COMMISSIE
COGEM
GENETISCHE
MODIFICATIE

Aan de minister van
Volkshuisvesting, Ruimtelijke
Ordening en Milieubeheer
Mevrouw dr. J.M. Cramer
Postbus 30945
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DATUM 14 april 2008

KENMERK CGM/080414-01

ONDERWERP Renewal application cultivation of genetically modified maize MON810

Geachte mevrouw Cramer,

Naar aanleiding van een adviesvraag betreffende de hernieuwing van de vergunning voor de teelt van genetisch gemodificeerde maïs MON810 van Monsanto Company, deelt de COGEM u het volgende mee.

Samenvatting

De COGEM is gevraagd te adviseren over een hernieuwing van de vergunning voor de teelt van de genetisch gemodificeerde maïslijn MON810. In deze maïslijn komt het *cry1Ab* gen tot expressie waardoor de maïslijn resistent is tegen onder meer de Europese maïsboorder. In 1996 heeft de COGEM een positief advies gegeven voor deze maïslijn en sinds 1998 is MON810 toegelaten voor teelt in Europa. In 2003 werd MON810 voor het eerst geteeld in Europa.

Onlangs werd de COGEM gevraagd te adviseren naar aanleiding van het verschijnen van een Frans rapport waarin wordt geconcludeerd dat nieuwe gegevens vragen oproepen over het bestaan van mogelijke risico's voor mens en milieu bij het telen van MON810. De COGEM heeft deze gegevens bestudeerd en kwam tot de conclusie dat de risico's verbonden aan de teelt van MON810 verwaarloosbaar klein zijn en dat zij geen redenen ziet de vergunning voor teelt in te trekken.

De aanvrager heeft voor de hernieuwingsaanvraag het MON810 dossier op een aantal punten aangevuld in overeenstemming met de geldende Europese richtlijnen. Deze gegevens zijn door de COGEM uitvoerig bestudeerd. Daarnaast heeft de aanvrager een aantal recente monitoringsrapporten aangeleverd uit Europese landen waar MON810 is geteeld.

Op basis van deze gegevens en de overwegingen die naar voren zijn gebracht in dit advies, komt de COGEM tot de conclusie dat de risico's voor mens en milieu bij de teelt van maïslijn MON810 verwaarloosbaar klein zijn. De COGEM ziet daarom geen redenen om de vergunning voor teelt van MON810 niet te verlengen.

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
Dr. I. van der Leij

Renewal application cultivation of genetically modified maize MON810
COGEM advcice CGM/080414-01

Summary

This notification concerns the renewal of the authorization for continued cultivation of genetically modified maize line MON810. Maize line MON810 contains the cry1Ab gene causing the plant to be resistant to certain lepidopteran insects such as the European corn borer (Ostrinia nubilalis).

Previously, COGEM advised positively on the import as well as the cultivation of maize line MON810. In 1996 COGEM gave a positive recommendation on the market authorisation of this line. MON810 was authorised by the United States in 1996 and has been approved for import and cultivation in Europe since 1998. MON810 was first commercially cultivated in Europe in 2003. In 2006 this maize line was cultivated in six European countries (Spain, France, Czech Republic, Germany, Portugal and Slovakia). COGEM also advised positively on the import of hybrid maize lines with MON810 such as NK603 x MON810 and MON88017 x MON810.

Recently, COGEM was requested to advice on the report 'Projet d'avis sur la dissemination du MON810 sur le territoire français' by the French Comité de préfiguration d'une haute autorité sur les organismes génétiquement modifiés. This report concludes that new facts about the genetically modified maize line MON810 raise questions about the consequences of MON810 for human health and the environment. In reaction to the French report, COGEM analysed the data and concluded that risks associated with the cultivation of MON810 are negligible. Consequently, COGEM assessed there is no reason to rescind the authorisation of MON810.

In this renewal application, the MON810 dossier has been updated to comply with the current European guidelines. The applicant provided amongst others a more up-to-date molecular characterisation and series of monitoring reports which have been composed over the years MON810 has been cultivated in Europe.

