Cultivation of maize line MON89034 x MON88017

COGEM advice CGM/091222-02

This notification concerns the cultivation of genetically modified maize line MON89034 x MON88017. This maize line expresses the genes cry1A.105, cry2Ab2, cry3Bb1 and cp4epsps conferring resistance to certain lepidopteran and coleopteran insects and tolerance to glyphosate containing herbicides.

Previously, COGEM advised positively on the import of both parental maize lines MON88034 and MON88017 and on the import of hybrid maize line MON88034 x MON88017. COGEM advised negatively on the cultivation of parental maize line MON88017 because the studies provided were insufficient to conclude there are no adverse effects to be expected on NTOs.

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, in Europe the appearance of volunteers is very rare.

COGEM is of the opinion that the molecular characterization of MON89034xMON88017 is adequately performed. With regard to potential adverse effects of MON89034xMON88017 on non-target organisms (NTOs), the applicant refers to laboratory, greenhouse and field studies. None of the laboratory experiments have been carried out with MON89034 x MON88017. In most cases either Cry1Ab.105, Cry2Ab2 or Cry3Bb1 pure protein was used. In other cases a different maize line was used, for example MON863 which expresses a cry3Bb1 protein which differs by one amino acid from cry3Bb1 in MON88017. COGEM is of the opinion that the applicant did not sufficiently demonstrate that the Cry1A.105, Cry2Ab2 and Cry3Bb1 proteins do not interact. Furthermore, most of the NTOs that were studied do not occur in the European Union and the applicant did not explain why these organisms are relevant to European maize fields. The laboratory experiments also exhibit other shortcomings: the statistical power of the experiments and the obtained P value are not given, the choice for the statistical test is not explained and the applicant did not provide an explanation for the high mortality (over 15%) in certain control groups.

The applicant presented two studies that describe MON89034xMON88017 field trials. However, these field trials investigate the effect of MON89034xMON88017 on target and pest organisms. None of the field trial studies investigates the effect of ecologically relevant NTO's in Europe.

COGEM is of the opinion that the data provided are insufficient to allow a conclusion that cultivation of MON89034xMON88017 exerts negligible effects on NTOs. In conclusion, COGEM cannot advise positively on cultivation of maize line MON89034xMON88017. COGEM is of the opinion that additional data from laboratory experiments and field trials have to be supplied to be able to make a reliable environmental risk analysis on cultivation of maize line MON89034xMON88017.

Introduction

The scope of the present notification (EFSA/GMO/BE/2009/71) by Monsanto Company, as represented by Monsanto Europe S.A., concerns the cultivation of genetically modified maize line MON89034 x MON88017.

MON89034 x MON88017 was produced by crossing the two genetically modified parental maize lines MON89034 and MON88017 using traditional breeding methods. The hybrid maize line contains the *cry1A.105*, *cry2Ab2*, and *cry3Bb1* genes, which confer resistance to certain insect pests. In addition, this line contains the *cp4epsps* gene, which confers tolerance to glyphosate containing herbicides. *Cry1A.105* and *cry2Ab2* are derived from MON89034 and confer resistance against the European corn borer (ECB, *Ostrinia nubilalis*), the Mediterranean corn borer (MCB, *Sesamia nonagrioides*) and other Lepidopteran pests. The c*ry3Bb1* and *cp4epsps* genes are derived from MON88017. *Cry3Bb1* confers resistance against the coleopteran corn rootworm larvae (CRW, *Diabrotica* spp.).

Previous COGEM advice

In 2009 COGEM advised positively on import and processing for use in food and feed of parental maize line MON89034.[1] To date, COGEM has not yet been asked to issue an advice on the cultivation of maize line MON89034.

