Bioveiligheidsraad
Conseil de Biosécurité

Secretariaat
Secrétariat

17-11-2009

O./ref.: WIV-ISP/15/BAC/2009_01510[1]

Title: Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/RX-MON810 from Monsanto under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/RX-MON810 was submitted by Monsanto on 29 June 2007 for the renewal of authorisation of the insect resistant genetically modified (GM) maize MON810 for food and feed applications, including cultivation under Regulation (EC) No. 1829/2003[2].

The application EFSA/GMO/RX-MON810 was officially acknowledged by EFSA on 29 January 2008. On the same date EFSA started the formal three-month consultation of the Member States, in accordance with Articles 6.4 and 18.4 of Regulation (EC) No. 1829/2003 (consultation of national Competent Authorities within the meaning of Directive 2001/18/EC designated by each Member State in the case of genetically modified organisms (GMOs) being part of the products).

Within the framework of this consultation, the Belgian Biosafety Advisory Council, under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts chosen from the common list of experts drawn up by the Biosafety Advisory Council and the Division of Biosafety and Biotechnology (SBB) to evaluate the dossier. Nine experts answered positively to this request and formulated a number of comments to the dossier, which were edited by the coordinator. See Annex I for an overview of all the comments and for the list of comments actually placed on the EFSAnet on 13 May 2008.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 15 June 2009 (The EFSA Journal, 2009, 1149, 1-84)[3], and published together with the responses of the EFSA GMO Panel to comments submitted by the experts during the three-month consultation period.

On 2 July 2009 the opinion of EFSA was forwarded to the Belgian experts. They were invited to give comments and to react if needed to the answers given by the EFSA GMO Panel, in particular in case the comments formulated in their initial assessment of the dossier were not taken into account in the opinion of EFSA.

The comments formulated by the experts together with the opinion of EFSA including the answers of the EFSA GMO Panel form the basis of the advice of the Biosafety Advisory Council given below.

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[1] revised version of document BAC_2009_1490
[2] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. (OJ L 268, 18.10.2003, p.1)
[3] See: http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902628240.htm

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Scientific evaluation

1. Environmental risk assessment

According to the Biosafety Advisory Council no major risks were identified concerning the environment, except for the development of resistance to lepidopteran target pests. Case-specific monitoring for occurrence of insect resistance is therefore considered necessary.

2. Molecular characterisation

According to the Biosafety Advisory Council the molecular characterisation data are considered as sufficient.

3. Assessment of food/feed safety and nutritional value

The Biosafety Advisory Council agrees with the conclusion of EFSA on the compositional equivalence of MON810, consanguinous events and non-GM comparators. However the council has criticism on the use of non-isogenic controls in the comparative assessments which is not fully in line with recent EFSA guidance..

Following the comments submitted by the Belgian experts, the Biosafety Advisory Council considers that the compositional analysis lacks the analysis on dietary fibre. The Biosafety Advisory Council recommends the analysis on dietary fibre since this concept is widely accepted in human food studies.

4. Monitoring

General surveillance is advised to follow-up unanticipated allergenicity aspects since the allergenicity of the whole GM maize has not been tested.

Conclusion

Based on the scientific assessment of the dossier done by the Belgian experts, taking into account the opinion of EFSA, the answers of the EFSA GMO Panel to the questions raised by the Belgian experts, the answers of the notifier to the EFSA GMO Panel questions and considering the data presently available, the Biosafety Advisory Council,

Agrees with the GMO panel of EFSA that
a) No major risks concerning the environment were identified.
b) No major risks for human and animal health were identified.

In addition, the Biosafety Advisory Council recommends:

(1) That the applicant describes a monitoring plan for case specific monitoring of resistance development in the target pest. This monitoring plan should use methods widely accepted by the community of scientists and practitioners.

(2) That the IRM plan is evaluated on a regular basis, taking into account the adoption rates and/or changes in cropping practices, and adjusted if needed, in order to keep the IRM at an effective level.

(3) To conduct comparative trials in a sound scientific way.

p.o.

Prof. D. Reheul
President of the Belgian Biosafety Advisory Council

Annex: Full comments of experts in charge of evaluating application EFSA/GMO/RX-MON810 *(ref. BAC_2008_0742) and comments submitted on the EFSAnet (ref: BAC_2008_743)*

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13 May 2008

Application EFSA/GMO/RX-MON810
Comments submitted on the EFSAnet on mandate of the Biosafety Council

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 07 March 2008
Coordinator: Prof. Dirk Reheul
Experts: Pascal Cadot (Consultant), Patrick De Clercq (UGent), Leo Fiems (ILVO), Rony Geers (KUL), Jean-Luc Hofs (FUSAGx), André Huyghebaert (UGent), Peter Smet (Consultant), Frank Van Breusegem (VIB)
Domains of expertise of experts involved: Genetics, genome analysis, genetic engineering, toxicology, immunology, alimentary allergology, animal nutrition, traceability of alimentary chain, analysis of food/feed, industrial processing, agronomy, ecology, plant-insect relations, bio-diversity, insect resistance, effect on non-target species, impact on bio-diversity, risk analysis, biosafety research, nature conservation, maize
Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman

INTRODUCTION

Dossier **EFSA/GMO-RX-MON810** concerns an application of the company **Monsanto** for the renewal of authorisation of the genetically modified **maize MON810** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 29 January 2007.
The scope of the application is:
☐ GM plants for food use
☐ Food containing or consisting of GM plants
☒ Food produced from GM plants or containing ingredients produced from GM plants
☒ GM plants for feed use
☒ Feed produced from GM plants
☒ Import and processing (Part C of Directive 2001/18/EC)
☒ Seeds and plant propagating material for cultivation in European Union (Part C of Directive 2001/18/EC)

Comments posted on the EFSAnet

The comments are structured according to the "Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed" (EFSA Journal (2004), 99, 1-94). The comments below are those that were posted on the EFSAnet. It should be noted that all the comments received from the experts are considered in the evaluation of this dossier and in formulating the final advice of the Biosafety Advisory Council. The compilation of all the comments that were received from the experts (including the references) is given in a separate document.

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Point 7: the existence of a General Surveillance (GS) monitoring plan and Insect resistance management (IRM) plan should be mentioned.

B. INFORMATION RELATING TO THE RECIPIENT OR (WHERE APPROPRIATE) PARENTAL PLANTS

Comments/Questions of the expert(s)

Section B.1. : Information relating to the name of recipient is correct and complete. The breeding origin of the MON810 should be specified (inbred pure line or population…).

Section B.2., B3, B4:

Scientific information about maize pollen dissemination and probable cross-pollination improved since 1998. As an application for renewal, the report should have mentioned new European scientific studies: Messean *et al*. (2006), Brunet (2006), Klein *et al*. (2003), Sanvido *et al*. (2007), Mazzoncini *et al*. (2007), … There is still a need for pollen dispersal monitoring.

Survivability of maize in Europe is very limited but should be monitored in Southern Europe where winter mean temperatures are close to 15°C (South West Spain and SW Sicily).

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

No comments

D. INFORMATION RELATING TO THE GM PLANT

D.1 DESCRIPTION OF THE TRAITS AND CHARACTERISTICS WHICH HAVE BEEN INTRODUCED OR MODIFIED

No comments

D.2. INFORMATION ON THE SEQUENCES ACTUALLY INSERTED OR DELETED

No comments

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Pollen is a major plant part to be in contact with non-target insects. Why didn't Monsanto complete the report with accurate pollen Cry protein levels?
Other measurements are missing: toxin concentration in roots, tiller, ear leaf, silk and cob.

D.4. INFORMATION ON HOW THE GM PLANT DIFFERS FROM THE RECIPIENT PLANT IN: REPRODUCTION, DISSEMINATION, SURVIVABILITY

No comments

D5. GENETIC STABILITY OF THE INSERT AND PHENOTYPIC STABILITY OF THE GM PLANT

No comments

D.6. ANY CHANGE TO THE ABILITY OF THE GM PLANT TO TRANSFERR GENETIC MATERIAL TO OTHER ORGANISMS

No comments

D.7. INFORMATION ON ANY TOXIC, ALLERGENIC OR OTHER HARMFUL EFFECTS ON HUMAN OR ANIMAL HEALTH ARISING FROM THE GM FOOD/FEED

D.7.1 Comparative assessment

Comments/Questions of the expert(s)

The results presented have been issued in 1994-1995. Does it mean that neither assessment nor monitoring has been carried out since that time?

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

These presented data have already been reviewed before, by the EFSA GMO panel.
Some important remarks and observations :

1. The range of nutrients covered is limited in comparison to similar dossiers. Information on mineral composition is rather limited and restricted to calcium and phosphorous. Vitamins are completely absent in the analysis.
The importance of particular minerals and vitamins is substantial in food and feed. If maize is used as animal feed, minor nutrients like minerals and vitamins are generally added to concentrates; hence low concentrations in the maize may be overcome by these supplements.
In case maize is used as a human food, minerals and vitamins play an important role, especially for particular consumer groups, among others consumers with a high intake of maize derived foods.

2. Maize is known to be rather sensitive to particular moulds, with the risk of production of mycotoxins, The dossier does not deal with this, although quite a lot of scientific information is available.

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

The CrY1Ab protein was produced by recombinant E. coli (P. 92-96 of Technical dossier), because it may be practically impossible to obtain a sufficient amount of plant derived protein. It has been mentioned that testing bacterial surrogate proteins should not substitute for testing the plant-expressed proteins (Freese and Schubert, 2004).

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

The information on the variability in the results is not straightforward available in the study with broilers (Taylor, 2001).