In general, no wild relatives of maize are present in the Netherlands 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 Dutch conditions.

COGEM is of the opinion that the molecular characterization of MON810 was adequate and that that the risk for human health or the environment with the modification of maize MON810 is negligible. Furthermore, based on the available scientific literature, COGEM is of the opinion that cultivation of maize MON810 poses negligible risks to non-target organisms.

Based on the history of safe use of maize line MON810 and the considerations put forward in this advice, COGEM is of the opinion that the cultivation of maize line MON810 poses a negligible risk the environment. Therefore, COGEM is of the opinion that the authorization for cultivation of MON810 can be renewed.

1. Introduction

This notification concerns the renewal of the authorization for cultivation of genetically modified maize line MON810. This maize line contains the *cry1Ab* gene causing the plant to be resistant to certain lepidopteran insects such as the European corn borer (*Ostrinia nubilalis*).

2. History genetically modified maize MON810

MON810 is a genetically modified maize line which is resistant to the European corn borer. In 1996 COGEM gave a positive recommendation on the market authorisation of this line[1]. MON810 was authorised by the United States in 1996 and has been approved for import and cultivation in Europe since 1998. MON810 was first commercially cultivated in Europe in 2003. In 2006 this maize line was cultivated in six EU countries (Spain, France, Czech Republic, Germany, Portugal and Slovakia). The largest area planted with MON810 (50,000 hectares) was in Spain[2]. COGEM also advised positively on the import of hybrid maize lines with MON810 such as NK603 x MON810 and MON88017 x MON810.

Since the introduction of MON810 numerous publications have appeared on research into the environmental safety of this maize line, some of which provoked objections on the authorization of this maize line. In 2005, a number of European member states invoked the safeguard clause on amongst others MON810[3]. This safeguard clause provides that, where a Member State has justifiable reasons to consider that a genetically modified organism which has received consent for placing on the market, constitutes a risk to human health or the environment, it may provisionally restrict or prohibit the use and/or sale of that GMO on its territory. The EFSA's Scientific Panel on Genetically Modified Organisms (GMO panel) published a report in reaction to the specific questions posed by the Member States on this maize line. In this report, the GMO panel affirmed its conclusions with respect to the potential impact of Cry1Ab on biodiversity, that MON810 maize is unlikely to have adverse effects on human and animal health or the environment.

In 2006, Greece invoked the safeguard clause to provisionally prohibit the cultivation of the authorized genetically modified maize MON810 on its territory. The EFSA investigated the evidence presented in the Greek submission. The EFSA's GMO Panel concluded that, in terms of risk to human health and the environment, no new scientific evidence was presented that would invalidate the risk assessment of genetically modified maize MON810[4].

More recently, COGEM was requested to advice on the report 'Projet d'avis sur la dissemination du MON810 sur le territoire français' by the French *Comité de*

préfiguration d'une haute autorité sur les organismes génétiquement modifiés. This report concludes that new facts about the genetically modified maize line MON810 raise questions about the consequences for humans and the environment of using MON810[5]. COGEM was asked whether the French report gives cause to revise its opinion on MON810. In reaction to the French report, COGEM analysed the data presented in the report and concluded on this basis that any risks associated with the cultivation of MON810 are negligible. Consequently, COGEM sees no reason to rescind the authorisation of MON810[6].

Another French report was issued in reaction to the analysis of the French *Comité de préfiguration d'une haute autorité sur les organismes génétiquement modifiés*. This report was composed by Bergé and Ricroch[7]. In their opinion, based on the extensive amount of research (around 7000 publications) done over the years on the environmental impact of maize MON810; there are no sound scientific reasons to expect any adverse effects of this maize event on human health or the environment. Therefore, it was concluded that an intended ban on maize MON810 is not justified by the information presented in scientific literature on this maize event.

In March of this year, Monsanto lost an appeal of the French ban on modified corn at France's highest court. A few days later, Romania also announced to put a ban on the cultivation and import of MON810[8].

The renewal application for the continued cultivation of genetically modified maize MON810 is now being assessed by the European member States, including the Netherlands. Therefore, COGEM was asked to issue an advice on this application.

