SCIENTIFIC OPINION

Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2010[1]

EFSA Panel on Genetically Modified Organisms (GMO)[2, 3]

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ABSTRACT

Following the request from the European Commission, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) assessed the monitoring report for the 2010 growing season of maize MON810 provided by Monsanto Europe S.A. On 7 September 2011, the EFSA GMO Panel already adopted a scientifc opinion on the 2009 monitoring report of maize MON 810. The EFSA GMO Panel followed the same approach as for the assessment of the 2009 monitoring report and assessed, in close collaboration with the EFSA Unit for Scientific Assessment Support, the methodology applied by the applicant for the Case-Specific Monitoring and General Surveillance of maize MON 810 in 2010. Concerning the Case-Specific Monitoring (CSM), the EFSA GMO Panel considered the plan for Insect-Resistant Management mainly based on the "high dose/refuge strategy", monitoring of target pest resistance and education of farmers. Concerning General Surveillance (GS), the EFSA GMO Panel paid particular attention to the design and analysis of the farmer questionnaires. The EFSA GMO Panel notes similar shortcomings in the methodology for CSM and GS as in the 2009 monitoring report. Hence, the EFSA GMO Panel reiterates the same recommendations for improvement of the methodology for the post-market environmental monitoring of maize MON 810 as in its scientific opinion on the 2009 monitoring report of maize MON 810. However, from the data submitted by the applicant in its 2010 monitoring report, the EFSA GMO Panel does not identify adverse effects on the environment, human and animal health due to maize MON810 cultivation during the 2010 growing season. The outcomes of the 2010 monitoring report do not invalidate the previous EFSA GMO Panel's scientific opinions on maize MON 810.

KEY WORDS

GMO, PMEM, annual report, cultivation, case-specific monitoring, general surveillance, insect-resistance management, maize, MON 810

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SUMMARY

On 7 September 2011, in response to a request from the European Commission, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) adopted a scientific opinion on the monitoring report of maize MON 810 for the 2009 growing season provided by Monsanto Europe S.A.. During its assessment of the 2009 monitoring report, the EFSA GMO Panel identified shortcomings in the methodology for both Case-Specific Monitoring (CSM) and General Surveillance (GS) of maize MON 810 and hence provided recommendations for improvement of the Post-Market Environmental Monitoring (PMEM) of maize MON 810.

Following a similar request from the European Commission to assess the monitoring report of the same maize MON 810 for the 2010 growing season, the EFSA GMO Panel firstly noted that the applicant followed the same approach as in its 2009 monitoring report.

The EFSA GMO Panel assessed, in close collaboration with the EFSA Unit for Scientific Assessment Support, the methodology applied by the applicant for the CSM and GS of maize MON 810 in 2010. The methodology used for the monitoring of maize MON 810 in 2010 is similar to what was done in 2009. Therefore, the EFSA GMO Panel mostly focused on the new 2010 datasets, i.e., data from farmer questionnaires, from a survey in Spain on refugia compliance and from monitoring resistance development in target pests.

The EFSA GMO Panel notes similar shortcomings in the methodology for CSM and GS as in the 2009 monitoring report. Hence, the EFSA GMO Panel reiterates the same recommendations for improvement of the methodology for the PMEM of maize MON 810 as in its scientific opinion on the 2009 monitoring report of maize MON 810.

However, from the data submitted by the applicant in its 2010 monitoring report, the EFSA GMO Panel does not identify adverse effects on the environment, human and animal health due to maize MON810 cultivation during the 2010 growing season. The outcomes of this 2010 monitoring report do not invalidate the previous EFSA GMO Panel's scientific opinions on maize MON 810.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION AND EFSA

Genetically Modified (GM) maize MON 810 (notification reference C/F/95/12-02) was authorised under Directive 90/220/EEC (EC, 1990) in the European Union (EU) for all uses (with the exception of food uses) by the Commission Decision 98/294/EC (EC, 1998). A final consent was granted to the applicant (Monsanto Europe S.A.) by France on 3 August 1998. Food uses of maize derivatives were notified according to Article 5 of the Novel Food Regulation (EC) No 258/97 on 6 February 1998.

