbiciden met als werkzame stof glufosinaat-ammonium.

Maïs heeft in Nederland geen wilde verwanten en opslag van maïsplanten is in Nederland nagenoeg uitgesloten. Verwildering van de maïsplant is in Nederland nooit waargenomen. Er zijn geen redenen om aan te nemen dat de modificaties het verwilderingspotentieel vergroten. De COGEM is verder van mening dat de moleculaire karakterisering van de maïslijn volledig is.

De COGEM is echter van mening dat de gegevens betreffende de effecten op niet-doelwitorganismen onvoldoende zijn. Zowel de laboratoriumexperimenten als de gegevens van de veldexperimenten zijn onvoldoende van kwaliteit om een volledige risico-analyse te kunnen uitvoeren. De COGEM is derhalve van mening dat zij geen positief advies kan geven en stelt voor de aanvrager te verzoeken om aanvullende gegevens te overleggen.

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. Dr. ir. B.P. Loos
Dr. I. van der Leij

Cultivation of genetically modified maize line 59122

COGEM advice CGM/070612-07

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.).

Previously, COGEM advised positively on the import of maize line 59122. 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. In the opinion of COGEM, the molecular analysis is adequate.

Based on the studies on non-target organisms provided, COGEM has some serious reservations about the conclusion of the applicant that maize line 59122 exerts no negative effects on non-target organisms.

Concerning the general surveillance and monitoring plan, COGEM is of the opinion that more attention should be paid to the possible development of a dominant form of Bt resistance.

COGEM is of the opinion that based on the studies and data provided, she can not perform a reliable environmental risk analysis with regard to the cultivation of this maize variety. Therefore, COGEM can not issue a positive advice on the cultivation of maize line 59122.

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* show 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.

During the last few years, COGEM was asked repeatedly to issue advice on applications concerning the cultivation of various genetically modified (GM) maize varieties. To obtain permission for cultivation of a gm maize line, an environmental risk

assessment has to be carried out by the applicant. The objective of the risk assessment is to identify and evaluate potential adverse effects (direct and indirect, immediate or delayed) of the genetically modified maize line on human health and the environment.
The environmental risk analyses focuses amongst others on the potential of the gm maize variety to establish feral populations; its potential to outcross with wild relatives and the effects of outcrossing on the environment; its potential to cause effects on non-target organisms and its potential to cause effects on the soil ecosystem and risks associated with incidental consumption by humans and animals.

To identify and evaluate these points, the crop characteristics, the molecular characterization of the gm plant (e.g. location of the insert and characteristics of the inserted genes) and the environment in which the plant is introduced (e.g. wild relatives, geographical and climatological conditions), are taken into account. The base line used to determine whether or not identified risks are negligible, is the current agricultural practice, including application of permitted insecticides to control the corn rootworm.

In the case of maize, COGEM has repeatedly stated that maize is not able to run wild in Northwest Europe, and that no wild relatives are present in Europe.

Previous COGEM advices

Previously, COGEM advised positively on the import of maize line 59122 (9).

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 dormacy, 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

Maize line 59122 was produced by *Agrobacterium*-mediated transformation. An overview of the sequences introduced is given below:

Components of the insert in maize 59122:

- ubi1ZM promotor, derived from *Zea mays L.*

- *cry34Ab1* gene, from *Bacillus thuringiensis* strain PS149B1; confers resistance to coleopteran insects
- PINII, terminator sequence from *Solanum tuberosum* proteinase inhibitor II gene
- TA peroxidase promotor, derived from *Triticum aestivum*
- *cry35Ab1* gene, from *B. thuringiensis* strain PS149B1; confers resistance to coleopteran insects
- CaMV *35S* promotor, derived from *Cauliflower mosaic virus (*CaMV*)*
- *Pat* gene, derived from *Streptomyces viridochromogenes*; confers tolerance to glufosinate ammonium herbicide
- CaMV 35 terminator, derived from CaMV

Properties of the introduced genes conferring herbicide tolerance

Maize line 59122 was genetically modified by the introduction of the *pat* gene, encoding the enzyme phosphinothricin acetyltransferase protein (PAT). Expression of PAT confers tolerance to glufosinate-ammonium herbicides.

The active ingredient in glufosinate-ammonium herbicide is L-phosphinothricin (L-PPT), which binds to glutamine synthetase in plants. The detoxification of excess ammonia is thereby prevented, leading to plant death. Maize line 59122 expresses the *pat* gene which catalyses the conversion of L-PPT into an inactive form, which does not bind glutamine synthetase. The application of glufosinate-ammonium herbicides to maize line 59122 will therefore be ineffective since ammonia is detoxified.

The PAT protein does not exhibit amino acid homology to known allergens or toxins.