D.7.5 Product specification

No comments

D.7.6 Effect of processing

No comments

D.7.7 Anticipated intake/extent of use

No comments

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

At this moment, there seems to be no direct toxicological, nor ecotoxicological danger. Nevertheless, keeping in mind the precautionnary principle, **no massive cultivation** can be allowed as long as major doubts remain. Besides permanent monitoring of existing fields, major efforts are needed to generate valuable scientific data.
Taking the additional information - concerning possible environmental effects - into consideration, some remarks are important

- "*The Bt maize produces 1500-2000 times as much Bt-toxin as is released through a single treatment in conventional crop protection, with the chemical called DIPEL, which contains Bt toxin.*" [(1)] "*Other experiments have found that the residues of Bt plants are slower to decompose than their isogenic lines. Some 8% of the toxin produced by the plant remained in the field after harvesting. Indeed, a substantial share of this active toxin quantity could be identified in the soil 11 months later.*" (EFSA, 2005) . The dossier should be completed with available information re the environmental impact of these facts.
- "*Impact of Bt toxin on non-target organisms*" (EFSA, 2004) Permanent monitoring of existing fields and new scientific research should be conducted, in order to have first-class data on which appropriate decisions can be based. Further comments in D.9.4.
- "*Impact of MON810 maize on the large-scale beekeeping industry in Greece*" (EFSA, 2006) : The question that should be answered is whether bees are sensitive to this kind of toxin. Little

literature is available and does not provide a clear answer to the problem. Immediate action should be undertaken to clear this item.

Comment 2

Chowdhury et al. (2003) reported that only traces of Cry1Ab survived the passage through the gastrointestinal tract of calves. This was confirmed by the fact that Lutz et al. (2005) found that Cry1Ab protein was degraded during digestion in cows. However, small fragments of Cry1Ab were detected in blood, liver, spleen and kidney of animals MON810 maize (Mazza et al., 2005).<u>What is the medium or long term effect of this ?</u>

Comment 3

The study on Ladybird beetles (Hoxter, 1992b) does not provide information on the variability within the results so that the power of the analysis cannot be evaluated, which is also the case for the study on earthworms (Palmer, 1995). The number of animals per treatment is sufficient to find differences in the experiments with rats (Lemen and Dudek, 2001) and with mice (Naylor, 1992), but it is not the case for the study referred to as Monsanto Company (1996).

D. 7.8.1 Safety assessment of newly expressed proteins

No comments

D.7.8.2 Testing of new constituents other than proteins

No comments

D.7.8.3 Information on natural food and feed constituents

No comments

D.7.8.4 Testing of the whole GM food/feed

No comments

D.7.9 Allergenicity

Comments/Questions of the expert(s)

The applicant states that Cry1Ab is only a small part of the total protein content as an argument to confirm Cry1Ab as being non allergenic. However, only the titration of the protein of interest is valuable, not the determination of its relative content. In this respect, the levels of Cry1Ab are described at around 0.3 µg/g in the maize grain. This means that the ingestion of 300 g of non-concentrated maize-derived food product gives 90 µg of Cry1Ab, which lies in the lowest levels currently observed to be able to elicit allergic reactions.

Simulated gastric fluid and simulated intestinal fluid (P.107-109, Technical dossier) were used to test allergenicity. However, Bannon et al. (2003) and Herman et al. (2006) concluded that the use of the SGF technique to predict the allergenic status of the proteins remains uncertain.

Assessment of allergenicity of the whole GM plant.

This has not been evaluated in the application. The reviewer wishes to emphasize that the rationale of this section is to evaluate, due to the introduction of the new traits, possible changes in the allergenicity of the recipient plant when this plant is known as an allergenic source.

Although not frequent, food allergy to maize exists and allergens have been determined (Pastorello et al. 2003; Pasini et al. 2002, Weichel et al. 2006). The introduction and expression in the plant of Cry1Ab might interfere with other maize proteins, including allergens, and modify their expression levels. Care must be taken that food allergy to maize grain does not become more frequent due to the introduction of new traits and the interferences thereof. For that reason, it is relevant to analyze whether the expression levels of known major allergens is increased in genetically modified MON810 maize grains. Patient IgE binding to maize grain extract or titration of known major allergens of maize should be carried out for the GMO and natural counterpart.

Given that the application also deals with cultivation in the E.U, another concern is maize pollen allergy. Although literature on that subject is scarce, allergy to maize pollen is well known in the allergy outpatient departments of the clinics and of the independent allergologists. It results from cross-reactivity with grass pollen, and is a major allergy problem in children living near maize fields. The most known cross-reacting allergens are Zea m 1 and Zea m 13, that cross-react with the group 1 and 13 allergens of grasses (Petersen et al. 2006). Therefore, the expression level of those major allergens should be determined in the pollen of genetically modified maize MON810.

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

P.65, Table 10 (Technical dossier) tryptophan concentration in MON 810 is different from MON 818, although means are identical, but ranges are somewhat different. It looks like 0.6 is not the mean for MON818 with a range from 0.4 to 0.6; This may be verified.

Compositional data for protein, fat, ash, ADF, NDF, fat, carbohydrates and dry matter for MON 810 were similar to the control, MON 820. How is dry matter in Table (Technical dossier)14 expressed? Units are lacking.

D.7.11 Post-market monitoring of GM food/feed

No comments

D.8. MECHANISM OF INTERACTION BETWEEN THE GM PLANT AND TARGET ORGANISMS (IF APPLICABLE)

No comments

D.9. POTENTIAL CHANGES IN THE INTERACTIONS BETWEEN THE GM PLANT WITH THE BIOTIC ENVIRONMENT RESULTING FROM THE GENETIC MODIFICATION

D.9.1. Persistence and invasiveness

No comments

D.9.2 Selective advantage or disadvantage

No comments

D.9.3 Potential for gene transfer

No comments

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

Baseline susceptibility and resistance allele frequency of the ECB is not uniform and depends on the sampling structure (population and region) (Meise and Langenbruch, 2007). Variations in toxin expression according to the plant age and environmental conditions can disturb the “high dose” strategy (Dutton et al., 2004). In addition, the monitoring of Bt expression shows that concentrations vary strongly between different plant individuals of Mon810 (Nguyen et al., 2007).So more studies should still be done to reduce uncertainty about resistance acquisition. As the risk of a resistance outbreak related to ECB and *Sesamia* is probable, IRM plans must be carefully respected in the scope of the Industry IRM Working Group (Alcalde *et al*., 2007).

Toxin resistance is a major threat in the environmental risk assessment and recent results show that it is appearing in another species in Africa. As published recently (Van Rensburg, 2007), toxin resistance may evolve and present a tangible risk which must be addressed with a specific monitoring. In that study, which has been conducted after reports of severe damage caused by *Busseola fusca* (stem borer), substantial numbers of larvae from the Bt derived population survived over the entire period of the bioassay.

D.9.5 Interactions of the GM plant with non-target organism

Comments/Questions of the expert(s)

Many studies have demonstrated no or little direct effects of the Cry1Ab-endotoxin on non-target invertebrate organisms, including non-pest herbivores, pollinators and carnivorous natural enemies. Not surprisingly, Bt environment affects non-target fauna differently compared with non sprayed agro-system (Marvier et al., 2007).

Only few studies have reported adverse effects of Cry1Ab toxin on non-target organisms. Lövei & Arpaia (2005) and Romeis et al. (2006) provide reviews of the literature on side effects of Cry proteins to beneficial arthropods. Laboratory studies by Hilbeck et al. (1998, 1999) revealed some negative effects on the predatory insect *Chrysoperla carnea*, but later studies showed that these adverse effects were not direct toxic effects but were mediated by (nutritional) quality of intoxicated prey, i.e. an *indirect* effect (Romeis et al., 2004; Rodrigo-Simon et al., 2006). Lower prey quality was also believed to be partly or wholly responsible for adverse effects observed in the generalist carabid predator *Poecilus cupreus* and the parasitoid *Cotesia marginiventris* offered prey or hosts that were fed Bt maize (CryIAb) (Meissle et al., 2005; Vojtech et al., 2005); the experiments in Meissle et al. (2005) showed that the ground beetle did not avoid Bt-containing prey, which means that exposure in the field may occur. However, in a study on transgenic canola expressing CryIAc on another carabid beetle as a non-target indicator organism (Ferry et al., 2006), the results suggested that behavioural preferences of the predator (i.e., rejection of contaminated prey) would mitigate adverse indirect effects of reduced prey quality caused by consumption of Bt-canola plants.

The degree of exposure suffered by non-target organisms has been the subject of different studies with variable outcome. The applicant may be criticized for only listing studies in the literature review in Annex 3.1 showing that exposure of non-target organisms is negligible or non-existent. For instance, the applicant refers to a study by Harwood et al. (2006) showing that there was no evidence for uptake of Cry1Ab endotoxins by a carabid predator in laboratory and field experiments. However, the applicant fails to mention other studies by the same authors that do indicate such uptake and suggest that the toxin may transfer into higher order trophic levels of food chains (Harwood et al., 2005, 2007). Similar findings (i.e., that Bt toxins can be transferred to predatory arthropods) were reported in a field study by Obrist et al. (2006). Likewise, Alvarez-Alfageme et al. (2008) showed that Cry1Ab toxin was transferred in undegraded form from Bt-maize to the predatory coccinellid *Stethorus punctillum* via its tetranychid prey, but that the predator did not suffer adverse effects from the toxin.

The applicant refers to a number of field studies supporting the safety of Cry1Ab containing crops to a wide range of beneficial insects. The overall conclusion of these studies, that there are little differences in non target communities in Bt corn and non-Bt corn (where conventional insecticides are used), is well interpreted by the applicant. This conclusion is corroborated by two 3-year farm-scale studies in Spain focusing on arthropod predators (de la Poza et al., 2005; Farinos et al., 2008). On the other hand, a study by Pilcher et al. (2005) demonstrated lower densities of a specialist parasitoid in Bt corn plots, as a result of the lower abundance of its host, the target pest *Ostrinia nubilalis*. A study by Bourguet et al. (2002) also indicated that indirect effects on populations of certain more specific natural enemies of the different lepidopteran target pests would be expected if pest populations would be reduced as the result of the efficient control exerted by the expression of Cry-endotoxins (EFSA, 2006). Although similar effects are likely to occur also in non-transgenic maize where conventional pesticides are being used, I expect that the effect in transgenic maize may be less transient given that the toxin is expressed at very high levels (>99% level of efficacy, Pilcher et al. 2005) and continuously throughout the crop's cultivation period. Large-scale adoption of the transgenic maize may exacerbate these effects. Therefore, it is imperative to install sufficient refuges consisting of non-transgenic maize to avoid adverse impacts on these specific natural enemies.