Following the request by the applicant for the renewal of the authorisation for placing maize MON 810 on the market, the EFSA GMO Panel adopted a scientific opinion on the renewal under Regulation (EC) No 1829/2003 of maize MON 810 for import, processing for food & feed uses and cultivation in June 2009 (EFSA, 2009). The EFSA GMO Panel concluded that « *maize MON 810 is unlikely to have any adverse effect on the environment in the context of its intended uses, especially if appropriate management measures are put in place in order to mitigate possible exposure of non-target (NT) Lepidoptera* ». The EFSA GMO Panel recommended that, especially in areas of abundance of non-target Lepidoptera populations, the adoption of the cultivation of maize MON 810 be accompanied by management measures in order to mitigate the possible exposure of these species to maize MON 810 pollen. In addition, the EFSA GMO Panel advised that resistance management strategies continue to be employed and that the evolution of resistance in lepidopteran target pests continues to be monitored in order to detect potential changes in resistance levels in pest populations. The EFSA GMO Panel agreed with the overall approach and methodology proposed by the applicant for GS, but advised the applicant to describe in more detail how information will be collected that could be used to assess if the intended uses of maize MON 810 are having unanticipated adverse environmental effects.

From 2005 onwards, the applicant submitted to the European Commission monitoring reports on the cultivation of maize MON 810 according to the requirements laid down in Directive 2001/18/EC (EC, 2001).

On 4 November 2010, the EFSA GMO Panel received a first request from the European Commission to assess the monitoring report submitted by Monsanto on the cultivation of maize MON 810 in 2009. EFSA therefore established a 'Standing Working Group on the annual PMEM reports' in charge of the assessment of annual PMEM reports of GM crops cultivated in the EU.

On 7 September 2011, the EFSA GMO Panel adopted a scientific opinion on the 2009 monitoring report on maize MON 810 (EFSA, 2011b). The EFSA GMO Panel noted shortcomings in the methodology for CSM and GS and hence made recommendations for improvement of the PMEM of maize MON 810. However, from the data submitted by the applicant, the EFSA GMO Panel did not identify adverse effects on the environment, human and animal health due to maize MON 810 cultivation in 2009.

On 8 November 2011, the EFSA GMO Panel received from the European Commission a request to assess the following monitoring report submitted by Monsanto on the cultivation of maize MON 810 in 2010 (hereafter referred to as '2010 MON 810 report'). On 21 December 2011, and according to the EFSA GMO Panel scientific opinion providing guidance on PMEM of GM plants (EFSA, 2011a), the applicant was required to provide raw data (i.e., farmer responses to questionnaires). The missing data were received on 24 January 2012.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION AND EFSA

On 8 November 2011, the EFSA GMO Panel received a request from the European Commission (EC) '*to assess the maize MON 810 monitoring report for the 2010 cultivation season provided by Monsanto and the related comments from Member States. This assessment should be reported through the adoption of an opinion which should include the analysis of the appropriateness of the methodology of implementation and also clearly indicate the potential consequences of this assessment on the safety of the GMO in question. The European Commission asked the EFSA GMO Panel to adopt a scientific opinion by January 2012.*'

ASSESSMENT

1. INTRODUCTION

Maize MON 810 was developed by the applicant, Monsanto Europe S.A., to express the Cry1Ab protein, derived from *Bacillus thuringiensis* subsp. *kurstaki* (Bt), which confers protection against the lepidopteran target pests European corn borer (ECB; *Ostrinia nubilalis* Hübner) and Mediterranean corn borer (MCB; *Sesamia nonagrioides* Lefebvre). Maize MON 810 is currently cultivated in the EU in countries such as the Czech Republic, Poland, Spain, Portugal, Romania and Slovakia. The applicant reports to the EC and Member States on an annual basis the results of its monitoring activities on the cultivation of maize MON 810 in the EU.

As was the case for the 2009 monitoring report of maize MON 810, the EFSA GMO Panel was asked by the EC to assess the monitoring report of maize MON 810 for the subsequent growing season (hereafter referred to as the "2010 MON 810 report"). The applicant[4] adopted the same PMEM approach as it had in its 2009 MON 810 report and:

(1) reported the results of its insect resistance management (IRM) plan, including data related to the implementation of non-Bt refugia and the evolution of the target pests' resistance, as well as information on farmer education;

(2) reported the results of its GS monitoring programme, including the analysis of the questionnaires answered by selected farmers in the EU Member States where maize MON 810 was cultivated in 2010;

(3) submitted a review of peer-reviewed publications on the safety of maize MON 810 and the Cry1Ab protein.

In preparing the present scientific opinion, the EFSA GMO Panel made the best use of its previous experience with the 2009 MON 810 report. It also took into account various sources of information such as comments from Member States on the 2010 MON 810 report, the most recent scientific data and relevant peer-reviewed publications. On 21 December 2011, the EFSA GMO Panel asked the applicant to provide raw data (i.e. farmer responses to questionnaires), which was received on 24 January 2012.