In 2007 COGEM issued a positive advice on import and processing for use in food and feed of parental maize line MON88017.[2] In 2008 COGEM advised negatively on the cultivation of this maize line.[3] The applicant conducted several laboratory and field studies to assess possible adverse effects of MON88017 on non-target organisms (NTOs). Only one of these tests was conducted with the exact protein as expressed in MON88017. Most laboratory tests were performed with different Cry3Bb1 proteins or with different variants of in maize expressed *cry3Bb1* genes. Furthermore, COGEM placed remarks on the selection of NTOs used in the experiments and noticed that the general surveillance plan gave no guarantees that sufficient data were obtained. In 2007 COGEM issued a negative advice on import and processing for use and feed of MON89034 x MON88017 due to an initially incomplete molecular characterization of parental maize line MON89034.[4] Recently, COGEM reconsidered the elements of the molecular characterization that are needed for the environmental risk analysis and formulated revised criteria for the molecular characterization of commercial releases of GM crops.[5] Based on these revised criteria, COGEM advised positively on the import of MON89034 in 2009. Therefore, the argumentation for the initially negative advice on MON89034 x MON88017 is superseded.[6, 1]

Aspects of the crop

Maize (*Zea mays L.*) is a member of the grass family *Poaceae.* Maize is cultivated as an agricultural crop, originating from Central America. Although insect pollination can not be completely excluded, maize is predominantly wind pollinated.[7,8] According to literature, pollen viability varies between 30 minutes and 9 days.[8,9,10] In Europe, no wild relatives of maize are present and, therefore, hybridization with other species cannot 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.[7] Establishment of maize plants in the wild has never been observed in the Netherlands.

Molecular characterization MON89034 x MON88017

The genetically modified maize line MON89034 x MON88017 was produced by crossing the two parental genetically modified maize lines MON89034 and MON88017 using traditional breeding methods. The molecular characterization of maize MON89034 and MON88017 was previously evaluated by COGEM.[1,2] It was concluded that the molecular characterization of both parental lines was adequate. An overview of the construction and inserted genetic elements of both parental lines is given below.

Parental maize line MON89034

The genetically modified maize line MON89034 was produced by *Agrobacterium tumefaciens*-mediated transformation using *A. tumefaciens* strain ABI. This strain contained the PVZMIR245 vector, which consisted of two T-DNA regions and the vector backbone. The T-DNA I region contained the *cry1A.105* and the *cry2Ab2* genes whose encoded proteins provide protection to certain lepidopteran insects. The T-DNA II region contained the neomycin phosphotransferase II (*nptII*) gene, which confers resistance to certain aminoglycoside antibiotics, such as neomycin, kanamycin and paromycin. The T-DNA I and T-DNA II regions were both flanked by so-called right and left border sequences which allow the T-DNA regions to be inserted independently. After transformation paromycin resistant plants were selected. These plants contained the T-DNA II region or the T-DNA I and II regions. During subsequent breeding the T-DNA I and T-DNA II regions which were integrated at different loci segregated. The plants that contained the T-DNA II region were eliminated and only the plants containing the T-DNA I region were selected. An overview of the introduced T-DNA I sequences is given below:

- Right border region derived from the Ti-plasmid of *A. tumefaciens* used for transfer of the T-DNA;
- *e35S* promoter providing constitutive expression, which was derived from *Cauliflower mosaic virus* (CaMV) and contains the duplicated enhancer region;
- *Cab* leader, leader region from the chlorophyll a/b-binding protein from wheat;
- *Ract1* intron, intron from the rice actin gene;
- Cry1A.105 coding sequence, coding sequence for the *cry1A.105* gene which is a modified version of the *cry1A* gene from *Bacillus thuringiensis. Cry1A.105* encodes Cry1A.105, a modified Cry1A protein, which consists of domains I and II from Cry1Ab/Cry1Ac, domain III from Cry1F and substantially the entire Cterminal domain of Cry1Ac. The codon usage of *cry1A.105* has been optimized for expression in monocots.
- *Hsp17* terminator from the wheat heat shock protein 17.3, which ends transcription and directs polyadenylation;