Effects on non-pest lepidopterans feeding on maize would be expected, but the crop does not constitute an important resource of food for indigenous butterflies in Europe; pollen is only shed by Bt-maize plants during a limited window of time and it remains in the immediate vicinity of the crop, so possible adverse effects are expected to be transient and local. In the USA, mainly effects on the monarch butterfly (which does not occur in Europe) were studied. E.g. according to their results Prasifka et al. (2007) assume that "Monarch larvae exposed to Mon810 anthers behave differently and that ingestion may not be the only way Bt can affect non-target insects".
Although some of these studies showed some adverse fitness effects, they were not considered likely to pose a significant risk to the monarch butterfly populations in North America (see Dively et al., 2004; Anderson et al., 2005 and references therein). Little is known on the distribution of European lepidopteran species in agricultural landscapes and their potential exposure to Bt maize (e.g., Gathmann et al., 2006). However, negative impacts on native butterflies were reported by Darvas et al. (2003, 2004 a,b). According to latter studies Cry1Ab toxins may kill some 20% of hatching *Inachis io* caterpillars on nettle plants within 5 m of MON810 Bt maize. However, it should be stressed that:

- these Hungarian papers and the data therein were inaccessible to me
- two of these references are merely abstracts of symposia
- none of these data were, as far as I could retrieve, ever subjected to peer review, or at least disseminated into the international scientific literature; this is all the more striking given the relevance of the reported findings

A study by Rosi-Marshall et al. (2007) published in the renowned journal PNAS showed that species of Trichoptera (caddisflies) occurring in headwater stream systems suffered negative effects of Cry1Ab from Bt maize in laboratory feeding trials. This study has been used to underline that an environmental risk assessment of Bt maize should also take into account water-dwelling insects. However, this paper has received serious criticism for its inappropriate methodology and unfounded conclusions in two letters to PNAS (Beachy et al., 2008; Parrott, 2008) and a scientific opinion paper in Trends in Biotechnology (Miller et al., 2008). The arguments laid out in these latter papers were entirely followed by EFSA (2007).

Interestingly, however, negative effects of Cry1Ab were recently reported for another water dwelling organism, the water flea *Daphnia magna* (Bøhn et al., 2008); the laboratory experiments indicated a toxic effect rather than a lower nutritional value of the Bt-maize.

Manachini et al. (2003) and Bourguet et al. (2002) found a decrease of the biocontrol function among specialist antagonist of target pest.
Daly et al. (2005) found a decrease of natural enemy abundance of Nabis sp.
Variable effects have been observed on earthworms (Zwahlen et al., 2003; Clark and Coats, 2006) (more details in D.9.8)

The application for renewal states that (page 126) "Cry1Ab has a selective toxicity towards certain Lepidopteran pests but not against other orders". According to the arguments here-above, this statement should be moderated because there is an <u>uncertainty</u> about the Cry1Ab activity on some non-target organisms through the "gene *x* environment" interaction. So accurate monitoring plans must be put in place.

D.9.6 Effects on human health

No comments

D.9.7 Effects on animal health

No comments

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

Comment 1

The role of cultivation practices on the impact of Bt in the environment hasn't been taken enough into account:

- Fu *et al*.(2008) report the occurrence of interactions between inorganic salts (contained in mineral fertilisers) and Bt toxin adsorption that impact the fate of Bt toxins in the soil.
- Icoz et stotzky (2008) report that microbial process play a major role in the dissipation of CryA toxins and this process depends, in turn, on soil type, seasons, cultivar, crop practices…

Bt concentrations in the soil don't always decrease as fast as described in section D 9.5. Marchetti *et al*. (2007) report a DT_{50} of 10 or 11 days for sandy and clay soil, which is higher than the 1.5 day mentioned in the application for renewal.

Additional concentrations of Bt toxins in the soil due to GM cultivation affects the GRAM+/GRAM$^-$ ratio compared with a non-GM crop (Xue *et al*., 2005). Most of the results published in this area are still preliminary (Evans *et al*. 2002; Zwahlen *et al*. 2003) and need more complements. Nevertheless, at this stage of the knowledge, current studies show no significant or acute detrimental effects of CryA toxins on soil microorganisms or soil microbiology.

Comment 2

The applicant dossier and the studies referred to therein have reported no or negligible effects of the expressed Cry1Ab proteins on detritivorous organisms living in and on the soil. For instance, there are no reported effects of the Cry1Ab toxin on the annelid *Eisenia fetida* (an epigaeic compost worm that is usually not found in maize fields) nor on the collembolan *Folsomia candida*. A number of studies have reported no negative effects of Cry1Ab expressing maize on other soil organisms (discussed in EFSA 2006).

On the other hand, there are reports on sublethal effects of the Cry1Ab expressing GM maize on immatures and adults of the anecic earthworm *Lumbricus terrestris* when fed on litter of the GM maize, although the adverse effects could not be confirmed in higher-tier (small scale) field trials (Zwahlen et al., 2003). In a laboratory study, Vercesi et al. (2006) reported no detrimental effects of Bt maize residues on development and fecundity of the earthworm *Aporrectodea caliginosa* (which is a more relevant species in an agricultural setting), with the exception of a slight decrease in cocoon hatchability. However, the authors questioned whether this effect would have any ecological significance in the field. Wandeler et al. (2002) studied the consumption of Bt- and non-Bt-maize by the woodlouse *Porcellio scaber* and reported that the woodlouse fed less on the Bt maize than on the corresponding non-Bt control variety. They also found that the woodlouse was effectively exposed to the toxin, but made no mention of adverse effects on the organism. In a glasshouse experiment, Griffiths et al. (2006) reported some effects of Cry1Ab expressing maize on soil microbial and faunal communities but these effects were all minor and comparable to those of conventional ("current best practice") insecticide treatments.

It needs emphasis again that so far no published studies have described the consequences of long-term cultivation of Bt corn on earthworms and other soil dwelling animals.

D.9.9 Impacts of the specific cultivation, management and harvesting techniques

Comments/Questions of the expert(s)

Mon810 is currently a reliable means to control ECB but, as mentioned in section D 9.4, there is a risk that target insects develop CryA toxin resistance in the medium or long term.

The report states that Mon810 "reduces the use of chemical insecticides and reduces applicator exposure to dangerous active ingredients" but doesn't mention the number of insecticide sprays that can be saved.
ECB and *Sesamia* are among the major pests in maize fields and may cause 13% yield losses. ECB reinforces its presence in southern France and progresses rapidly up north where two generations can grow in a season. In France, infestation hotspots are seen in the Rhone region and South-West. However, in most of the regions spray numbers are still low: in Poitou-Charentes in France (see Agreste Report, 2003), where an average larvae density is about 0,8 larvae/plant, only 42% of the maize fields are sprayed (mostly with pyrethroïds) and receive 1.4 sprays on average. Insecticide quantities, mostly Cypermethrin and Lamda Cyhalothrin, respectively average 0.08 and 0.01 kg/ha, which is not an exaggerated load for the environment in comparison with other crops. Similar statistics are found in Germany and Spain (Brookes, 2002). This information is confirmed by the Mon810 monitoring report (Schmidt and Tinland, 2006).
In conclusion, the impact of the pesticide reduction due to Bt maize on human health or environment still remains weak and doesn't represent a major benefit.

No reference is made about coexistence measures. However specific lay-out, spatial organisation and separate chain processing may be required in some areas (Messean, 2006b). These measures generate additional economic costs, which should be discussed.

Mon810 should be recommended in a IPM management system, which should be detailed by the Monsanto Company.

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

No comments

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

The applicants describe an insect resistance management (IRM) plan for case-specific monitoring of resistance development in corn rootworms that is generally based on methods that have up to now been widely accepted by the community of scientists and practitioners.

However, care should be taken to meticulously implement and continuously evaluate and, if needed, adjust the recommended measures in the plan, particularly if large scale adoption of the Bt/herbicide tolerant maize would change existing cropping methods (e.g., related to crop rotation or tillage) possibly affecting abundances of the target pests, other herbivores and natural enemies.

Also, some of the key assumptions for the high-dose refuge strategy which forms the basis of the environmental monitoring plan, may not be entirely fulfilled.

Farinós et al. (2004) highlighted the need to adapt the insect resistance management strategies for certain areas. It has been shown that 1) *Sesamia nonagrioides* females mate before they move for oviposition, so that females emerging from refuge would rarely mate with potential resistant moths emerging from Bt maize fields and vice versa, and 2) *Ostrinia nubilalis* mobility is also reduced before oviposition in irrigated maize fields, which corresponds to the agronomic practices of most maize growing areas in Spain. These findings need to be considered in the IRM plan.

Dalecky et al. (2006) further pointed out that:

- the behavioural ecology of stem borers, as related to the timing between dispersal and mating, may differ from species to species (and so may be different between *Ostrinia nubilalis* and *Sesamia* spp.)
- the high dose refuge strategy, in which refuges are situated a few hundred meters from Bt maize fields, may not ensure complete mixing between susceptible and resistant *Ostrinia nubilalis* moths, because some pre-dispersal mating occurs for both males and females (up to about 57% of newly emerged females have been found to mate locally)
- Bt-resistance alleles in resistant stem borers may be associated with fitness costs, decreasing dispersal and mating success; as such, intermixing between susceptible and resistant moths may be further compromised

Eizaguirre et al. (2006) showed that in *Sesamia nonagrioides*:

- a high proportion of larvae may move to plants in adjacent rows, favouring the survival of partially resistant individuals; this suggests that mixing of Bt and non-Bt maize in the same field would not be a recommendable strategy to delay resistance

- surviving adults that have been exposed to sublethal concentrations of the Bt toxin have an asynchronous development compared to individuals originating from non Bt refuges which limits the random mating between susceptible and resistant individuals
- individuals exposed to sub-lethal levels of Bt maize may have lower fitness (e.g., as related to their responsiveness for diapause inducing and terminating cues)

Although these studies show that the main prerequisite for random mating in the high-dose refuge strategy proposed in the resistance monitoring plan may not be fulfilled, there have been no field reports to date of resistance in lepidopteran stem borers towards Bt-maize expressing Cry1Ab, or other Cry toxins.