In response to the mandate of the EC, the EFSA GMO Panel, in close collaboration with the EFSA Unit for Scientific Assessment Support (EFSA SAS Unit), assessed the appropriateness of the methodology (e.g. farmer survey). Considering the timeline, the EFSA GMO Panel acknowledges that the applicant could not have fully implemented the Panel's recommendations on PMEM, as referred to in its scientific opinion on the 2009 MON 810 report, in the 2010 monitoring scheme for maize MON 810 (EFSA, 2011b).

Considering the unchanged methodology for PMEM of maize MON 810 in 2010, the EFSA GMO Panel mostly focused its assessment on the new 2010 datasets (i.e. farmer responses to questionnaires, figures from a Spanish survey on refugia compliance and 2010 data on monitoring resistance development in target pests). Hence, for forthcoming monitoring reports, the EFSA GMO Panel invites the applicant to highlight the parts of the reports that contain additional datasets and novel methodology, if any, compared with the report for the previous year.

In the following chapters of this scientific opinion, the EFSA GMO Panel describes its assessment of the 2010 MON 810 report.

[4] The 2010 MON 810 report submitted by Monsanto is publicly available on the webpage of the EC Directorate General for Health and Consumers, at http://ec.europa.eu/food/food/biotechnology/index_en.htm

2. CASE-SPECIFIC MONITORING (CSM)

2.1. Summary of the information provided by the applicant

As in its 2009 MON 810 report, the applicant submitted an IRM plan developed from the approach[5] described by the industry-based EU Working Group on Insect Resistance Management. The IRM plan for maize MON 810 consists of:

(1) a strategy based on a high dose of Cry protein accompanied by non-Bt refugia in order to delay the potential development of resistance of the target pests (ECB and MCB) to maize MON 810;

(2) resistance monitoring and baseline studies on target pests' susceptibility;

(3) communication with and education of farmers (e.g. a technical user guide[6]) and a proactive education programme for farmers on compliance with implementation of refugia (e.g. letters, interviews and press articles, leaflets).

More details on these key elements of the IRM plan are described below:

(1) The applicant[7] asked farmers planting more than 5 ha of maize MON 810 to plant a refuge area with maize that does not express Cry1Ab protein within 750 m of the maize MON 810 field and corresponding to at least 20 % of the area planted with maize MON 810. The applicant specified that this 5 ha threshold relates to the total area of Bt maize, within or among fields, planted by one grower and is independent of the size of the individual fields or the total land area managed by the grower. As a consequence, the requirement for refugia can only be applicable to farm sizes of more than 5 ha. In Spain, farmer satisfaction and compliance with refugia implementation were assessed through a survey[8] sponsored by ANTAMA (a Spanish foundation supporting the use of new technologies in agriculture).

(2) Monsanto referred to a number of studies to measure the baseline susceptibility of ECB and MCB to the Cry1Ab protein. According to the approach of the aforementioned EU Working Group on Insect Resistance Management[9], bioassays should be performed on F_1 progeny whenever possible and 200–300 insects should be collected from each sampling location. The methodology used in these assays should follow those described in published work by Marçon *et al*. (1999, 2000) and Gonzalez-Nuñez *et al*. (2000). Specifically, the study should use seven to nine concentrations of each Cry protein in a diet-overlay format. Estimates for each concentration should be based on no fewer than 60 individuals per treatment/concentration using an appropriate experimental design with replication.

The applicant stated that ECB and MCB were monitored for potential development of resistance[10]. In 2010, owing to the limited number of MCB larvae required for the bioassay, the applicant used F_2 progeny from field-collected larvae. Furthermore, samples of MCB larvae collected from south-west Spain and Portugal were pooled for the first time in 2010. The applicant claimed that, in order to be effective, resistance monitoring focused *on geographical areas with considerable commercial growing of MON 810 varieties*. The applicant stated that the monitoring plan should be able to detect whether the frequency of the resistance allele remains below 5 %.

[5] MON 810 2010 PMEM report, Appendix 6.
[6] MON 810 2010 PMEM report, Appendix 3.
[7] MON 810 2010 PMEM report, Appendix 6.
[8] MON 810 2010 PMEM report, section 3.2.1.1.
[9] MON 810 2010 PMEM report, Appendix 6.
[10] MON 810 2010 PMEM report, Appendices 7 and 8.