3. 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[9,10]. According to literature, pollen viability varies between 30 minutes and 9 days[10,11,12]. 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[9]. Establishment of maize plants in the wild has never been observed in the Netherlands or other European countries.

4. Molecular characterization

Maize line MON810 was produced using particle acceleration transformation. An overview of the introduced sequences is given below:

4.1 Components of the insert in MON810

- e35S, promoter, originating from the Cauliflower mosaic virus
- Zmhsp70 gene, stabilizes the level of gene transcription
- Cry1Ab, DNA sequence originating from *Bacillus thuringiensis* encoding for a Cry1Ab protein.

4.2 Properties of the introduced genes conferring insect resistance

MON810 was genetically modified with the *cry1Ab* gene derived from *B. thuringiensis* (subsp. *kurstaki*). The produced Cry1Ab, a δ-endotoxin, is lethal to insects of the lepidopteran order, including larvae of the European corn borer (*O. nubilalis*) and the pink borer (*Sesamia cretica*). The δ-endotoxin selectively binds to receptors located in the midgut of susceptible insects. Following binding, 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[13, 14].

The larvae of the European corn borer cause severe damage to corn crops by feeding on the stalks and creating boreholes. This results in weakened plants, eventually causing the plant to fall over. The damaged plants are also more susceptible to molds and rot. Furthermore, larvae can feed on the kernel causing a reduction of grain quality. The European corn borer is a pest insect in the United States and Canada. In the Netherlands, this insect species is not of agronomic interest because the crop consists mainly of fodder maize. Together with the fodder maize, the pupae of the corn borer are chopped during harvesting. Consequently, the corn borer population is not able to establish itself. In addition, the climate in the Netherlands is not optimal for the European corn borer.

4.3 Molecular analysis

The molecular characterization of maize line MON810 has previously been assessed by COGEM when the application was initially presented in 1996. In a report by Kania *et al.* (1995)[15] provided with the initial application it was stated that MON810 contained the e35S promoter, the Hsp70 intron and a *cry1Ab* coding sequence sufficient to encode an insecticidally active Cry1Ab protein. Furthermore, it was noted that no backbone sequences from plasmids were detected in the genome of MON810.

A few years later, additional experiments by Borokov *et al* (2001), Rigden *et al*. (2003) and Hernandez *et al* (2003)[16, 17, 18] reported that the MON810 insert contains a fragment of the 3'end of the e35S promoter and a truncated *cry1Ab* gene. Furthermore, it was concluded that the 3'maize genome junction region showed no homology with known sequences. Data also confirmed that no backbone sequences from plasmid PV-ZMBK07 or PV-ZMGT10 were present in MON810.

In the renewal application the insert in MON810 was recharacterized using more sensitive methods. Data analyzed by Scanlon *et al.* (2007)[19] in the renewal application indicate that MON810 contains a single copy, consisting of the e35S promoter, Hsp70 intron, and the *cry1Ab* coding sequence. Additional experiments showed that only 307 bp of the 3' end of the e35S promoter is present compared to the original e35S promoter, 314 bp at the 5' end of the s35S is absent. The *cry1Ab* sequence is 2448 bp long sufficient to encode an insecticidally active Cry1Ab protein. MON810 does not contain the T-NOS transcriptional termination sequence present in the original expression cassette, which was lost during the insertion together with the 3'end of the *cry1Ab* gene. The molecular characterization by Scanlon *et al*. confirmed once more that no backbone sequences from the plasmids used in the transformation were present in MON810. According to the applicant, PCR and DNA sequence analyses thus confirmed the data previously reported in Kania *et al* (1995). Furthermore, the applicant provides more sequence data on the 5'and 3' region flanking the insert in MON810: 1109 bp of the 5' flanking region and 464 bp of the 3' region.