- *FMV* promoter providing constitutive expression from *Figwort Mosaic Virus* (FMV);
- *Hsp70* intron from the heat shock protein 70 gene of maize;
- *SSU-CTP* targeting sequence, chloroplast targeting sequence of the small subunit of ribulose 1,5- bisphosphate carboxylase from maize;
- Cry2Ab2 coding sequence, coding sequence for the *cry2Ab2* gene, which encodes the Cry2Ab2 protein. The Cry2Ab2 protein has been isolated from *B. thuringiensis* var. *kurstaki*. The codon usage of *cry2Ab2* has been optimized for expression in monocots;
- *nos* terminator sequence from the nopaline synthase gene of *A. tumefaciens,* which ends transcription and directs polyadenylation;
- Left border region derived from the Ti-plasmid of *A. tumefaciens* used for transfer of the T-DNA.

Parental maize line MON88017

The genetically modified maize line MON88017 was produced by *Agrobacterium tumefaciens*-mediated transformation using *A. tumefaciens* strain ABI. This strain contained the PVZMIR39 vector, which consisted of a T-DNA region and the vector backbone. The T-DNA region contained the *cp4 esps* and *cry3Bb1* gene whose encoded proteins provide tolerance to glyphosate and protection to certain coleopteran insects respectively. The T-DNA region was flanked by so-called right and left border sequences which allow the T-DNA region to be inserted. After transformation and subsequent breeding glyphosate tolerant plants were selected. An overview of the introduced sequences is given below:

- Left border region derived from the Ti-plasmid of *A. tumefaciens* used for transfer of the T-DNA;
- P-ract/ ract1 intron, promoter and intron derived from *O. sativa*, intron promotes transcription;
- *Ctp2* gene from *Arabidopsis thaliana*; encoding the N-terminal chloroplast transit peptide;
- *cp4epsps* gene from *A. tumefaciens* CP4; encoding 5-enolpyruvlshikimate-3-phosphatesynthase (epsps);
- NOS 3', terminator from *A. tumefaciens*; terminates transcription;
- P-e35S, originating from CaMV, promoter with a duplicated enhancer region;
- Wt-CAB, originated from *Triticum aestivum* (wheat); 5' untranslated leader of the wheat chlorophyll a/b binding protein;
- Ract1 intron, derived from *Oryza sativa* (rice); promotes transcription;
- *cry3Bb1* gene, originating from *Bacillus thuringiensis subsp. kumamotoensis*, DNA sequence coding for a genetic variant of CryBb1 protein;
- Tahsp 17 3', wheat heat shock protein derived from *T. aestivum*; stops transcription and induces the polyadenylation;
- Right border region derived from the Ti-plasmid of *A. tumefaciens* used for transfer of the T-DNA.

Properties of the introduced genes

Maize line MON89034 x MON88017 was genetically modified by the insertion of the *cry1A.105, cry2Ab2, cry3Bb1*, and *cp4epsps* genes. The *cry1A.105*, *cry2Ab2*, and *cry3Bb1* genes encode δ-endotoxins specific for insects of the order Lepidoptera (*cry1A.105*, *cry2Ab2*) and Coleoptera (*cry3Bb1*), respectively. The δ-endotoxins are solubilized in the midgut of susceptible insects and are activated by midgut proteases to release a toxin fragment. The toxin fragment binds to specific receptors on the epithelial surface of the midgut. Subsequently, pores are formed in the membranes of the gut cells of the insect, enabling midgut bacteria to enter the body cavity, which leads to septicemia and death.[11]

The *cp4esps* gene encodes the CP4 EPSPS protein. EPSPS is a natural occurring enzyme involved in the biosynthesis of aromatic amino acids. Glyphosate inhibits EPSPS, resulting in a lack of amino acids essential for growth and development of plants. The CP4 EPSPS protein is not inhibited by glyphosate which results in plants tolerant to glyphosate containing herbicides.[12]

Environmental risk assessment

In the opinion of COGEM, there is no reason to assume that the traits introduced in maize MON89034 x MON88017 will increase the potential of maize to establish feral populations. With regard to potential adverse effects of MON89034 x MON88017 on non-target organisms (NTOs), the applicant refers to laboratory, greenhouse and field studies. These studies will be discussed below. Since *cp4epsps* is a gene resulting in herbicide tolerance when expressed, there are no target organisms for the CP4 ESPSPS protein. Therefore, target organisms for the combined trait product are the same as those for the CryA.105, Cry2Ab2, and Cry3Bb1 proteins together.