2.2. Assessment by the EFSA GMO Panel

2.2.1. High-dose/refuge strategy

2.2.1.1. High dose

As in its scientific opinion on the 2009 MON 810 report (EFSA, 2011b), the EFSA GMO Panel agrees with the applicant that appropriate IRM strategies are capable of delaying the evolution of resistance under field conditions (Alstad and Andow, 1995; Andow, 2008; Tabashnik *et al*., 2008, 2009). Furthermore, the EFSA GMO Panel is not aware of new information on Cry1Ab expression levels in maize MON 810 that would invalidate the efficiency of the "high-dose/refuge strategy" for the two major European target pests, namely *O. nubilalis* and *S. nonagrioides*. However, for some regionally important lepidopteran pests (e.g. *Helicoverpa armigera*), the Cry1Ab protein might not be expressed in relevant plant tissues at sufficiently high toxicity doses, meaning that one of the underlying assumptions contributing to the success of the 'high-dose/refuge strategy in delaying resistance evolution is not fulfilled for maize MON 810 for those species (see section 2.3 and EFSA, 2009).

2.2.1.2. Implementation of non-Bt refugia

The EFSA GMO Panel analysed the results of the survey by ANTAMA addressing the implementation of non-Bt refugia by 100 Spanish farmers (from the Ebro Valley) who cultivated maize MON 810 in 2010. It concluded that 12 % of the farmers[11] growing maize MON 810 in 2010 did not plant a refuge area. The reasons given by the farmers for not planting a refuge area were (1) ECB causes significant economic losses; (2) sowing is easier (with Bt maize); (3) they consider their farms as small farms (i.e. less than 5 ha and therefore no refuge is required). The EFSA GMO Panel points out some inconsistencies in reporting figures of the ANTAMA survey and therefore recommends the applicant to provide the raw data of this survey in relation to the compliance with non-Bt refugia.

The 2010 MON 810 report shows partial non-compliance with the implementation of non-Bt refugia in Spain, which was further confirmed by the farmer questionnaires[12].

The EFSA GMO Panel stresses that non-compliance with refugia requirements is deemed to be one of the main reasons for the onset of resistance to Bt maize in target insects in other areas of the world (Kruger *et al*., 2012). Hence, the EFSA GMO Panel considers that the non-Bt refugia strategy should be implemented to ensure that, in any situation, there would be sufficient refuge areas to inhibit resistance evolution in target pests. The current IRM plan does not necessarily meet this requirement, as clusters of small MON 810 fields belonging to different farmers with an aggregate area higher than 5 ha might not include refugia. Hence, the EFSA GMO Panel reiterates the recommendation made in its 2009 scientific opinion on the renewal of maize MON 810 for cultivation (EFSA, 2009): "*In the case of a cluster[13] of fields with an aggregate area greater than 5 ha of Bt maize, there should be refugia equivalent to 20 % of this aggregate area, irrespective of individual field and farm size*".

Considering the current adoption rate of Cry1Ab-expressing maize in the EU, the susceptibility of target pests to the Cry1Ab protein produced by that maize is unlikely to significantly decline in many of the cropping systems in the EU. However, in hotspot areas[14], where there is high uptake and repeated cultivation of Bt maize in a region, especially where associated with more than one generation of the target pests per year, there is an increased probability of a decrease in susceptibility, indicating possible resistance evolution in target pests. The applicant focused the sampling in areas

[11] Instead of 19 % reported in the 2009 MON 810 report.
[12] MON 810 2010 PMEM report, Appendix 1, section 3.4.3.
[13] In the present document, a "cluster of fields" is defined by a group of adjacent MON 810 fields that can be from different farms.
[14] In the present document, "hotspot area" is defined as an area of high adoption of maize MON 810 and the presence of multivoltine target pests.

with high uptake of maize MON 810 but no detailed description of possible hotspots could be retrieved from the 2010 MON 810 report. The EFSA GMO Panel is of the opinion that such information should be provided. Such information could assist risk managers to identify regions where non-compliance with refugia might pose a greater risk in relation to resistance evolution.

Finally, some Member States, having commented the 2010 MON 810 report, suggested that the "refuge-in-a-bag[15] (RIB)" technique could be an alternative to non-Bt refugia in response to the partial compliance of farmers with refugia implementation. The EFSA GMO Panel does not consider the "refuge in bag" an appropriate strategy for managing resistance evolution in the EU, considering the biology of the target pests. Rather, the Panel recommends that the applicant improves communication with farmers and take steps to educate them on the need to comply with refugia implementation.