D.11.2 Interplay between environmental risk assessment and monitoring

No comments

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

1) Interactions between the GM plant and target organisms, case-specific monitoring **with risk management.**

The IRM plan is complete. To delay the risk of insect resistance and reinforce the IR management, Mon810 should be introduced when a certain average pest pressure threshold (example: 0.8 larvae/plant) is reached in a given area and should not be adopted below (see Hochberg *et al*. (2006) for more details).

2) Additional case-specific monitoring **without risk management**

Contradictory results (pointed in section 9.5 and 9.8) should be re-assessed by the scientific community (Itps, EFSA….) on a multi-disciplinary base, under standard process and methods.

D.11.4 General surveillance of the impact of the GM plant

Comments/Questions of the expert(s)

Is there a parallel field/farm monitoring plan put in place by the public sector (INRA or others) or does field data rely only upon Monsanto monitoring network? This information doesn't appear clearly in the report.

<u>Farm questionnaire: not accurate enough for non-target arthropods ?</u>

- The observation data related to the monitoring character is quality based and leads sometimes to inaccurate results, whatever reliable the non parametric statistical analysis can be.
- Data related to fertiliser application is not accurate and doesn't consider doses/ha.
- Insecticides are simply mentioned without indication of doses, quantity or number of sprays during the agricultural season. This avoids the comparative evaluation of the biocide charge in

the environment (a farmer can decrease the number of sprays but increase the quantity of insecticide).

- Main weed species occurrence should be mentioned in comparison with non-Bt fields.
- The questionnaire focuses mainly on target and non-target pests, when non-target neutral arthropods and beneficials are not.

The observed data should be based on counts of bioindicators belonging to the different existing functional groups (ECB parasitoids, predators, neutral species, earthworms, amphibians, birds…) through sampling or trap monitoring, according to a precise protocol (see methods in Delos *et al*, 2006).

Interactions between the GM plant and non-target organism

Contradictory results have been reported about predatory insects (see introduction in Marvier *et al*., 2007) because of deficient protocols or analysis. The interaction between Bt toxin and the quality of the target prey must be more deeply investigated and should be included in an ERA plan: would non-Bt starving preys induce the same effect as Bt infected preys?

Uncertainties due to the difficulty of interpretations present in the conclusions of impact studies as seen in Candolfi et al. (2004), related to the impact of Bt toxin on lepidopteran, dipteran and hymenopteran species in the field in particular, should be cleared within an accurate monitoring plan. This plan could be assimilated to a case-specific monitoring, in the long term, to test the hypothesis of an indirect adverse effect of Bt toxin on the non-target fauna. Results of the 2006 monitoring report (Schmidt and Tinland, 2006) indicating a non-expected effect on Diptera and Araneae support this suggestion.

Toxin expression

Since the combination of non-biotic stresses may affect toxin concentration in the plant (Dutton et al., 2004), the results of the GS plan (target pest pressure, presence or absence of irrigation) should be systematically linked with local climatic data, to complete the monitoring of the efficacy of Mon810 on target insects and the high-dose & refuge IRM.

Areas to be monitored (section 11.4.3.3.)

There is an inconsistence at line 10-12: “the **Glyphosate** trait has utility in a wide range of agricultural environment. Therefore, the introduction of Mon810 is not confirmed to specific geographical zones”
It should be: the **Bt trait** has utility….

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

The general surveillance is adapted for a broad agricultural survey focused on potential and additional benefit to the farming unit. On the other hand, it seems to be less adapted to environmental impacts.

In order to address specific impact issues of Mon810, GM-fields should theoretically be compared with non-GM fields of the same farm or agrosystem. In the GS monitoring reports (2005 and 2006) presented in Annexe II of the dossier, data from small GM-fields (less than 5 ha, without obligation to plant refuge) have been processed with data from large GM-fields. Especially in the case of Spain, where refuges are not often respected and where Mon810 plantings are the majority (see the rate of all maize/Mon810 acreage), no direct comparison between Bt and non-Bt maize could be made in the same farming unit. Introduction of bias and misinterpretation of data may occur:

- If the GM farmer made a comparison with one of its neighbours' non-GM field (without knowledge of the cropping history),
- If the farmer refers to non-GM data from previous years.

Some of the results of the GS reports should also be analysed and presented separately at a regional or national scale (highlighting treatment x local environment). This is the case for the incidence of diseases, which are sometimes specific to a region. The interaction between Bt and the environment or agrosystem should be more often taken into account.

Finally, only two general surveillance reports have been presented separately in the annexes (2005, 2006). It would have been interesting to assess to results of previous years and have them compiled in order to it to study multi-annual trends.

References

see document BAC_2008_742 in annex

Bioveiligheidsraad
Conseil de Biosécurité

Secretariaat
Secrétariat

May 2008

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Compilation of comments of experts in charge of evaluating the application EFSA/GMO/RX-MON810

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 07 March 2008
Coordinator: Prof. Dirk Reheul
Experts: Pascal Cadot (Consultant), Patrick De Clercq (UGent), Leo Fiems (ILVO), Rony Geers (KUL), Jean-Luc Hofs (FUSAGx), André Huyghebaert (UGent), Jean-Pierre Maelfait (UGent), Peter Smet (Consultant), Frank Van Breusegem (VIB)
Domains of expertise of experts involved: Genetics, genome analysis, genetic engineering, toxicology, immunology, alimentary allergology, animal nutrition, traceability of alimentary chain, analysis of food/feed, industrial processing, agronomy, ecology, plant-insect relations, bio-diversity, insect resistance, effect on non-target species, impact on bio-diversity, risk analysis, biosafety research, nature conservation, maize
Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman

INTRODUCTION

Dossier **EFSA/GMO-RX-MON810** concerns an application of the company **Monsanto** for the renewal of authorisation of the genetically modified **maize MON810** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 29 January 2007.
The scope of the application is:
☐ GM plants for food use
☐ Food containing or consisting of GM plants
☒ Food produced from GM plants or containing ingredients produced from GM plants
☒ GM plants for feed use
☒ Feed produced from GM plants
☒ Import and processing (Part C of Directive 2001/18/EC)
☒ Seeds and plant propagating material for cultivation in European Union (Part C of Directive 2001/18/EC)

Depending on their expertise, the experts were asked to evaluate the genetically modified plant considered in the application on its 1) molecular, 2) environmental, 3) allergenicity, 4) toxicity and/or 5) food and feed aspects. It was expected that the expert should evaluate if the information provided in the application is sufficient in order to state that the marketing of the genetically modified plant for its intended uses, will not raise any problems for the environment or human or animal health. If information is lacking, the expert was asked to indicate which information should be provided and what the scientifically reasoning is behind this demand.

The comments are structured as in the "Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed" (EFSA Journal (2004), 99, 1-94).

List of comments received from the experts

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comment 1

The Cry1Ab protein, produced by MON 810, has been reviewed and considered safe by the Scientific Committee on Plants (SCP, 1998). MON 810 has been used as a commercial product since 1997 and so far, no toxic, allergenic or other harmful effects to human health or the environment have been reported. This means that MON 810 can be safely used.

Comment 2

No comments/questions

Comment 3

Point 7: the existence of a General Surveillance (GS) monitoring plan and Insect resistance management (IRM) plan should be mentioned.

Comment 4

Marketing of this transgenic MON810 maize expressing the Cry1Ab toxin to control lepidopteran pests like *Ostrinia nubilalis* and *Sesamia nonagrioides* is revelant to large parts of Europe where these pests are abundant, but not or less so to some areas where these lepidoperans are currently absent or not economically important (e.g., in Belgium, where maize is mainly cultivated as a fodder crop and *Ostrinia nubilalis* is not a pest problem). However, climate change may stimulate northward range expansion of these insects in the future or accelerate population build up and as such increase their pest status.

B. INFORMATION RELATING TO THE RECIPIENT OR (WHERE APPROPRIATE) PARENTAL PLANTS

Comments/Questions of the expert(s)

Comment 1

No comments/questions

Comment 2

Section B.1. : Information relating to the name of recipient is correct and complete. Nevertheless the breeding origin of the MON810 should be specified (inbred pure line or population…).

Section B.2., (a) reproduction (p.15): information about pollen viability reflects minimum or regular pollen field life but do not consider maximum measurements. Brunet (2006) showed that maize pollen grains are transported by air convection at a non negligible altitude where thermo-hydric conditions allow an increase of their viability by hours.

(b) sexual compatibility: Luna *et al.*(2001) calculated a theoretical maximum pollen viability distance of 32 km.

Section B.3.: Survivability of maize in Europe is very limited but should be monitored in Southern Europe where winter mean temperatures are close to 15°C (South West Spain and SW Sicily).

Section B.4. : Theoretical and natural pollen transportation was calculated by Luna *et al*. (2001). Brunet (2006) mentioned maize gene flow over several kilometres with average hybridization rates of 0.25%. Messean *et al*. (2006) recommended a 400 m. isolation distance to reach a 0.05% out-crossing, which is slightly different from Luna et al. (2001), reference mentioned in the report (p.18). Even though Brunet's measurements are below the European standard threshold for product consumption, they get closer to purity thresholds of the seed multiplication industry. In addition Brunet (2006)'s experimentation was not conducted in "full open field" and more parameters should be investigated in more dissemination favourable conditions.

Sections B.5., B.6., B.7. provide correct and valuable information.

Conclusion: scientific information about maize pollen dissemination and probable cross-pollination improved since 1998. Although no essential or critical facts have been brought about an eventually increase of the dissemination potential, there is still a need for pollen dispersal monitoring.

As an application for renewal, the report should have mentioned new European scientific studies: Messean *et al*. (2006), Brunet (2006), Klein *et al*. (2003), Sanvido *et al*. (2007), Mazzoncini *et al*. (2007)…

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Comment 1

No comments/questions

Comment 2

The genetic modification is described in detail with appropriate reference and a physical and genetic map of functional elements is provided according to EFSA requirement.