In a recent article, Rosati *et al* (2008)[20] describes a more extensive molecular analysis of the 3' end of the transgene insert of MON810is. They confirmed the absence of the complete NOS terminator sequence and part of the 3' end of the *cry1Ab* gene. The *cry1Ab* gene is 2448 bp long lacking a translation stop codon and genuine transcription termination signals. Bioinformatics indicates that the 3' flanking sequence of the MON810 insert is homologous to a HECT E3 ubiquitin ligase gene from *Oryza sativa*.
A comparison with a maize BAC clone, containing the putative HECT E3 ubiquitin ligase gene, indicates that the MON810 transgene cassette is inserted at the end of exon 8 of the HECT gene. Relative to the transcription of the HECT gene, transcription of the *cry1Ab* gene occurs in opposite orientation.

Further experiments were conducted to investigate the expression of the *cry1Ab* insert. cDNA was made from RNA from MON810 and PCR analysis using forward primers on the *cry1Ab* gene and reverse primers on the genomic region downstream of the 3' inert resulted in the identification of fragments with different lengths. To identify the origin of

these fragments, sequence analysis was performed. This indicated that chimeric transcripts are made consisting of *cy1Ab* sequences and antisense sequences of the HECT gene (exon 8). Several of the RNAs contained deletions compared to the genomic sequence, suggesting splicing. Rosati *et al*. (2008) reported that translation of an RNA from the truncated *cry1Ab*-antisense HECT fusion gene results in a protein that contains extra 2 amino in addition to the Cry1Ab protein. This is also mentioned in the renewal application and reported by Scanlon *et al* (2007). In addition, Rosati *et al*. also reported a possible fusion protein which contains 18 additional amino acids to the *cry1Ab* part. The mRNA giving rise to this putative fusion protein originates most likely from alternative splicing. In silico analysis of the 3' flanking region of the *cry1Ab* insert, using the NetPlantGene server for splice site prediction, indicates that it contains several putative splice sites. However, bioinformatics showed that translation of the *cryAb1* RNAs does not give rise to fusion proteins with significant homology to known protein domains.

It showed to be impossible to amplify sequences from the insertion locus in non-transgenic maize using primers from the 5'end and 3'flanking sequence of the MON810 maize insert. Therefore, the integration of the MON810 insert has probably caused DNA rearrangements or insertion of additional DNA. However, bioinformatics reveals no matches of putative fusion proteins encoded by the junction between the e35S promoter and the 5' flanking sequence with known allergens or toxins.

COGEM is of the opinion that based on the data published in recent articles and data presented the renewal application of MON810, the insertion of the *cry1Ab* insert involved two truncation events leading to partial loss of the e35S promoter and part of the *cry1Ab* sequence (including the entire NOS terminator). Furthermore, during the insertion DNA rearrangements have occurred. However, the observed truncations and rearrangements are unlikely to have given rise to fusion proteins with adverse effects for human health or the environment. Nor does the loss of genomic sequences of the *cry1Ab* fragment interfere with the activity of the Cry1Ab protein and the vigor and yield of this maize event. Therefore, COGEM is of the opinion that the molecular characterization of MON810 was adequate and that the risk for human health and the environment as a consequence of the modification of maize MON810 is negligible.

5. Environmental risk assessment

Since the introduction of MON810 numerous publications have appeared on research into the environmental safety of this maize line[7]. In reaction to the published report by the French *Comité de préfiguration d'une haute autorité sur les organismes génétiquement modifiés* titled 'Projet d'avis sur la dissemination du MON810 sur le territoire français'

COGEM prepared a response to the French report on the 1st of February 2008. Additionally to this reaction COGEM planned to produce a more extensive response at a later date. This additional assessment is included below and will examine literature on possible adverse affects of MON810 on non-target organisms.

MON810 expresses the Cry1Ab δ-endotoxin (Bt toxin), which is specific to lepidopteran insects, such as the European corn borer (*O. nubilalis*), the Mediterranean corn borer (*Sesamia nonagrioides*) and the fall armyworm (*Spodoptera frugiperda*). Several studies have been performed to assess possible adverse effects of maize lines that express Cry1Ab, such as MON810, Bt11 and Bt176, on non-target organisms.

Non-target organisms might be exposed to Cry1Ab when Cry1Ab maize is incorporated in soil or when feeding on maize tissues. In addition, lepidopteran larvae may eat maize pollen if it is deposited on their host plants. Predators and parasitoids may be exposed to Cry1Ab when their hosts have fed on Cry1Ab maize. Studies that have examined adverse effects of Cry1Ab maize on these non-target organisms are discussed.