2.2.2. Baseline susceptibility studies and resistance monitoring of target pests

The applicant focused its resistance monitoring scheme on two major European target pests, namely ECB and MCB. The 2010 MON 810 report did not refer to other pests. However, in its 2009 scientific opinion (EFSA, 2009), the EFSA GMO Panel considered that other lepidopteran pests present in some areas might also be subject to resistance evolution due to exposure to the Cry1Ab protein expressed in maize MON 810 (see Bergé and Ricroch, 2011). The EFSA GMO Panel therefore reiterates its 2009 recommendation to the applicant that, in areas where lepidopteran pests other than ECB and MCB are important pests of maize, these species should also be considered in the context of both the CSM for IRM strategy (Alcalde *et al.*, 2007) and GS through farmer questionnaires (Tinland *et al.*, 2007; Schmidt *et al*., 2008; EFSA, 2009).

In its scientific opinion on the 2009 MON 810 report (EFSA, 2011b), the EFSA GMO Panel had already assessed the overall approach for IRM and paid particular attention to the key aspects of the IRM plan (i.e. baseline set-up, ECB and MCB sampling plan, methodology of bioassays and the monitoring protocol designed for early detection of resistance evolution). In preparing this scientific opinion, the EFSA GMO Panel considered the new 2010 datasets from bioassays with ECB and MCB larvae.

Susceptibility[16] of ECB and MCB to Cry1Ab protein was assessed by the applicant in a laboratory colony and in larval samples collected from refugia areas and fields adjacent to Bt maize in some EU countries. In 2010, ECB larvae were sampled in Slovakia, Portugal and Spain (south-west Iberia) whereas MCB larvae were collected in central and south-west Iberia (including areas of Spain and Portugal). To detect changes in susceptibility to maize MON 810 in ECB and MCB populations, the applicant used the same methods employed in its 2009 MON 810 report: mortality assessed to determine the lethal concentrations (LC) and/or growth inhibition assessed for the molting inhibition concentrations (MIC). For MCB, only growth inhibition data (MIC values) were provided to reflect changes in susceptibility to Cry1Ab protein. For ECB, both mortality (LC) and MIC values could be used for the same purposes. Finally, the applicant concluded that "the IRM plan proposed by the industry is still valid since no change in susceptibility to Cry1Ab was observed". The EFSA GMO Panel acknowledges that the available dataset does not show evidence of resistance evolution in these target pests.

Given the similarities (e.g. methodology) with the 2009 MON 810 report, the EFSA GMO Panel refers to its assessment of the 2009 MON 810 report and its conclusions and recommendations in the 2011 scientific opinion (EFSA, 2011b). As in the previous MON 810 report, the EFSA GMO Panel evaluated to what extent the monitoring protocols designed by the applicant "allow for early detection of potential pest resistance before field failures occur and therefore enable additional management measures to be effectively implemented in a timely manner". In this respect, the EFSA GMO Panel reiterates the importance of the sampling scheme for target pests, e.g. sampling over time in hotspot

[15] RIB consisting in sowing mixed Bt and non-Bt seeds.
[16] MON 810 2010 PMEM report, Appendix 8.

areas with high uptake of maize MON 810 and multivoltine target pests, and sampling within maize MON 810 fields where the likelihood of detecting potentially resistant target pests is high (for further details, see EFSA, 2011b).

The EFSA GMO Panel had already considered the relevance of the resistance allele ranging from 1 % to 5 % for early detection of resistance evolution (see Appendix 2 of EFSA, 2011b) and pointed out that a level of detection of an allele frequency of 5 % does not allow the necessary time for taking any adaptive response, either for multivoltine strains of ECB or for MCB. The EFSA GMO Panel is of the opinion that the monitoring plan should aim to detect allele frequencies clearly below 5 %.

Furthermore, the EFSA GMO Panel identified the following shortcomings specific to the 2010 MON 810 report.

- The bioassays were performed with the F_2 generation from field-collected larvae whereas the F_1 generation was used in 2009. This was accepted by the EFSA GMO Panel as no change in susceptibility was expected.

- Even though MIC values are more sensitive and precise than LC values, the EFSA GMO Panel recommends that the applicant provides both LC and MIC values in future monitoring reports.

- Samples of MCB larvae collected in south-west Iberia (including areas of Spain and Portugal) were pooled. The EFSA GMO Panel does not support this pooling of data as it does not permit detection of interpopulation variation in the susceptibility of target pest populations.

2.2.3. Communication with and education of farmers

From the ANTAMA survey[17] in the Spanish Ebro valley, where a high concentration of maize MON 810 was planted in 2010, all the farmers planting maize MON 810 were aware of the recommendation to plant a non-Bt refuge. Of the surveyed farmers, 80 % considered that they were well informed about refugia implementation and about 25 % thought that the implementation was “little easy/not easy”.