D. INFORMATION RELATING TO THE GM PLANT

D.1 DESCRIPTION OF THE TRAITS AND CHARACTERISTICS WHICH HAVE BEEN INTRODUCED OR MODIFIED

Comments/Questions of the expert(s)

Comment 1

No comments/questions

Comment 2

According to the EFSA Guidance Document information or reference to another section should be provided about non-target; otherwise information is complete.

D.2. INFORMATION ON THE SEQUENCES ACTUALLY INSERTED OR DELETED

Comments/Questions of the expert(s)

Comment 1

No comments/questions

Comment 2

Mon-810 doesn't express a marker gene. Southern blot and Western blot/PCR analysis indicate that the genes for Glyphosate tolerance (CP4EPSPS) and antibiotic resistance (nptII) were not transferred into the plant. In addition, bioinformatic analysis of flanking data is provided as well, which is a positive element in the assessment.

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1

No comments/questions

Comment 2

Pollen is a major plant part to be in contact with non-target insects. Why Monsanto didn't complete the report with accurate pollen Cry protein levels?
Other measurements are missing: toxin concentration in roots, tiller, ear leaf, silk and cob.

D.4. INFORMATION ON HOW THE GM PLANT DIFFERS FROM THE RECIPIENT PLANT IN: REPRODUCTION, DISSEMINATION, SURVIVABILITY

Comments/Questions of the expert(s)

Comment 1

No comments/questions

Comment 2

Full agreement with the provided information.

D5. GENETIC STABILITY OF THE INSERT AND PHENOTYPIC STABILITY OF THE GM PLANT

Comments/Questions of the expert(s)

Comment 1

No comments/questions

Comment 2

In agreement with the provided information.

D.6. ANY CHANGE TO THE ABILITY OF THE GM PLANT TO TRANSFERR GENETIC MATERIAL TO OTHER ORGANISMS

Comments/Questions of the expert(s)

Comment 1

No comments/questions

Comment 2

Full agreement with the provided information.

D.7. INFORMATION ON ANY TOXIC, ALLERGENIC OR OTHER HARMFUL EFFECTS ON HUMAN OR ANIMAL HEALTH ARISING FROM THE GM FOOD/FEED

D.7.1 Comparative assessment

Comments/Questions of the expert(s)

Comment 1

Agreement with the provided information, which doesn't differ from the previous application.
The results presented have been issued in 1994-1995. Does it mean that neither assessment nor monitoring has been carried out since that time?

Comment 2

Compositional analyses include forage and grain from MON 810 grown under different field conditions in the USA and the EU. Control forage and grain from conventional maize was also analyzed. Results were analyzed statistically and also compared with literature data.
No further comment.

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1

No comment

Comment 2

Information includes results of the compositional analysis of several field trials.

Grain from MON 810 and a control of the US field trial in 1994; proximates, amino acid composition, fatty acid composition, fibre, anti-nutrient and minerals were analyzed The fibre analysis is limited to crude fibre. As an anti-nutrient phytic acid was assessed. Mineral analysis was limited to calcium and phosphorus.
The applicant concludes that the grain from MON 810 is comparable to the control.

Grain from MON 810 and a control of the EU field trial in 1995. Analysis include proximates, amino acid and fatty acid composition, fibre as NDF and ADF.
The applicant concludes that the compositional data of MON 810 are comparable to the control and within the literature data ranges of commercial hybrids.

Forage from MON 810 and a control from the EU field trial in 1995. Analysis include proximates and fibre as NDF and ADF.
It is concluded that forage from MON 810 and the control 820 are similar in composition.

Grain and forage analysis of progeny of MON 810 field trials in the EU in 1995. Grains were analyzed according to proximates, ADF, NDF, fatty acids and amino acids. Forage analysis by NIR methods includes, in addition to proximates, soluble sugars and in vitro digestibility.
The applicant concludes that measured ranges are similar to literature data.
As a general conclusion the applicant proposes compositional equivalence.

Comment
These data have already been reviewed before, by the EFSA GMO panel.

However I would like to make some observations:
1. The range of nutrients covered is limited in comparison to similar dossiers. Information on mineral composition is rather limited and restricted to calcium and phosphorous. Vitamins are completely absent in the analysis.
I would like to emphasize the importance of particular minerals and vitamins. I accept that this observation is less important for maize as animal feed as minor nutrients like minerals and vitamins are generally added to concentrates.
In case maize is used as a human food, minerals and vitamins play an important role, especially for particular consumer groups, among others consumers with a high intake of maize derived foods.

2. Maize is known to be rather sensitive to particular moulds, with the risk of production of mycotoxins, among others aflatoxins. I found no information about this subject in the dossier. Taking into account the experience during recent years is there any information about an increased or reduced sensitivity to mycotoxins?

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1

The CrY1Ab protein was produced by recombinant E. coli (P. 92-96 of Technical dossier), because it may be practically impossible to obtain a sufficient amount of plant derived protein. It has been mentioned that testing bacterial surrogate proteins should not substitute for testing the plant-expressed proteins (Freese and Schubert, 2004).

Comment 2

No comment

Comment 3

See above, no further comment.

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1

The information on the variability in the results is not straightforward available in the study with broilers (Taylor, 2001).

Comment 2

No comment

Comment 3

No comment

D.7.5 Product specification

Comments/Questions of the expert(s)

Comment 1

No comment

Comment 2

No comment

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1

Full agreement with the provided information.

Comment 2

Main applications as animal feed, human food and industrial applications are reviewed. The applicant states that processing characteristics are not changed, as the introduced trait is of agronomic interest and compositional equivalence is demonstrated.
Based upon the information obtained, I agree with this statement.

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1

General agreement with the provided information.

Comment 2

The applicant states that the anticipated intake will not be changed due to the introduction of MON810. The genetic modification will not affect current usage patterns.
I agree wit this conclusion.

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

Based on the information provided by the applicant, the dossier seems to be complete and the product seems to excert no toxic effects (see D.7.8.1 and D.7.8.4 below).

Taking the additional information - concerning possible environmental effects - into consideration, I would like to make the following remarks.

- "*The Bt maize produces 1500-2000 times as much Bt-toxin as is released through a single treatment in conventional crop protection, with the chemical called DIPEL, which contains Bt toxin.*" (EFSA, 2005) : None or insufficient information is given. To my point of view, this item can quite easily be examined by means of scientific experiments based on analytical techniques. Immediate action should be undertaken to clear this item.
- "*Impact of Bt toxin on non-target organisms*" (EFSA, 2004) : According to current available literature, no direct danger seems to exist. However, permanent monitoring of existing fields and new scientific research should be conducted, in order to have first-class data on which appropriate decisions can be based.
- "*Impact of MON810 maize on the large-scale beekeeping industry in Greece*" (EFSA, 2006b): The question that should be answered is whether bees are sensitive to this kind of toxin. Little literature is available and does not provide a clear answer to the problem. Immediate action should be undertaken to clear this item.
- "*Other experiments have found that the residues of Bt plants are slower to decompose than their isogenic lines. Some 8% of the toxin produced by the plant remained in the field after harvesting. Indeed, a substantial share of this active toxin quantity could be identified in the soil 11 months later.*" [(1)] : this problem is closely related to the impact on non-target soil organisms.

Conclusion: At this moment, taking into account the currently available literature, there seems to be no direct toxicological, nor ecotoxicological danger. Nevertheless, keeping in mind the precautionnary

principle, **no massive cultivation** can be allowed as long as major doubts remain. Besides permanent monitoring of existing fields, major efforts are needed to generate valuable scientific data.
It should be kept in mind that the conclusions made for this dossier, will have severe consequences for a lot of other dossiers also.

Comment 2

As the level of Cry1Ab protein in progeny of MON810 is rather low, and because of a NOAEL for Cry1Ab in mice of 4000 mg/kg (Cockburn, 2002), the use of MON810 may not provoke safety problems. Chowdhury et al. (2003) reported that only traces of Cry1Ab survived the passage through the gastrointestinal tract of calves. This was confirmed by the fact that Lutz et al. (2005) found that Cry1Ab protein was degraded during digestion in cows. However, small fragments of Cry1Ab were detected in blood, liver, spleen and kidney of animals MON810 maize (Mazza et al., 2005). Lutz et al. (2005) concluded that Cry1Ab protein was degraded during digestion in cows.

Comment 3

The study on Ladybird beetles (Hoxter, 1992b) does not provide information on the variability within the results so that the power of the analysis cannot be evaluated, which is also the case for the study on earthworms (Palmer, 1995). The number of animals per treatment is sufficient to find differences in the experiments with rats (Lemen and Dudek, 2001) and with mice (Naylor, 1992), but it is not the case for the study referred to as Monsanto Company (1996).

Comment 4

The (medium and long term) effect of Bt toxins on the animal and human metabolism is little know and is not discussed in the application for renewal.

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

<u>6) Safety assessment of newly expressed proteins.</u>

Since this dossier is intended as a renewal of an existing application, no further testing is recommended. All necessary tests have been performed previously.

<u>6a) Bioinformatics analysis of the CrylAb protein, utilizing the AD5, TOXIN5 and ALLPEPTIDES databases (Roderick and Silvanovich, 2004).</u>

No biologically relevant structural similarities to allergens, toxins, or pharmacologically active proteins were observed for the CrylAb protein sequence.

Potential structural similarities shared between the CrylAb protein and proteins in the toxin database were evaluated using the FASTA sequence alignment tool. Identified proteins were ranked according to their degree of similarity. The best similarity observed was to the *Bacillus thuringiensis* pesticidal crystal protein CrylAb, demonstrating *99.9%* identity over an 817 aa window with an *E* score of zero. This alignment is not surprising, since the CrylAb protein is listed within GenBank as an insecticidal toxin and, as such, was included in the TOXIN5 database during its construction. The CrylAb protein did not demonstrate any structural similarity with any proteins that may present toxicity to humans and animals.

Potential structural similarities shared between the CrylAb protein and proteins in the ALLPEPTIDES database were evaluated using the FASTA sequence alignment tool. Identified proteins were ranked according to their degree of similarity. The best similarity observed was to the *Bacillus thuringiensis* pesticidal crystal protein CrylAb, demonstrating an overlap of 817 aa, with 99.9% identity and an *E* score of zero. The remaining alignments with significant E scores are to Cry protein homologues. As a result, the CrylAb protein does not share any structural similarity with any proteins that may have adverse biological activity toward humans and animals.