Properties of the introduced genes conferring insect resistance

The introduced genes c*ry34Ab1* and *cry35Ab1* act together to control certain coleopteran insect pests, among others the larvae of the Western corn rootworm (*Diabrotica virgifera virgifera*), Northern corn rootworm (*Diabrotica barberi*) and the Southern corn rootworm (*Diabrotica undecimpunctata howardi*).

With the insertion of the two genes, the plant is able to produce δ-endotoxins (Bt toxin). The δ-endotoxin selectively binds to receptors located in the midgut of susceptible insects (11). After this binding to receptors, the gut is perforated, enabling enterobacteria from the midgut to enter the body, causing the insect to die from poisoning within 48 to 120 hours (8).

The corn rootworm is an economically important pest insect, which causes major crop losses. Larvae of this insect feed on maize roots, resulting in the interference of the plant's ability to absorb water and nutrients and in the reduction of the stability of the plant. As a consequence, damaged plants may lodge, making harvesting difficult. The corn rootworm was accidentally introduced in the mid-nineties in Bosnia presumably by

military air traffic and became established shortly after introduction. The pest is still spreading at regular rate of about 40 km per year, but is infamous for rapid spread over large distances by (air) traffic. In 2003 this insect was first discovered near the airport of Schiphol in the Netherlands, but eradicated successfully. If the corn rootworm is able to establish and spread in the Netherlands, extensive crop damage can be expected (10). The Cry34Ab1 and Cry35Ab1 proteins do not exhibit amino acid homology to known allergens or toxins.

Molecular analysis
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 the opinion of COGEM, the risk is negligible that maize line 59122 becomes environmentally persistent or invasive resulting in unintended spreading trough increased weediness.

COGEM is of the opinion that the studies on the effects on non-target organisms (NTOs) provided, contain some serious shortcomings. COGEM has repeatedly criticized the quality of NTO-studies and underlines the importance of a thorough and representative study on non-target organisms once more in this advice. COGEM realizes that standardized criteria for the relevant studies are still lacking. Therefore, COGEM urges that EU-wide, standardized criteria are formulated. To facilitate this, COGEM has taken the first preliminary steps by commissioning a research project on the determination of effects of insect-resistant transgenic crops on non-target arthropods (13).

As far as the effects on non-target organisms in this specific case are concerned, COGEM has some serious questions and remarks regarding the conclusions of the applicant on the safety of maize 59122. These points will be discussed below, starting with remarks on the laboratory studies which are followed by a discussion on the quality of the Spanish field study that the applicant provided.

Laboratory studies
The first point concerns the laboratory study on the effect of maize 59122 on ladybird beetles (*Coleomegilla maculate*) in Annex 21. COGEM questions the conclusion of the applicant that no negative effects are found. Results from this study indicate that *C.*

maculata larvae fed with a Cry34Ab1 and Cry35Ab1 containing diet (at a level 10 times the expected environmental concentration), no significant difference in mortality was measured. However, sub-lethal effects appear to be significant in this study expressed in a reduction in larval weight resulting in a slower development. Another 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.

In the opinion of COGEM it is not clear how the realistic exposure rate of Cry34Ab1 and Cry35Ab1 for ladybird beetles has been determined. Ladybird beetles do not feed directly on the plant, but on other insects, which might accumulate Cry34Ab1 and Cry35Ab1 by feeding of the maize. In relation to the sub-lethal effect found 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. Therefore, it can not be concluded that the reduction in larval weight is an insignificant effect.

COGEM has some remarks related to the statistical relevance of amongst others annex 21. Because of the small number of replications, COGEM considers it questionable whether generalized statements can be made on the significance of the effects observed. For example, the number of insects used in this study is only 30 per treatment. In the control group, the mortality rate is 3% while this percentage in the group fed with the Cry34Ab1 and Cry35Ab1 containing diet is 10 to 20%. Although the increased mortality rate is not statistically significant, it does not mean that a study with a larger amount of insects would confirm the biological insignificance of the effects observed. In a group of only 30 insects, no significant conclusions can be drawn from the effect of the consumption of Cry34Ab1 and Cry35Ab1. Therefore, COGEM can not conclude that maize 59122 has no negative effects on these NTOs. In general the data of the experiments presented lack statistical power, which seriously hampers the generalized conclusions drawn.

Furthermore, COGEM draws attention to the choice of NTOs in Annex 24. The use of *Nasonia vitripennis* as a representative of the parasitic *Hymenoptera* in maize is questionable. *N. vitripennis* is a parasitic hymenopteran which parasitizes fly pupae in bird's nests. *N. vitripennis* is not present in maize. COGEM is therefore of the opinion that the conclusions of the study on *N. vitripennis* are not relevant to the maize ecosystem.