As described in section 2.2.1.2 above, the EFSA GMO Panel noted that a certain percentage of farmers growing maize MON 810 did not comply with the implementation of non-Bt refugia in 2010.

Even though the EFSA GMO Panel acknowledges the initiatives taken by the applicant in 2010 to improve the education of farmers (i.e. by means of farmer interviews on refugia compliance in local newspapers and by revising the user’s manual of the farmer questionnaire), further efforts should be made to optimise communication with farmers.

The EFSA GMO Panel remains of the opinion that special attention should be paid to refugia implementation in those areas in which the likelihood of resistance evolution is higher. Therefore, the EFSA GMO Panel reiterates its previous recommendation to the applicant to ensure further education and training of farmers on their obligations to inhibit the evolution of insect resistance (EFSA, 2011b). In particular, applicants need to inform farmers that, where adoption of Cry1Ab-expressing maize is high, then there is a need to consider the total areas of Bt maize cultivation, independently of farm and field sizes, and to adopt refugia accordingly.

2.3. Conclusions and recommendations on CSM

The EFSA GMO Panel assessed the results of the implementation of the CSM/IRM plan in 2010, as provided by the applicant in the 2010 MON 810 report. Based on the information available in this report, the EFSA GMO Panel did not identify changes in the methodology, compared with the 2009

[17] MON 810 2010 PMEM report, Section 3.2.1.1

MON 810 report, or evidence of resistance evolution in target pests. No new data from CSM/IRM of maize MON 810 grown in 2010 were provided that would invalidate previous evaluations by the EFSA GMO Panel of maize MON 810 (EFSA, 2009, 2011b).

However, during the evaluation of the 2010 CSM/IRM results, the EFSA GMO Panel identified similar shortcomings (mostly in the methodology) as in the previous monitoring report. Hence the EFSA GMO Panel reiterates the recommendations made to the applicant in its scientific opinion on the 2009 MON 810 report (for further details, see section 2.3 of EFSA, 2011b).

(1) Related to the implementation of non-Bt refugia and education of farmers:

- to consider non-Bt refugia for all clusters of fields of Bt maize with an aggregate area greater than 5 ha, irrespective of individual field and farm size, and to invite farmers to collaborate in joint implementation of non-Bt refugia;

- to report on maize cropping density and frequency, maize MON 810 adoption rate and the number of target pest generations on a geographical scale that is relevant to IRM in order to identify hotspot areas;

- to further educate farmers on the need to comply with refugia implementation and to inform them about situations that increase the probability that resistance to the Cry1Ab protein may evolve in target pests and other regionally important lepidopteran pests, thus threatening the efficacy of maize MON 810.

(2) Related to resistance monitoring of target pests:

- to focus the sampling of target lepidopteran pests in hotspot areas over time to increase the likelihood of detecting resistance evolution. Sampling in areas with a lower adoption rate of maize MON 810 is also required but at a lower frequency in order to establish susceptibility baselines;

- to include in the sampling surviving target lepidopteran pests within maize MON 810 fields in order to detect potentially resistant individuals. The sampling should be mainly done as late as possible in the growing season in order to increase the likelihood of detecting surviving individuals;

- to consider regionally important lepidopteran pests (other than ECB and MCB) of maize MON 810 in the context of CSM for IRM strategy (EFSA, 2009) and, where appropriate, adjust the design and implementation of the IRM plan accordingly;

- in hotspot areas, to revise the monitoring protocol, aiming to detect a resistance allele frequency between 1 % and 3 %. The EFSA GMO Panel recommends increasing the number of larvae collected or using F_2 screening.

In addition, in response to shortcomings specific to the 2010 MON 810 report, the EFSA GMO Panel recommends that in future monitoring reports the applicant:

- provides the raw data from the survey by ANTAMA in relation to compliance with non-Bt refugia;

- does not pool samples from different populations of target pests in the monitoring of resistance evolution in target pests in order to allow detection of interpopulation variation in the susceptibility of target pest populations;

- provides both LC and MIC values for each target pest.

The shortcomings identified in the methodology do not invalidate the conclusion on the absence of evidence of resistance evolution in target pests.

3. GENERAL SURVEILLANCE (GS)

3.1. Summary of the information provided by the applicant

The applicant reported the results of the GS plan for the 2010 growing season of maize MON 810, mainly by analysing the results of questionnaires answered by selected farmers in the EU Member States where maize MON 810 was cultivated in 2010. As for the 2009 growing season, the 2010 plan for GS[18] of maize MON 810 consisted of four elements: (1) a survey of 271 farmers conducted by interviewers following a written questionnaire; (2) the data gathered from publications related to maize MON 810; (3) company stewardship activities; and (4) alerts on environmental issues by authorities and existing networks.