5.1 Effects of Cry1Ab on soil organisms

Soil organisms may be exposed to Cry1Ab when maize residues are left on the field, degrade and enter the soil. Cry1Ab proteins are degraded, but a fraction of the Cry1Ab may persist in the soil[21,22]. The concentration of Cry1Ab in soil samples collected during the growing period and after harvesting was below the biological active concentration[23].

Field studies carried out to study the effect of Cry1Ab maize on the number of protozoa, nematodes, and the bacterial community observed no[22,25] or a transient effect[24]. Several laboratory studies have examined the effect of Cry1Ab maize on fungi. Cry1Ab maize did not have an effect on the number of fungi[25]. In addition, the mycorrhizal colonization of Cry1Ab MON810 maize roots and the mycorrhizal infectivity of Cry1Ab MON810 rhizosphere soil was similar to that of a near-isogenic maize line[26]. However, a lower level of mycorrhizal colonization (*Glomus mosseae*) of Cry1Ab Bt176 maize roots, but not of Cry1Ab Bt11 maize roots was reported and a lower level of mycorrhizal colonization of soil containing Cry1Ab Bt11 maize residues, but not Cry1Ab Bt176 maize residues was observed [27]. No consistent effect on mycorrhizae was observed in the Cry1Ab maize lines. Therefore, COGEM is of the opinion that the observed effects are not caused by the Cry1Ab, but are probably due to differences in plant compound composition.

In laboratory experiments Cry1Ab maize did not have an adverse effect on mortality or development of earthworms *Lumbricus terrestris*[25,28] and *Aporrectodea caliginosa*[29]. However, sublethal effects on weight of *L. terrestris* adults[28] and a decreased hatching success for *A. caliginosa*[29] were observed. The authors suggested that the observed differences may be due to differences in plant compound composition[28] or questioned the ecological significance of their effects under field conditions[29] .

During laboratory experiments that were carried out to assess the effect of Cry1Ab maize on soil arthropods no negative effects were observed on collembola[26,30], woodlice (*Porcellio scaber*)[31] or on the soil mite population[26,32]. In addition, during a field experiment the effect of Cry1Ab maize on the abundance of mites and collembolans was assessed. Although some adverse effects were detected, these effects were small and comparable to changes observed between different maize varieties[33]. Another field study assessed the decomposition process of Cry1Ab maize and identified organisms that were present in the soil. Collembolans (Isotomidae, Tullbergiidae, Entomobryidae), mites (Gamasina, Oribatida) and anellids (Enchytraeidae) were extracted most frequently. Although at certain time points during the experiment significantly less individuals were extracted from Cry1Ab maize litter for two of these groups (Tullbergiidae and Enchytraeidae) at the end date of the experiment no significant differences were observed[34]. Any effect of Cry1Ab maize on the soil arthropod community is therefore a transient effect.

Overall, it can be concluded that any effect of Cry1Ab maize on soil organisms was significantly lower than the effect of crop type, and land management practices[24].

5.2 Effects of Cry1Ab on non-target arthropods

Non-target arthropods may ingest Cry1Ab when feeding on Cry1Ab maize. The non-target arthropods that were collected from Cry1Ab maize fields possessed varying amounts of Cry1Ab. Negligible amounts of Cry1Ab were found in aphids, thrips and leafhoppers[35]. In field trials, the number of aphids (*Metopolophium dirhodum*, *Rhopalosiphum padi* and *Sitobion avenae*) and thrips varied between different time points, but no adverse effect of Cry1Ab maize was observed[36].