Laboratory and greenhouse studies

The applicant performed several experiments to study whether MON89034 x MON88017 has an adverse effect on NTOs. Studies on collembola (*Folsomia candida*), soil microorganisms and the northern bobwhite quail (*Colinus virginianus*) were carried out with plant material of parental maize line MON89034 or maize lines resembling parental maize line MON88017 (eg MON853, MON854, MON855, and MON859). Moreover, a study on lady bird beetle (*Coleomegilla maculate*) was carried out with maize line MON863, resembling parental maize line MON88017. The *cry3Bb1* gene in MON88017 differs by one amino acid (Aspragine (D) instead of Glycine (G)) from *cry3Bb1* in MON863. The applicant does not demonstrate possible consequences of this substitution regarding the effect on non-target organisms. Only two target organisms were tested to conclude that there were no statistical differences in biological activity between the two Cry3Bb1 proteins. COGEM is of the opinion that the application of a similar but not identical Cry3Bb1 protein in these studies is a serious demerit. All other laboratory experiments used either the Cry1A.105, Cry2Ab2 or Cry3Bb1 purified protein. In none of the laboratory experiments or greenhouse studies hybrid maize line MON89034 x MON88017 was used. COGEM is of the opinion that experiments to study the effect of MON89034 x MON88017 on NTOs should be carried out with MON89034 x MON88017 and not with other maize lines (e.g. MON89034,

MON 853, MON 854, MON 855, MON 859, or MON 863). Whenever possible, instead of pure proteins plant material of MON89034 x MON88017 should be used.
As stated above, the majority of the laboratory experiments were carried out with either Cry1A.105, Cry2Ab2 or Cry3Bb1 pure protein. Only one study using target organisms (the European corn borer, the corn earworm, and the Colorado potato beetle) was presented to demonstrate the absence of interaction between these three proteins. Preferably, studies to assess the interaction between proteins should be carried out with non-target organisms as well. Moreover, in the study that examined the potential for interaction between the Cry1A.105, Cry2Ab2, and Cry3Bb1 proteins the Cry2Ab2.820 protein was used. According to the applicant, the Cry2Ab2.820 protein contains three additional chloroplast transit peptide amino acids at the N-terminus. Furthermore, the Cry2Ab2.820 protein includes an additional amino acid after the cleavage site of the protein. As the Cry2Ab2.820 protein appears to be different from the Cry2Ab2 protein that is present in maize MON89034 x MON88017, the applicant should provide information to show that Cry2Ab2.820 is biologically identical to Cry2Ab2. Because the applicant did not demonstrate that Cry2Ab2.820 is biologically identical to Cry2Ab2 the information from the study on the interaction between Cry1A.105, Cry2Ab2.820 and Cry3Bb1 may not be used to conclude that the Cry1A.105, Cry2Ab2 protein, and Cry3Bb1 do not interact. In conclusion, COGEM is of the opinion that the applicant did not sufficiently demonstrate that the Cry1A.105, Cry2Ab2, and Cry3Bb1 proteins do not interact. Therefore, the combination of both Cry1A.105, Cry2Ab2, and Cry3Bb1 proteins should have been used in the laboratory experiments that are carried out with pure proteins instead of testing each protein by itself.