Comment 2

No comment

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

Comment 1

No comment

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Comment 1

No comment

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

7) Testing of the whole GM food/feed.
Since this dossier is intended as a renewal of an existing application, no further testing is recommended. All necessary tests have been performed previously.

Comment 2

Agreement with the provided information.
Nevertheless metabolomic studies should be carried out in order to complete the existing results (see examples in Paris *et al*. 2006; Manetti *et al*., 2006).

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

Simulated gastric fluid and simulated intestinal fluid (P.107-109, Technical dossier) were used to test allergenicity. However, Bannon et al. (2003) and Herman et al. (2006) concluded that the use of the SGF technique to predict the allergenic status of the proteins remains uncertain. On the other hand, the Cry1Ab protein represents a very small portion of the total protein in MON 810.

Comment 2

Assessment of allergenicity of the introduced traits.
One remark: the applicant states that Cry1Ab is only a small part of the total protein content as an argument to confirm Cry1Ab as being non allergenic. However, only the titration of the protein of interest is valuable, not the determination of its relative content. In this respect, the levels of Cry1Ab are described at around 0.3 µg/g in the maize grain. This means that the ingestion of 300 g of non-concentrated maize-derived food product gives 90 µg of Cry1Ab, which lies in the lowest levels currently observed to be able to elicit allergic reactions.
This might have been an issue in the case of Cry1Ab being allergenic. However, Cry1Ab was already described as non-allergenic, and there is no new data to contest this.

Assessment of allergenicity of the whole GM plant.
This has not been evaluated in the application. The reviewer wishes to emphasize that the rationale of this section is to evaluate, due to the introduction of the new traits, possible changes in the allergenicity of the recipient plant when this plant is known as an allergenic source.
Although not frequent, food allergy to maize exists and allergens have been determined (Pastorello et al. 2003; Pasini et al. 2002, Weichel et al. 2006). The introduction and expression in the plant of Cry1Ab might interfere with other maize proteins, including allergens, and modify their expression levels. Care must be taken that food allergy to maize grain does not become more frequent due to the introduction of new traits and the interferences thereof. For that reason, it is relevant to analyze whether the expression levels of known major allergens is increased in genetically modified MON810 maize grains. Patient IgE binding to maize grain extract or titration of known major allergens of maize should be carried out for the GMO and natural counterpart.
Given that the application also deals with cultivation in the E.U, another concern is maize pollen allergy. Although literature on that subject is scarce, allergy to maize pollen is well known in the allergy outpatient departments of the clinics and of the independent allergologists. It results from cross-reactivity with grass pollen, and is a major allergy problem in children living near maize fields. The most known cross-reacting allergens are Zea m 1 and Zea m 13, that cross-react with the group 1

and 13 allergens of grasses (Petersen et al. 2006). Therefore, the expression level of those major allergens should be determined in the pollen of genetically modified maize MON810.

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1

P.65, Table 10 (Technical dossier) tryptophan concentration in MON 810 is different from MON 818, although means are identical, but ranges are somewhat different. It looks like 0.6 is not the mean for MON818 with a range from 0.4 to 0.6; This may be verified.
Compositional data for protein, fat, ash, ADF, NDF, fat, carbohydrates and dry matter for MON 810 were similar to the control, MON 820. How is dry matter in Table (Technical dossier)14 expressed? Units are lacking. Compositional equivalence has been established for MON 810; therefore, nutritional equivalence can be assumed (Clark and Ipharraguerre, 2001; Flachowsky and Aulrich, 2001).

Comment 2

Full agreement with the provided information.

D.7.11 Post-market monitoring of GM food/feed

Comments/Questions of the expert(s)

Comment 1

Full agreement with the provided information.

D.8. MECHANISM OF INTERACTION BETWEEN THE GM PLANT AND TARGET ORGANISMS (IF APPLICABLE)

Comments/Questions of the expert(s)

Comment 1

No comment related to this section.

D.9. POTENTIAL CHANGES IN THE INTERACTIONS BETWEEN THE GM PLANT WITH THE BIOTIC ENVIRONMENT RESULTING FROM THE GENETIC MODIFICATION

D.9.1. Persistence and invasiveness

Comments/Questions of the expert(s)

Comment 1

The likelihood for maize to persist or being invasive in Europe is very low and non-significant. Nevertheless recommendations made in section B3 should be considered in a routine GS plan.

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

Comment 1

In general, Cry1Ab toxin doesn't confer a significant and sustainable selective advantage to maize in natural habitats since maize is sensitive to other externalities as temperature, water stress, non-target pests and diseases. In general, in Europe, maize has a very low competitiveness or fitness in natural environment due to the effects of non-biotic stress, and mostly night temperature below 15°C and frost (Davies *et al*., 1996). Nevertheless there is an increased risk in the South western region of Spain and Sicily.

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

Comment 1

See comment in section B.4.
At the landscape level, confirmation of Brunet's (2006) study should be made but detected hybridization rates are below the 0.9% labelling threshold.
The most recent studies (Husken *et al*., 2007; Sanvido *et al*., 2007) confirm that a separation distance of 50 meters is sufficient to contain maize pollen dispersal under a 0.9% labelling threshold.

A reasonable distance of 50 m. between non-GM labelled field and GM field should be recommended to comply with coexistence measures.

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

Comment 1

Toxin resistance is a major threat in the environmental risk assessment and recent results show that it is appearing in another species in Africa. As published recently (Van Rensburg, 2007), toxin resistance may evolve and present a tangible risk which must be address with a specific monitoring. In that study, which has been conducted after reports of severe damage caused by *Busseola fusca* (stem borer), substantial numbers of larvae from the Bt derived population survived over the entire period of the bioassay.
Variations in toxin expression according to the plant age and environmental conditions can disturb the "high dose" strategy (Dutton et al., 2004). In addition, the monitoring of Bt expression shows that concentrations vary strongly between different plant individuals of Mon810 (Nguyen et al., 2007).

Baseline susceptibility and resistance allele frequency of the ECB is not uniform and depends on the sampling structure (population and region) (Meise and Langenbruch, 2007), so more studies should still be done to reduce uncertainty about resistance acquisition.
As the risk of a resistance outbreak related to ECB and *Sesamia* is probable, IRM plans must be carefully respected in the scope of the Industry IRM Working Group (Alcalde *et al*., 2007).

Comment 2

See my comment in D.11

D.9.5 Interactions of the GM plant with non-target organism

Comments/Questions of the expert(s)

Comment 1

In Romeis *et al*. (2006) the effect of purified toxin has been reviewed on four non-target species belonging to three families (Chrysopidae, Anthocoridae, Coccinellidae) in a contained environment. Some of these studies are not recent (1997-2004) and the authors (Pilcher *et al*., 1997; Al-Deeb *et al*., 2001; Pons *et al*. 2004) didn't find significant effects of the toxin on the mortality or development of these insects. Based on the above studies and some others the application for renewal states that (page 126) "Cry1Ab has a selective toxicity towards certain Lepidopteran pests but not against other orders". This statement should be moderated because there is an uncertainty about the Cry1Ab activity on some non-target organisms through the "gene *x* environment" interaction:

- According to their results Prasifka *et al*. (2007) assume that "Monarch larvae exposed to Mon810 anthers behave differently and that ingestion may not be the only way Bt can affect non-target insects".
- Bt environment affects non-target fauna compared with non sprayed agro-system (Marvier *et al.*, 2007).

- Manachini *et al*. (2003) and Bourguet *et al*. (2002) found a decrease of the biocontrol function among specialist antagonist of target pest.
- Daly *et al*. (2005) and Pilcher *et al*. (2005) found a decrease of natural enemy abundance of *Nabis sp*.
- Variable effects have been observed on earthworms (Zwahlen *et al*., 2003; Clark and Coats, 2006).

Existence of interactions between the quality of the preys and the Bt toxin may cause mortality of some predator species (Hilbeck 1998; Dutton *et al*., 2002; Meissle *et al*., 2005). Although Bt has negligible direct effect on the non-target insects, indirect effects are suspected. Furthermore, slight but significant effects are observed in the abundance of lepidopteran, dipteran and hymenopteran species (Candolfi *et al*., 2004). Hilbeck and Schmidt (2006) reported that "the key experiments explaining the mode of action in most non-target species (as *Chrysoperla carnea*) are still missing. Considering the steadily increasing global production areas of Bt crops, it seems prudent to thoroughly understand how Bt might affect non-target organisms". At this stage and given that: 1- scientific studies are not completed and didn't fully demonstrate the innocuousness of Bt toxin on non-target organisms; 2- continuous expression in the plant and its uncertain effect on non-target organism, studies (case-specific or general, this should be determined at EU decision levels) on predator/parasitoid and neutral arthropod bioindicators should be carried on (through specific monitoring in the fields).

Mon810 cropping with the change of control practices can have an indirect effect on non-target pests. Increasing non-target pest (Aphids and leafhoppers) pressure has been reported in Bt Maize (Eizaguirre *et al*., 2006) and deserves additional field monitoring and updated control recommendations.

Conclusion: It is accepted that a rational Mon810 management may reduce pesticide use and doesn't pose unreasonable adverse effects to non-target wildlife in comparison to full pesticide management. The deregulation of Mon810 may be re-conducted provided that accurate monitoring plans will be put in place.

Comment 2

I can agree with the applicant that many studies have demonstrated no or little *direct* effects of the Cry1Ab-endotoxin on non-target invertebrate organisms, including non-pest herbivores, pollinators and carnivorous natural enemies.