Other non-target arthropods, such as spider mites (*Tetranychus urticae*) did contain high levels of Cry1Ab[35], but are unaffected by Cry1Ab[37]. Possible effects on higher trophic levels are discussed in the following paragraph. The amount of Cry1Ab present in non-target arthropods reflects their feeding behaviour. Adults of a chrysomelid beetle (*Oulema melanopus*) feed on maize from late May to late June, but they become less active and stop feeding later in the season. Considerable concentrations of Cry1Ab were detected at the end of June, but no Cry1Ab was detected at later sampling dates. The Cry1Ab was detected in the omnivorous mirids (*Trigonotylus* spp.) collected from Cry1Ab maize fields. Apparently, mirids substantially fed on Cry1Ab maize leaves and possibly on pollen. Although Cry1Ab is detected in these non-target organisms, there are no indications that they are negatively influenced by Cry1Ab.

5.3 Effects of Cry1Ab on predators

The presence of Cry1Ab in these non-target arthropods indicates that their predators may be exposed to Cry1Ab. The level in which they are exposed to Cry1Ab depends on their food sources and reflects the presence of Cry1Ab in these food sources. Some arthropod

predators (hemerobiids, *Nabis* sp., *Hippodamia* sp. and *Demetiras* sp.) that were collected in Cry1Ab maize fields contained no or negligible concentrations of Cry1Ab. Other predators (*Orius* spp, *Chrysoperla* spp. and *Stethorus* sp.) contained Cry1Ab when maize pollen or spider mites were available[35]. The level of Cry1Ab was highest in *Stethorus punctillum*, a specialist predator that feeds exclusively on spider mites, a herbivore which is not affected by Cry1Ab[37]. The field study also showed that *Chrysoperla* spp. is exposed to Cry1Ab[35]. However, results from other studies show that Cry1Ab is not directly toxic to the green lacewing (*Chrysoperla carnea*)[37,38]. Similarily to the green lacewing, field studies show that *Orius* spp. is exposed to Cry1Ab[35], but the Cry1Ab does not affect development, fecundity and survival of *Orius albidipennis*[39].

In addition, a field study carried out to assess the abundance of predators (Anthocoridae, Araneae, Carabidae and Coccinellidae) showed that the number of predators varied between years and between locations, but the abundance of predators in Cry1Ab maize fields was similar to the abundance of predators found in maize fields of the near-isogenic maize line[40]. These results confirm the results of another field experiment, in which no difference was observed between the number of predators, such as the bug *Orius insidiosus*, the syrphid *Syrphus corollae*, the ladybird *Coccinella septempunctata,* and the green lacewing *C. carnea*[36].

To summarize, although predators are often exposed to Cry1Ab, there are no indications that their abundance is influenced by the presence of Cry1Ab maize. However, if predators feed on target organisms of the Cry1Ab maize, e.g. on the European corn borer, they may be influenced by the reduced quality of the target organisms[37] or by a reduced presence of these target organisms.

5.4 Effects of Cry1Ab on parasitoids

Parasitoids may be exposed to Cry1Ab through their hosts. It is unlikely that Cry1Ab affects these parasitoids directly. However, if parasitoids parasitize the target organisms of Cry1Ab maize, they may be influenced by the quality and size of the target organisms or by a decreasing density of these target organisms. Studies have shown that parasitoids such as *Campoletis sonorensis* may be influenced negatively due to a reduced host quality[41,42]. In addition, the percentage of European corn borer larvae parasitized by tachinids (*Lydella thompsoni* and *Pseudoperichaeta nigrolineata*) was lower in Cry1Ab maize fields[36].

Although the number of parasitoid may be negatively influenced if they parasitize the target organisms of Cry1Ab maize, parasitoids are usually not confined to a single host species. Therefore it is unlikely that their persistence is threatened by the cultivation of Cry1Ab maize. Interestingly, Cry1Ab maize has been reported to positively affect *Cotesia marginiventris*, a parasitoid of lepidopteran pests[43].

5.5 Effects of Cry1Ab on butterflies

During pollen shed, pollen of Cry1Ab maize plants may be deposited on other plants in the margins of the maize fields. If pollen is deposited on their host plants, lepidopteran larvae may consume the maize pollen.