The applicant used several NTOs, namely collembola (*F. candida*), soil microorganisms, earthworm (*Eisenia fetida*), ladybird beetle (*Coleomegilla maculata*), minute pirate bug *(Orius insidiosus)*, honey bee *(Apis mellifera)*, parasitic wasp *(Ichneumon promissorius)*, Carabid beetle (*Poecilus chalcites*) and the bobwhite quail (*C. virginianus*), in laboratory experiments or greenhouse studies. Four of these NTOs, i.e. *C. maculata*, *O. insidiosus, I. promissorius,* and *C. virginianus* do not occur in the European Union. Subspecies of the Carabid ground beetle (*Poecilus chalcites)* do occur in Europe and are therefore a relevant non-target organism for this study according to the applicant. COGEM is of the opinion that NTOs that are relevant to the crop ecosystem in Europe should be used. Therefore, if non-European NTOs are used, the applicant should explain why these organisms are relevant to European maize fields. To facilitate the selection of relevant NTOs, a research report in which guidelines for the selection of NTOs relevant to the North-West European situation were developed, was written in commission of COGEM.[13,14] The guidelines in this report could be used to select NTOs relevant to the European situation.

In laboratory experiments that studied the effect of the Cry2Ab2 protein on minute pirate bugs and parasitic wasps the Cry2Ab2.820 protein was used. As this protein appears to be different from the Cry2Ab2 protein that is present in maize MON89034 x MON88017 and because the applicant did not show that Cry2Ab2.820 is biologically identical to Cry2Ab2, in COGEMs' view the

results obtained with this Cry2Ab2.820 protein cannot be used for conclusions on (the absence of) an effect caused by Cry2Ab2.

Most laboratory experiments were carried out with only three to six replicates with each replicate containing ten to fifty organisms. The number of replicates in combination with the variability within the experiment determines the ability to detect effects accurately. COGEM is of the opinion that an effect that is present should be detected in at least 80% of the cases, therefore experiments should have a statistical power of 0.8 or more. However, information on the statistical power of the experiments is not given and it is therefore unclear how well the experiments are able to detect an effect. If the statistical power of the experiments is below 0.8 the number of replicates should be increased to ensure an accurate detection of any effect that might be present. Different statistical tests have been used without explanation for the chosen method. COGEM is of the opinion that the applicant should clarify why a certain statistical test was chosen. In addition, in some of the experiments the obtained P value is not given. The applicant should give information about the obtained P-values.

In some of the laboratory experiments that used honey bees or ladybird beetles mortality in the control groups exceeded 15%. The applicant did not provide an explanation for the high mortality in some of the control groups. COGEM points out that a high mortality in control groups could indicate problems with the experimental setup and could mask an effect that is present.[15] Preferably, mortality in control groups should not exceed 15%.

Most of the laboratory experiments investigated sublethal effects (behaviour, weight and development to adult) in addition to mortality. Unfortunately, in most cases population growth was not studied. COGEM is of the opinion that it is important to study whether maize MON89034 x MON88017 has sublethal effects on non-target organisms because sublethal effects can affect population size significantly. In a previous advice COGEM stated that she considers measurements of population growth the method of choice when studying whether a genetically modified crop has an adverse effect on non-target organisms, because both mortality and sublethal effects are reflected in this parameter.[16]

Field studies

The applicant refers to a number of field studies with information on field trials. The majority of these studies referred to field trials carried out with other maize lines such as the parental maize lines MON89034 or MON88017 or to MON89034xNK603. Two field studies were carried out with MON89034 x MON88017; one in the US and one in Europe. COGEM considers the data from the US field study less relevant for the European situation.