More details on some of the elements of the GS plan are given here.

(1) Farmers planting maize MON 810 in 2010 were asked to record and report their observations and assessments in and around maize MON 810 fields in comparison with a baseline, either a non-GM maize crop or their historical local knowledge and experience[19]. Initially, the applicant had defined a total sample size of 2 500 questionnaires for the overall duration of the consent, namely 10 years. Therefore, the applicant planned to collect approximately 250 questionnaires per year. In 2010, a total of 271 questionnaires were received from farmers in six European countries (39 in the Czech Republic, 10 in Poland, 150 in Spain, 43 in Portugal, 25 in Romania and four in Slovakia). According to the applicant, the farmers/fields were randomly selected among the countries depending on the market penetration of maize MON 810. The farmer surveys were carried out by third parties with experience of conducting agricultural surveys, with the exception of Poland, where Monsanto representatives assisted the farmers to fill in the questionnaire[20]. In this respect, the 2010 MON 810 report states that the interviewers were trained to understand the background of the questions and were also provided with a "user manual" to assist them in filling in the questionnaires with the farmers. The questionnaires were completed between November 2010 and March 2011. The applicant explained that a database was developed for data management and storage. For each question, a variable was defined by a variable name and a variable label. All data were entered and checked for quality and plausibility before being considered for statistical analysis. In its report, the applicant concluded that the 2010 statistical analysis of the 271 questionnaires did not reveal any unanticipated adverse effects that could be associated with maize MON 810.

(2) A list of peer-reviewed publications on the safety of maize MON 810 and/or the Cry1Ab protein published between June 2010 and beginning of June 2011 was submitted. The applicant used specific key words and searched in journals included in the Web of Science database[21]. The first set of papers resulting from the search was screened for relevance to the ERA of maize MON 810. The applicant reported 12 publications on molecular and food/feed aspects and 25 publications related to the ERA of maize MON 810. The applicant concluded that the peer-reviewed literature did not raise any safety concerns for maize MON 810.

[18] The 2010 MON 810 report submitted by Monsanto is publicly available on the webpage of the EC Directorate General for Health and Consumers, at http://ec.europa.eu/food/food/biotechnology/index_en.htm
[19] MON 810 2010 PMEM report, Appendix 1.
[20] MON 810 2010 PMEM report, Appendix 1.
[21] MON 810 2010 PMEM report, section 3.1.6.

The applicant did not provide details on existing monitoring networks likely to be of use for GS of maize MON 810. Reference was made to the ongoing project by a Europabio Working Group to map the European existing networks and to set up a unique reporting system[22].

3.2. Assessment by the EFSA GMO Panel

3.2.1. Farmer questionnaires

The EFSA GMO Panel noted that the farmer questionnaire used in 2010 was identical to the one used in 2009. Considering the timeline, the Panel acknowledges that the applicant could not have implemented the previous recommendations on PMEM, as referred to in the Panel's scientific opinion on the 2009 MON 810 report (see section 3.3 of EFSA 2011b), in the 2010 monitoring scheme for maize MON 810.

According to the EFSA GMO Panel scientific opinion providing guidance on PMEM of GM plants (EFSA 2011a), raw data from the farmer survey were requested on 21 December 2011. The applicant provided the data on 24 January 2012.

According to the terms of reference of the mandate from the EC, the EFSA GMO Panel assessed the methodology followed by the applicant to analyse the farmer questionnaires. As for the assessment of the 2009 MON 810 report, the EFSA GMO Panel was assisted by the EFSA Unit for Scientific Assessment Support (EFSA SAS Unit) which provided methodological guidance for a systematic evaluation of the farmer questionnaires (see Appendix 1). Appendix 1 sets out a list of evaluation criteria (e.g. sample size, survey response rate, statistical analysis) that can be applied to farmer surveys in the context of GS of GM plants. Results on the appropriateness of the farmer questionnaire for maize MON 810, its design, it use and analysis are given in Appendix 1. The EFSA GMO Panel, assisted by the EFSA SAS Unit, identified weaknesses in the methodology and gives recommendations to the applicant (see section 3.3). However, from the 2010 analysis of the farmer questionnaires on maize MON 810, the EFSA GMO Panel concludes that no unanticipated adverse effect can be identified (see Appendix 1).