Laboratory and semi-field trials showed that Cry1Ab maize had a negative impact on development and survival of monarch butterfly larvae (*Danaus plexippus*)[44]. However, results observed during laboratory experiments cannot be directly extrapolated to field conditions. The amount of pollen to which lepidopteran larvae are exposed and the developmental stage in which they are exposed are important to determine whether adverse effects may occur under field conditions. The risk of Cry1Ab maize pollen of current varieties, such as MON810, on the monarch butterfly was determined to be negligible when the distribution, the overlap between maize pollen shed and the development of larvae, and the exposure to Cry1Ab maize pollen were considered[45]. Results from a semi-field study showed that Cry1Ab maize pollen of MON810 did not affect mortality or larval mass of the black swallowtail (*Papilio polyxenes*)[46]. In addition, the effect of Cry1Ab maize pollen of MON810 was assessed in German field trials. The number of the two most abundant lepidopteran species (*Plutella xylostella* and *Pieris rapae*) was similar in margins of MON810 maize fields when compared to margins of the near-isogenic maize fields[47]. These results indicate that pollen of current Cry1Ab maize varieties, such as MON810 do not negatively affect butterfly species.

5.6 Effects of Cry1Ab on honeybees

Honeybees may be exposed to Cry1Ab if they feed on pollen from Cry1Ab maize. Larvae are fed royal jelly, which is produced in the hypopharyngeal glands of nursing bees. Older larvae consume royal jelly and small amounts of pollen, while working bees of the nursing age consume large quantities of pollen not only for their own requirements, but also to produce royal jelly[48].

No Cry1Ab was present in the hypopharyngeal glands of honeybees that were fed Cry1Ab pollen and only traces of Cry1Ab were detected in the hypopharyngeal glands of honeybees which were fed a sugar solution to which Cry1Ab had been added[48]. Royal jelly, which is fed to the larvae, is produced in the hypopharyngeal glands. Therefore, the authors of this study concluded that larval stages of the honeybee are far less exposed to Cry1Ab than adults.

In addition, when honeybees were fed Cry1Ab pollen or a sugar solution to which Cry1Ab had been added survival and the development of the hypopharyngeal glands were not affected[48]. These results show that honeybees are not negatively influenced when exposed to Cry1Ab or Cry1Ab maize.

5.7 Effects of Cry1Ab on aquatic organisms

Aquatic organisms may be exposed to Cry1Ab when Cry1Ab maize residues enter streams. A laboratory study was set up to assess the effect of Cry1Ab maize on caddisflies (*Lepidostoma liba* and *Helicopsyche borealis*)[49]. No effect on mortality was observed when leaf-shredding caddisflies (*L. liba*) were fed Cry1Ab maize leaves. However, a reduction in growth rate of leaf-shredding caddisflies was detected. In addition, mortality of algal-scraping caddisflies (*H. borealis*) was higher when caddisflies were fed pollen at concentrations that were two to three times higher than the observed maximum aerial input rate[49]. It is unclear whether caddisflies are exposed to similar amounts of Cry1Ab in natural situations. There are no publications which report effects under natural conditions. However, in an abstract presented at the congress of the North American Benthological Society the authors of the above-mentioned study report that no significant adverse effects were observed in the field[50]. These data have not yet been published in a scientific journal and are therefore not verifiable.

Furthermore, as the results of the above-described laboratory study have been described in a recent publication, no experiments have been reported that confirm or repudiate these data. The experimental set up of the laboratory study has been questioned by other researchers[51,52], because the amount of Cry1Ab to which the caddisflies were exposed was not quantified and because improper controls were used.

On basis of the available data, COGEM is of the opinion that currently there are no reasons to assume that the risk of Cry1Ab maize to aquatic organisms is not negligible, although this cannot be fully excluded.

Since the introduction of MON810 numerous publications have appeared on research into the environmental safety of this maize line[7]. COGEM is of the opinion that none of these publications indicate that MON810 poses a risk to humans and the environment. In addition, publications on the possible effects of the Cry1Ab do not show that this toxin poses a risk to humans and the environment. Monitoring reports on the cultivation of MON810 or other maize lines that produce the Cry1Ab do not prove that the cultivation of these crops will lead to adverse effects.