Only few studies have reported adverse effects of Cry1Ab toxin on non-target organisms. Lövei & Arpaia (2005) and Romeis et al. (2006) provide reviews of the literature on side effects of Cry proteins to beneficial arthropods. Laboratory studies by Hilbeck et al. (1998, 1999) revealed some negative effects on the predatory insect *Chrysoperla carnea*, but later studies showed that these adverse effects were not direct toxic effects but were mediated by (nutritional) quality of intoxicated prey, i.e. an *indirect* effect (Romeis et al., 2004; Rodrigo-Simon et al., 2006). Lower prey quality was also believed to be partly or wholly responsible for adverse effects observed in the generalist carabid predator *Poecilus cupreus* and the parasitoid *Cotesia marginiventris* offered prey or hosts that were fed Bt maize (CryIAb) (Meissle et al., 2005; Vojtech et al., 2005); the experiments in Meissle et al. (2005) showed that the ground beetle did not avoid Bt-containing prey, which means that exposure in the field may occur. However, in a study on transgenic canola expressing CryIAc on another carabid beetle as a non-target indicator organism (Ferry et al., 2006), the results suggested that behavioural preferences of the predator (i.e., rejection of contaminated prey) would mitigate adverse indirect effects of reduced prey quality caused by consumption of Bt-canola plants.

The degree of exposure suffered by non-target organisms has been the subject of different studies with variable outcome. The applicant may be criticized for only listing studies in the literature review in Annex 3.1 showing that exposure of non-target organisms is negligible or non-existent. For instance, the applicant refers to a study by Harwood et al. (2006) showing that there was no evidence for uptake of Cry1Ab endotoxins by a carabid predator in laboratory and field experiments. However, the applicant fails to mention other studies by the same authors that do indicate such uptake and suggest that the toxin may transfer into higher order trophic levels of food chains (Harwood et al., 2005, 2007). Similar findings (i.e., that Bt toxins can be transferred to predatory arthropods) were reported in a field study by Obrist et al. (2006). Likewise, Alvarez-Alfageme et al. (2008) showed that Cry1Ab toxin was transferred in undegraded form from Bt-maize to the predatory coccinellid *Stethorus punctillum* via its tetranychid prey, but that the predator did not suffer adverse effects from the toxin.

The applicant refers to a number of field studies supporting the safety of Cry1Ab containing crops to a wide range of beneficial insects. The overall conclusion of these studies, that there are little differences in non target communities in Bt corn and non-Bt corn (where conventional insecticides are used), is well interpreted by the applicant. This conclusion is corroborated by two 3-year farm-scale studies in Spain focusing on arthropod predators (de la Poza et al., 2005; Farinos et al., 2008). On the other hand, a study by Pilcher et al. (2005) demonstrated lower densities of a specialist parasitoid in Bt corn plots, as a result of the lower abundance of its host, the target pest *Ostrinia nubilalis*. A study by Bourguet et al. (2002) also indicated that indirect effects on populations of certain more specific natural enemies of the different lepidopteran target pests would be expected if pest populations would be reduced as the result of the efficient control exerted by the expression of Cry-endotoxins (EFSA, 2006). Although similar effects are likely to occur also in non-transgenic maize where conventional pesticides are being used, I expect that the effect in transgenic maize may be less transient given that the toxin is expressed at very high levels (>99% level of efficacy, Pilcher et al. 2005) and continuously throughout the crop's cultivation period. Large-scale adoption of the transgenic maize may exacerbate these effects. Therefore, it is imperative to install sufficient refuges consisting of non-transgenic maize to avoid adverse impacts on these specific natural enemies.

Effects on non-pest lepidopterans feeding on maize would be expected, but the crop does not constitute an important resource of food for indigenous butterflies in Europe; pollen is only shed by Bt-maize plants during a limited window of time and it remains in the immediate vicinity of the crop, so possible adverse effects are expected to be transient and local. In the USA, mainly effects on the monarch butterfly (which does not occur in Europe) were studied. Although some of these studies showed some adverse fitness effects, they were not considered likely to pose a significant risk to the monarch butterfly populations in North America (see Dively et al., 2004; Anderson et al., 2005 and references therein). Little is known on the distribution of European lepidopteran species in agricultural landscapes and their potential exposure to Bt maize (e.g., Gathmann et al., 2006). However, negative impacts on native butterflies were reported by Darvas et al. (2003, 2004 a,b). According to latter studies Cry1Ab toxins may kill some 20% of hatching *Inachis io* caterpillars on nettle plants within 5 m of MON810 Bt maize. However, it should be stressed that:

- these Hungarian papers and the data therein were inaccessible to me
- two of these references are merely abstracts of symposia

- none of these data were, as far as I could retrieve, ever subjected to peer review, or at least disseminated into the international scientific literature; this is all the more striking given the relevance of the reported findings

A study by Rosi-Marshall et al. (2007) published in the renowned journal PNAS showed that species of Trichoptera (caddisflies) occurring in headwater stream systems suffered negative effects of Cry1Ab from Bt maize in laboratory feeding trials. This study has been used to underline that an environmental risk assessment of Bt maize should also take into account water-dwelling insects. However, this paper has received serious criticism for its inappropriate methodology and unfounded conclusions in two letters to PNAS (Beachy et al., 2008; Parrott, 2008) and a scientific opinion paper in Trends in Biotechnology (Miller et al., 2008). The arguments laid out in these latter papers were entirely followed by EFSA (2007) and I can agree with these.

Interestingly, however, negative effects of Cry1Ab were recently reported for another water dwelling organism, the water flea *Daphnia magna* (Bøhn et al., 2008); the laboratory experiments indicated a toxic effect rather than a lower nutritional value of the Bt-maize.

Comment 3 (received after EFSA deadline for comments)

The monarch butterfly is used as an example that Bt pollen is unlikely to pose any significant risk to the sustainability of the populations of butterflies living near maize fields. That the impact of Bt-maize on that American butterfly is negligible was indeed quite convincingly shown.
I have doubts however if that result can be extrapolated to the European situation.
In general the North-American and European agricultural landscapes are very different in spatial configuration, with the latter being much more fragmented and characterised by a fine-grained mixture of agricultural fields and natural and semi-natural elements, e.g. grassy field boundaries, hedgerows, margins of field roads, ditches, small nature reserves and other elements of the green infrastructure of the European agricultural landscape. The potential harmful effects of Bt-maize on Lepidoptera occurring in these landscape elements (possibly including threatened species) might therefore be much greater in Europe and hence need to be assessed. This was for instance done by Felke & Langenbruch (2005) in Bavaria for butterfly larvae. After two years of field observations they found that 26 diurnal and 53 nocturnal species of butterflies would be exposed to Bt maize pollen with high probability. They concluded: "Summarizing the results of the presented study we recommend only to permit cultivation of Bt-maize with negligible toxin expression in pollen to minimize potential risks for non-target butterflies. Despite this, transgenic maize fields should be surrounded by at least ten rows of non-transgenic maize hybrid to prevent dispersion of Bt-toxin containing pollen. Also cultivation of genetically modified maize should be prohibited near nature reserves". This could imply that the cultivation of Bt maize should be more strictly regulated in regions rich in nature as well within reserves as between agricultural fields, e.g. areas delimited on the basis of the Habitat and Bird directives.

D.9.6 Effects on human health

Comments/Questions of the expert(s)

Comment 1

EFSA (2006) concluded that there is no reason to believe that the continued placing on the market and MON810 maize is likely to cause any adverse effects for human health.

Comment 2

No detrimental effects on human health have been detected so far. Nevertheless risk assessment should be carried on using new methods such as metabolomics (Paris *et al*., 2006), which show in preliminary studies the metabolic variations by the presence of Bt transgene (Manetti *et al*., 2006).
In contrast Mon810 may improve grain quality by reducing levels of mycotoxins in the grains (Bakan *et al*., 2002; Papst *et al*., 2005; Hammond *et al*., 2006).

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1

EFSA (2006) concluded that there is no reason to believe that the continued placing on the market and MON810 maize is likely to cause any adverse effects for animal health.

Comment 2

No detrimental effects on animal health have been detected so far.

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

Comment 1

Accinelli et al. (2008) reported that there may only be a limited risk of accumulation of Cry1Ab protein in the soil due to the release from Bt-crops.

Comment 2

Risk assessments studies show, once again, conflicting information due to methodological limitations (Accinelli *et al*., 2008) or lack of multidisciplinary vision. The role of cultivation practices on the impact of Bt in the environment hasn't been enough taken into account:

- Fu *et al*.(2008) report the occurrence of interactions between inorganic salts (contained in mineral fertilisers) and Bt toxin adsorption that impact the fate of Bt toxins in the soil.
- Icoz et stotzky (2008) report that microbial process play a major role in the dissipation of CryA toxins and this process depends, in turn, on soil type, seasons, cultivar, crop practices…

Bt concentrations in the soil don't always decrease as faster as described in section D 9.5. Marchetti *et al*. (2007) report a DT_{50} of 10 or 11 days for sandy and clay soil, which is higher than the 1.5 day mentioned in the application for renewal.

Additional concentrations of Bt toxins in the soil due to GM cultivation affects the GRAM+/GRAM^{-} ratio compared with a non-GM crop (Xue *et al*., 2005). Most of the results published in this area are still preliminary (Evans *et al*. 2002; Zwahlen *et al*. 2003) and need more complements. Nevertheless, at this stage of the knowledge, current studies show no significant or acute detrimental effects of CryA toxins on soil microorganisms or soil microbiology.

Comment 3

The applicant dossier and the studies referred to therein have reported no or negligible effects of the expressed Cry1Ab proteins on detritivorous organisms living in and on the soil. For instance, there are no reported effects of the Cry1Ab toxin on the annelid *Eisenia fetida* (an epigaeic compost worm that is usually not found in maize fields) nor on the collembolan *Folsomia candida*. A number of studies have reported no negative effects of Cry1Ab expressing maize on other soil organisms (discussed in EFSA 2006).

On the other hand, there are reports on sublethal effects of the Cry1Ab expressing GM maize on immatures and adults of the anecic earthworm *Lumbricus terrestris* when fed on litter of the GM maize, although the adverse effects could not be confirmed in higher-tier (small scale) field trials (Zwahlen et al., 2003). In a laboratory study, Vercesi et al. (2006) reported no detrimental effects of Bt maize residues on development and fecundity of the earthworm *Aporrectodea caliginosa* (which is a more relevant species in an agricultural setting), with the exception of a slight decrease in cocoon hatchability. However, the authors questioned whether this effect would have any ecological significance in the field. Wandeler et al. (2002) studied the consumption of Bt- and non-Bt-maize by the woodlouse *Porcellio scaber* and reported that the woodlouse fed less on the Bt maize than on the corresponding non-Bt control variety. They also found that the woodlouse was effectively exposed to the toxin, but made no mention of adverse effects on the organism. In a glasshouse experiment, Griffiths et al. (2006) reported some effects of Cry1Ab expressing maize on soil microbial and faunal communities but these effects were all minor and comparable to those of conventional ("current best practice") insecticide treatments.