In Europe, field studies with MON89034 x MON88017 and MON89034 were established in 2007 at 8 sites located in Europe (three sites in Germany, 5 sites in Spain). Each plot used a randomized complete block design with three replications. A plot consisted of 6 rows of approximately 7 meters of length with a planting rate of 7-8 seeds per m. Two rows (corresponding with approximately 100 seeds) were designated for phenotypic and ecological interaction data. Ecological interactions were qualitatively assessed and compared to conventional

maize with similar background genetics. The organisms investigated were aphids, cutworm, wireworms, fruit flies, and the corn borer.
For the study in the US plots were established in 2004 at each of the five production sites in a randomized complete block design with three replications. Each plot consisted of 5 to 6 rows, approximately 20 ft in length, with a planting rate of 35 seeds per row. Plots at each site were qualitatively evaluated for differential response to observed biotic and abiotic ecological stressors. The biotic stressor organisms tested were wireworms, aphids, cutworms, corn borer, armyworm, corn rootworm, flea beetles, grasshoppers and japanese beetles. These organisms are all pest insects and some are target organisms specific for this maize line. Due to the qualitative and subjective nature of the ecological stressor observations, these data were not subjected to a statistical analysis. No overall differences were observed across sites between MON89034 x MON88017 and the control in their susceptibility or tolerance to the ecological stressors assessed. Therefore the investigators concluded that the observed ecological interactions for MON89034 x MON88017 were not altered as a result of the introduction of the combined lepidopteran protection, corn rootworm protection, and herbicide tolerance traits compared to the control.

The organisms tested in both relevant field trials carried out with MON89034 x MON88017 are all pest insects and target organisms and therefore these studies do not provide information to assess the effect of MON89034 x MON88017 on NTO's. COGEM is of the opinion that all relevant ecological groups (i.e. predators, parasitoids, pollinators/nectar feeders, soil organisms and protected/endangered butterflies) should be represented in field trials to test effects on NTOs. Furthermore, the maize lines in the field trials were planted in plots. It is unclear what the number of maize plants in a plot is. On basis of the data presented it cannot be excluded that the number of MON89034 x MON88017 maize plants in the plots is too low to draw legitimate conclusions on the effect of MON89034 x MON88017 on NTOs.

Overall, COGEM is of the opinion that the studies that have been carried out do not provide enough information to endorse the conclusion of the applicant that MON89034 x MON88017 does not adversely affect NTOs.

General surveillance

General surveillance has been introduced to be able to observe unexpected adverse effects of genetically modified crops on the environment. The setting or population in which these effects might occur is either not, or hardly predictable. The central tool for general surveillance in case of cultivation of MON89034 x MON88017 maize is an annual farmers' questionnaire which is addressed to a subset of farmers that cultivate MON89034 x MON88017 maize. In COGEM's view the questionnaire should not only contain questions about the performance of MON89034 x MON88017 maize on the field, but should also contain questions about unexpected effects of the MON89034 x MON88017 maize on the whole of the farmers' premises. COGEM is also of the opinion that the part of the farm questionnaire dealing with animals is too general. Birds, deer and insects are assigned to one category 'wildlife'. Information about the occurrence of wildlife should be obtained by different questions for specific groups of organisms (e.g. mammals,

(predatory) birds, and insects). In addition, the farmer should be asked whether unusual quantities of other animals were observed and whether dead animals were found. The questions in the farm questionnaire refer to the usual situation, but the usual situation is not well defined. It would be better to rephrase the questions to acquire data that can be used to detect negative or positive trends in populations of organisms relevant to the monitoring scheme.

Advice

The present application concerns the cultivation of the genetically modified maize line MON89034 x MON88017. This maize line expresses the genes cry1A.105, cry2Ab2, cry3Bb1 and cp4 epsps conferring resistance to certain lepidopteran and coleopteran insects and tolerance to glyphosate containing herbicides. In the past, COGEM advised positively on the import of maize line MON89034 x MON8817.

There are no wild relatives of maize in Europe and the appearance of volunteers is rare. Furthermore, there are no reasons to assume that the inserted traits will increase the now absent potential of the maize line to establish feral populations. COGEM is of the opinion that the molecular characterization is adequate.

The applicant conducted several laboratory and field studies and refers to these studies with regard to the absence of potential adverse effects of MON89034 x MON88017 on non-target organisms (NTOs).