Another point regarding annex 24 concerns again the statistical power of the experimental setup. It was concluded that Cry34Ab1 and Cry35Ab1 exert no significant effect on the *N. vitripennis.* In this experiment however, a high number of wasps in the control group drowned and this was not accounted for in the mortality calculation.

Another remark on the NTO-studies concerns the chemical used as a negative control in the nto-study in annex 26. In this study, concerning the effect of maize 59122 on honeybees, arsenic is used as a control. Arsenic is not allowed as an insecticide in agricultural environments in Europe. Although the use of arsenic as a control will undoubtedly be very effective, COGEM is of the opinion that the insecticide used as a control in NTO-experiments should be a representative maize insecticide registered and allowed in Europe.

Spain field study

COGEM has also some serious reservations concerning the quality of the field experiment which was performed in Spain (2005).

Regarding this field study on NTOs COGEM notices that the maize is planted at June 29th (2005). In Spain, maize is normally planted considerably earlier in the season. Because of the late start of the experiment, the development of maize will be a-synchronic with the course of development of NTOs. It is possible that relevant NTOs are no longer present by this time, while other insects are being attracted to this field because of the unusual presence of maize pollen. Secondly, cultivation at a later time, results in lower yields, which also can effect the presence of certain herbivorous insects.

The Spain field study compares four different events of maize to a control group. The methodological setup is not based on the effects of maize 59122 by itself, which does not contribute to the statistical power of the results of this study for the assessment of this particular maize variety.

In relation to the results of the field study, COGEM notices that certain types of insects (for example aphids, leafhoppers, *Orius ssp*.) appear to be abundant at date 1 in the experiment, followed by a strong decrease on dates 2 and 3. This effect is not explained nor noticed in the study. Also, this kind of effect results in statistically weak data.

Overall, COGEM is of the opinion that the quality of the field study provided is 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.

Finally, the applicant provided a series of dietary studies with rats and poultry. COGEM noticed that the overall liver yields in the poultry study *(Annex 15)* showed no significant effect. However, the liver yield in female chickens was higher compared to the non-transgenic control maize diet group. The applicant concluded that the values were still

within the tolerance range calculated for this study. Although food safety is not part of the legal task of COGEM, accidental consumption is part of the environmental risk analysis. Therefore, COGEM contacted the Dutch Institute of Food Safety (RIKILT) to verify the conclusions of the poultry feeding study. RIKILT had examined the results and concluded that there are no (biologically as well as statistically) significant effects of the poultry fed with maize 59122 compared to the non-transgenic control maize diet group.

Post-market monitoring plan / general surveillance

To obtain permission for cultivation of gm maize, a monitoring plan considering the environmental impact of cultivation is required. The applicant has formulated a general surveillance plan for maize 59122 in order to detect any unanticipated adverse effects on human health and the environment. In relation to the monitoring plan and the possible anticipated effects by the applicant, COGEM wants to draw attention to the potential development of resistance to Cry34Ab1 and Cry35Ab1 within the target pest population. In relation to the monitoring plan provided by the applicant, COGEM notes that the approach proposed will only be adequate in case of recessive inheritance of Bt resistance. In the application, insect resistant monitoring is completely based on the assumption that Bt-resistance is a recessive trait and that only homozygous individuals will be resistant. However, a dominant resistance allele can immediately increase in frequency under selective circumstances, such as the presence of Bt-toxin. In addition, literature shows that Bt-resistance in the European corn borer can also be partly dominant (12). However, as insect resistance management is of agronomical importance and not part of the environmental risk analysis, this aspect is not included in the conclusions of this advice.

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.

Concerning this notification, COGEM has some serious questions regarding the data presented concerning the testing of potential effects on NTOs. Based on the considerations put forward in this advice, COGEM is of the opinion that she can not

perform a reliable risk analysis with regard to the cultivation of this maize variety. Therefore, COGEM can not give a positive advice on the cultivation of maize line 59122.

COGEM is of the opinion that additional data from laboratory experiments and field studies have to be supplied for the European situation.
COGEM is of the opinion that an appropriate laboratory study includes a relevant statistical approach and a clear substantiated methodology. The NTOs that are taken into account in a study on non-target organisms, should be first of all relevant for the European situation. Secondly, the NTOs should be representative for the insects present in maize. COGEM proposes to carry out new study on effects on parasitic *Hymenoptera* with a species that is relevant to the maize ecosystem in Europe. Also, studies on a European ladybird beetle would be more relevant for this application.
In the opinion of COGEM, a relevant study is a field study which is comparable to the current agricultural practice, including application of commonly used insecticides to control the corn rootworm. A field experiment should therefore be performed at that time of year when maize is normally cultivated. Otherwise, no relevant conclusions can be drawn regarding the effects on NTOs. Also, the scope of field experiments should be preferably limited to one event against a control with sufficient numbers of replication, to contribute to the statistical power of the outcome.

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