It needs emphasis again that so far no published studies have described the consequences of long-term cultivation of Bt corn on earthworms and other soil dwelling animals.

D.9.9 Impacts of the specific cultivation, management and harvesting techniques

Comments/Questions of the expert(s)

Comment 1

Mon810 is currently a reliable means to control ECB but, as mentioned in section D 9.4, there is a risk that target insects develop CryA toxin resistance in the medium or long term.

The report states that Mon810 “reduces the use of chemical insecticides and reduces applicator exposure to dangerous active ingredients” but doesn’t mentions the number of insecticide sprays that can be saved.

ECB and *Sesamia* are among the major pests in maize fields and may cause 13% yield losses. ECB reinforces its presence in southern France and progresses rapidly up north where two generations can grow in a season. In France, infestation hotspots are seen in the Rhone region and South-West. However, in most of the regions spray numbers are still low: in Poitou-Charentes in France (see Agreste Report, 2003), where an average larvae density is about 0,8 larvae/plant, only 42% of the maize fields are sprayed (mostly with pyrethroïds) and receive 1.4 sprays on average. Insecticide quantities, mostly Cypermethrin and Lamda Cyhalothrin, respectively average 0.08 and 0.01 kg/ha, which is not an exaggerated load for the environment in comparison with other crops. Similar statistics are found in Germany and Spain (Brookes, 2002). This information is confirmed by the Mon810 monitoring report (Schmidt and Tinland, 2006).

In conclusion, the impact of the pesticide reduction due to Bt maize on human health or environment still remains weak and doesn’t represent a major benefit.

No reference is made about coexistence measures. However specific lay-out, spatial organisation and separate chain processing may be required in some areas (Messean, 2006b). These measures generate additional economic costs, which should be discussed.

Mon810 should be recommended in a IPM management system, which should be detailed by the Monsanto Company.

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

Comment 1

Cry1A toxin concentrations in water must be assessed as no accurate response about effects and degradation in water has been made (Rosi-Marshall *et al*., 2007). This calls for a deeper specific environmental assessment.

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

Comment 1

The applicants describe an insect resistance management (IRM) plan for case-specific monitoring of resistance development in corn rootworms that is generally based on methods that have up to now been widely accepted by the community of scientists and practitioners.

However, care should be taken to meticulously implement and continuously evaluate and, if needed, adjust the recommended measures in the plan, particularly if large scale adoption of the Bt/herbicide tolerant maize would change existing cropping methods (e.g., related to crop rotation or tillage) possibly affecting abundances of the target pests, other herbivores and natural enemies.

Also, some of the key assumptions for the high-dose refuge strategy which forms the basis of the environmental monitoring plan, may not be entirely fulfilled.

Farinós et al. (2004) highlighted the need to adapt the insect resistance management strategies for certain areas. It has been shown that 1) *Sesamia nonagrioides* females mate before they move for oviposition, so that females emerging from refuge would rarely mate with potential resistant moths emerging from Bt maize fields and vice versa, and 2) *Ostrinia nubilalis* mobility is also reduced before oviposition in irrigated maize fields, which corresponds to the agronomic practices of most maize growin areas in Spain. These findings need to be considered in the IRM plan.

Dalecky et al. (2006) further pointed out that:
- the behavioural ecology of stem borers, as related to the timing between dispersal and mating, may differ from species to species (and so may be different between *Ostrinia nubilalis* and *Sesamia* spp.)
- the high dose refuge strategy, in which refuges are situated a few hundred meters from Bt maize fields, may not ensure complete mixing between susceptible and resistant *Ostrinia nubilalis* moths, because some pre-dispersal mating occurs for both males and females (up to about 57% of newly emerged females have been found to mate locally)
- Bt-resistance alleles in resistant stem borers may be associated with fitness costs, decreasing dispersal and mating success; as such, intermixing between susceptible and resistant moths may be further compromised

Eizaguirre et al. (2006) showed that in *Sesamia nonagrioides*:
- a high proportion of larvae may move to plants in adjacent rows, favouring the survival of partially resistant individuals; this suggests that mixing of Bt and non-Bt maize in the same field would not be a recommendable strategy to delay resistance
- surviving adults that have been exposed to sublethal concentrations of the Bt toxin have an asynchronous development compared to individuals originating from non Bt refuges which limits the random mating between susceptible and resistant individuals

- individuals exposed to sub-lethal levels of Bt maize may have lower fitness (e.g., as related to their responsiveness for diapause inducing and terminating cues)

Although these studies show that the main prerequisite for random mating in the high-dose refuge strategy proposed in the resistance monitoring plan may not be fulfilled, there have been no field reports to date of resistance in lepidopteran stem borers towards Bt-maize expressing Cry1Ab, or other Cry toxins.

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

Comment 1

Although no acute negative effects of Mon810 on the environment have been detected, there is still numbers of uncertainties related to interactions between the GM plant and target organism and interactions between the GM plant and non target organism.

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

Comment 1

1) Interactions between the GM plant and target organisms, case-specific monitoring with risk management.

The IRM plan is complete. Nevertheless poor adoption rate of refuges in some regions should be improved through a best organisation within farming communities (see French cooperatives). To delay the risk of insect resistance and reinforce the IR management, Mon810 should be introduced when a certain average pest pressure threshold (example: 0.8 larvae/plant) is reached in a given area and should not be adopted below (see Hochberg *et al*. (2006) for more details).

2) Additional case-specific monitoring without risk management

Contradictory results (pointed in section 9.5 and 9.8) should be re-assessed by the scientific community (Itps, EFSA….) on a multi-disciplinary base, under standard process and methods.

D.11.4 General surveillance of the impact of the GM plant

Comments/Questions of the expert(s)

Comment 1

In a study on the associates of European Corn Borer, Freier *et al*. (2007) estimate the presence of a thousand arthropod species in maize fields. Impact study on each species will never be undertaken resulting in persisting shading areas of scientific knowledge. This complexity doesn't avoid an accurate monitoring through some bioindicators.

Question: Is there a parallel field/farm monitoring plan put in place by the public sector (INRA or others) or does field data rely only upon Monsanto monitoring network? This information doesn't appear clearly in the report.

Farm questionnaire: not enough accurate for non-target arthropods ?

- The observation data related to the monitoring character is quality based and leads sometimes to inaccurate results, whatever reliable the non parametric statistical analysis can be.
- Data related to fertiliser application is not accurate and doesn't consider doses/ha.
- Insecticides are simply mentioned without indication of doses, quantity or number of sprays during the agricultural season. This avoids the comparative evaluation of the biocide charge in the environment (a farmer can decrease the number of sprays but increase the quantity of insecticide).
- Main weed species occurrence should be mentioned in comparison with non-Bt fields.
- The questionnaire focuses mainly on target and non-target pests, when non-target neutral arthropods and beneficials are not.

The observation data should be based on counts of bioindicators belonging to the different existing functional groups (ECB parasitoids, predators, neutral species, earthworms, amphibians, birds…) through sampling or trap monitoring, according to a precise protocol (see methods in Delos *et al*, 2006).

Interactions between the GM plant and non-target organism

Contradictory results have been reported about predatory insects (see introduction in Marvier *et al*., 2007) because of deficient protocols or analysis. The interaction between Bt toxin and the quality of the target prey must be more deeply investigated and should be included in an ERA plan: would non-Bt starving preys induce the same effect as Bt infected preys?

Uncertainties due to the difficulty of interpretations present in the conclusions of impact studies as seen in Candolfi et al. (2004), related to the impact of Bt toxin on lepidopteran, dipteran and hymenopteran species in the field in particular, should be cleared within an accurate monitoring plan. This plan could be assimilated to a case-specific monitoring, in the long term, to test the hypothesis of an indirect adverse effect of Bt toxin on the non-target fauna. Results of the 2006 monitoring report (Schmidt and Tinland, 2006) indicating a non-expected effect on Diptera and Araneae support this suggestion.

Toxin expression

Since the combination of non-biotic stresses may affect toxin concentration in the plant (Dutton et al., 2004), the results of the GS plan (target pest pressure, presence or absence of irrigation) should be systematically linked with local climatic data, to complete the monitoring of the efficacy of Mon810 on target insects and the high-dose & refuge IRM.

Areas to be monitored (section 11.4.3.3.)

There is an inconsistence at line 10-12: “the **Glyphosate** trait has utility in a wide range of agricultural environment. Therefore, the introduction of Mon810 is not confirmed to specific geographical zones” It should be: the **Bt trait** has utility….

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

Comment 1

The general surveillance is adapted for a broad agricultural survey focused on potential and additional benefit to the farming unit. On the other hand, it seems to be less adapted to environmental impacts.

In order to address specific impact issues of Mon810, GM-fields should theoretically be compared with non-GM fields of the same farm or agrosystem. In the GS monitoring reports (2005 and 2006) presented in Annexe II of the dossier, data from small GM-fields (less than 5 ha, without obligation to plant refuge) have been processed with data from large GM-fields. Especially in the case of Spain, where refuges are not often respected and where Mon810 plantings are the majority (see the rate of all maize/Mon810 acreage), no direct comparison between Bt and non-Bt maize could be made in the same farming unit. Introduction of bias and misinterpretation of data may occur:

- If the GM farmer made a comparison with one of its neighbours’ non-GM field (without knowledge of the cropping history),
- If the farmer refers to non-GM data from previous years.

Some of the results of the GS reports should also be analysed and presented separately at a regional or national scale (highlighting treatment x local environment). This is the case for the incidence of diseases, which are sometimes specific to a region. The interaction between Bt and the environment or agrosystem should be more often taken into account.

Finally, only two general surveillance reports have been presented separately in the annexes (2005, 2006). It would have been interesting to access to results of previous years and compiled it to draw multi-annual trends.

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