None of the laboratory experiments have been carried out with MON89034 x MON88017. Some laboratory studies were carried out with the single lines or with similar lines expressing similar but not identical cry genes, for example MON863 which expresses a Cry3Bb1 protein which differs by one amino acid from Cry3Bb1 in MON88017. In most cases either Cry1Ab.105, Cry2Ab2 or Cry3Bb1 pure protein was used. COGEM is of the opinion that the applicant did not sufficiently demonstrate that the Cry1A.105, Cry2Ab2 and Cry3Bb1 proteins do not interact. In addition, four out of nine of the studied NTOs do not occur in the European Union and the applicant did not explain why these organisms are relevant to European maize fields. Furthermore, although the applicant did not show that Cry2Ab2.820 is biologically identical to Cry2Ab2 some of the experiments have been carried out with Cry2Ab2.820. In addition, the laboratory experiments also exhibit some other shortcomings: the statistical power of the experiments, the choice for a certain statistical test has not been explained and the applicant did not provide an explanation for the high mortality (over 15%) in certain control groups. Instead of providing an extensive list of all experiments that have been done with Monsanto GM corn, it would be recommendable if the applicant limits itself to the cultivars that are relevant for this application, which is in this case MON89034 x MON88017 and the separate parental lines. When representing results of studies in a table format COGEM prefers to see P-values and denote the size of the effect observed and whether it was positive or negative instead of an effect described as "non significant".

The applicant presented two studies that describe MON89034 x MON88017 field trials. These field trials were carried out in the USA and in Europe. However, both field trials studied the effect of MON89034 x MON88017 on target organisms instead of non-target organisms. COGEM is of

the opinion that the data provided are not sufficient to conclude that cultivation of MON89034 x MON88017 exerts negligible adverse effects on NTOs. Furthermore, the General Surveillance plan could be improved on several points.

Conclusion

COGEM is of the opinion that she cannot adequately perform a risk analysis with regard to the cultivation of MON89034 x MON88017. As a result of the concerns mentioned, COGEM currently cannot issue a positive advice on the cultivation of maize line MON89034 x MON88017.

COGEM is of the opinion that additional data should be submitted from laboratory experiments and field studies that study the effect of MON89034 x MON88017 maize on NTOs, from all relevant ecological groups (i.e. predators, parasitoids, pollinators/nectar feeders, soil organisms and protected/endangered butterflies) which are relevant to European maize fields.

Preferably, the additional data on laboratory experiments should refer to experiments with maize line MON89034xMON88017. If pure proteins were used in the laboratory experiments the applicant should demonstrate that the Cry1A.105, Cry2Ab2 and Cry3Bb1 proteins do not interact or the three Cry proteins should have been used in combination. Furthermore, if pure proteins were used the Cry proteins that are present in maize MON89034 x MON88017 should have been studied; in case other proteins were used the applicant should demonstrate that the studied Cry protein (e.g. Cry2Ab2.820 or Cry3Bb1 from MON863) is biologically identical to the Cry protein that is present in the maize line for which an application is submitted (e.g. Cry2Ab2 or Cry3Bb1).

The additional data on laboratory experiments should refer to studies with NTOs that are relevant to the crop ecosystem in Europe. If non-European organisms were used, the applicant should explain why these organisms are relevant to European maize fields. In addition, the additional data should refer to laboratory experiments with a statistical power of 0.8 or more, and the obtained P-values and an explanation for the statistical test should be presented.

Most importantly, additional data on field trials that were carried out in Europe with maize MON89034xMON88017 should be provided. COGEM considers the data from US field studies less relevant for the European situation. The additional data should refer to European field trials that study the effect of MON89034 x MON8807 on NTOs. In these field trials all relevant ecological groups (i.e. predators, parasitoids, pollinators/nectar feeders, soil organisms and protected/endangered butterflies) should be represented.

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