Overall, it can be concluded that Cry1Ab maize does not negatively affect non-target organisms. However, as the target organisms of Cry1Ab maize, such as the European corn borer are affected by Cry1Ab maize, predators or parasitoids of these target organisms may be indirectly affected.

Furthermore, a number of farmers have been requested to participate in environmental surveys through questionnaires of which the results were presented in the monitoring reports provided with the application. The applicant provided monitoring reports which have been composed over the recent years MON810 has been cultivated in respectively Spain (2003 and 2004), Czech Republic, France, Germany, Portugal and Spain (2005), Czech Republic, France, Germany, Portugal, Slovakia and Spain (2006). No adverse

effects on human or animal health or the environment as a result of cultivation or handling MON810 were reported. Additionally, there were no indications of resistance to Cry1Ab in field populations of *O. nubilalis* and *S. nonagrioides*.

6. Post-market monitoring plan / general surveillance

Since there were no reasons to expect any adverse effects on human health or the environment with the cultivation of MON810, case specific monitoring was not considered necessary by the applicant. A general surveillance plan was set up to report any unexpected adverse effects of MON810.

To obtain a permission to cultivate genetically modified maize, a monitoring plan considering the environmental impact of cultivation is required. The applicant has updated the general surveillance plan for MON810 maize to comply with the current European guidelines in order to detect any unanticipated adverse effects on human health and the environment. Key stakeholders and key networks were requested to inform the applicant in case of potential occurrence of any unanticipated adverse effects to health or the environment. Furthermore, general surveillance will take place trough farm questionnaires provided by the applicant to the growers and other users of MON810 maize.

COGEM is of the opinion that the monitoring plan provided by the applicant is sufficient to observe and register possible adverse effects of maize MON810, although not expected, as soon as possible.

Furthermore, the applicant is of the opinion that the party placing the GM plant on the market will primarily consider general surveillance in the areas where that specific GM plant is grown and monitor for any adverse effects of its cultivation at farm level. However; surveillance for adverse impacts of GM plants at regional and/or national levels is beyond the scope of farm monitoring or the direct capability of the party placing the GM plant on the market. Therefore, the general surveillance at this level is considered to be a national / European responsibility by the applicant.

In an advice previously issued by COGEM, the involvement of existing networks in post market monitoring of GM crops was already mentioned[53]. Therefore, COGEM agrees with this point of view and underlines the importance of using existing monitoring systems in other countries in general surveillance and including these monitoring systems in general surveillance. The Netherlands is in the process of implementing such a system including existing monitoring systems in general surveillance.

7. Advice

This notification concerns the renewal of the authorization for continued cultivation of genetically modified maize line MON810. This maize line contains the *cry1Ab* gene causing the plant to be resistant to certain lepidopteran insects such as the European corn borer (*O. nubilalis*). In 1996 COGEM gave a positive recommendation on the market authorisation of this line. MON810 was authorised by the United States in 1996 and has been approved for import and cultivation in Europe since 1998. MON810 was first commercially cultivated in Europe in 2003 and has a history of safe use.

In this renewal application the applicant updated the MON810 dossier with more detailed information regarding amongst others the molecular analysis and monitoring plan. Furthermore, a series of monitoring reports gathered over the years MON810 was cultivated, were provided.

In the renewal application the insert in MON810 was recharacterized by molecular analyses using more sensitive methods to assess the insert number, copy number, integrity of the inserted elements and absence of plasmid backbone sequences. COGEM is of the opinion that the molecular characterization of MON810 was adequate and that the risk for human health and the environment as a consequence of the modification of maize MON810 is negligible. Furthermore, a long history of safe use and the absence of adverse effects in feed and toxicity studies confirm that no harmful effects caused by incidental consumption of MON810 maize can be expected. Additionally, the provided monitoring reports confirm the safe use of maize event MON810.

Furthermore, based on the available scientific literature, COGEM is of the opinion that cultivation of maize MON810 poses negligible risks to non-target organisms.

In view of the considerations put forward in this advice, COGEM is of the opinion that the cultivation of genetically modified maize line MON810 poses a negligible risk to the environment. Therefore, COGEM is of the opinion that the authorization for cultivation of MON810 can be renewed.

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