3.2.2. Existing monitoring networks

The applicant referred to the ongoing project by a Europabio Working Group to map the existing European networks and did not deliver information on possible existing monitoring networks that could be involved in the GS of maize MON 810. Therefore, the EFSA GMO Panel acknowledges the same lack of relevant information that was identified in the 2009 MON 810 report. The EFSA GMO Panel is of the opinion that, in addition to farmer questionnaires, existing surveillance networks provide an additional tool for GS of GM plants that complement the farmer questionnaires. In this respect, the applicant should, where appropriate, use existing monitoring networks in its PMEM plan, as they are likely to collect relevant data for the GS of maize MON 810 (see EFSA, 2011a for further guidance).

3.2.3. Literature review

The EFSA GMO Panel acknowledges that the papers selected by the applicant were adequately discussed and put into the context of the overall safety assessment of maize MON 810.

However, the EFSA GMO Panel notes that relevant papers related to Bt maize/maize MON 810 and/or the Cry1Ab protein (published between June 2010 and beginning June 2011) were not reported by the

[22] MON 810 2010 PMEM report, section 3.1.2.3.

applicant. In addition to the three[23] review papers on Bt maize referred to in the 2010 MON 810 report[24], the following papers should have been listed and discussed:

> Batista R and Oliveira M, 2010. Plant natural variability may affect safety assessment data. Regulatory Toxicology and Pharmacology 58:S8-S12 (published December 2010).
>
> Farinós GP, Andreadis SS, de la Poza M, Mironidis GK, Ortego F, Savopoulou-Soultani M, Castañera P, 2011. Comparative assessment of the field-susceptibility of Sesamia nonagrioides to the Cry1Ab toxin in areas with different adoption rates of Bt maize and in Bt-free areas. Crop Protection 30, 902–906.
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> Huang F, Andow DA and Buschman LL, 2011. Success of the high-dose/refuge resistance management strategy after 15 years of Bt crop use in North America. Entomologia Experimentalis et Applicata 140, 1–16. doi: 10.1111/j.1570-7458.2011.01138.x (first published online 10 June 2011).
>
> Kruger M, Van Rensburg JBJ and Van den Berg J, 2012. Transgenic Bt maize: farmers' perceptions, refuge compliance and reports of stem borer resistance in South Africa. Journal of Applied Entomology 136, 38–50. doi: 10.1111/j.1439-0418.2011.01616.x (article first published online 1 March 2011).
>
> Zhu X, Chen L, Shen P, Jia J, Zhang D and Yang L, 2011. High sensitive detection of Cry1Ab protein using a quantum dot-based fluorescence-linked immunosorbent assay. Journal of Agricultural and Food Chemistry 59, 2184–2189 (epub 17 February 2011).

The new information available on maize MON 810 confirms the previous evaluation of the risk assessment performed by the EFSA GMO Panel (EFSA 2009a), which stated that maize MON 810 is as safe as its conventional counterpart with respect to potential effects on human and animal health and the environment.

3.3. Conclusions and recommendations on GS

From the data provided by the 2010 farmer survey on maize MON 810 and the literature review, no adverse effect of the cultivation of maize MON 810 in 2010 can be identified. However, the EFSA GMO Panel identified shortcomings similar to those found in its evaluation of the GS results for the 2009 growing season, and therefore makes the following recommendations to the applicant to reconsider the GS as it did in the scientific opinion on the 2009 MON 810 report (for further details, see Appendix 1):

- The sampling frame should be more detailed, including information on the source of the sampling frame.
- the cultivation areas with a high uptake of maize MON 810 and where maize MON 810 has been (repeatedly) grown in previous years should be over-represented in the sampling scheme.
- The sampling method should be described in more detail, including where relevant the statistical software and/or the program code used for this procedure.
- The losses to sampling should be fully documented, i.e. the number of farmers not participating in the survey and their reasons should be documented.

[23] De Vendomois *et al*., 2010; Yanni *et al*., 2010; Romeis and Meissle, 2011
[24] 2010 MON 810 report, section 3.1.6.

- Impartial and standardised interviews should be carried out by independent parties to reduce interviewer bias. In addition, effective quality and auditing procedures should be considered (EFSA, 2011a).

- The comparator field should be clearly identified beforehand (e.g. location: on the same farm?). If no comparator is being grown spatially or temporally close to the GM plant, then the rationale for selecting another comparator (e.g. maize grown in previous years) should be fully described (see EFSA, 2011a).

- additional questions to the farmer questionnaire should be considered to describe better the cultivation of Bt maize in the local area and/or in the previous years, and the receiving environment and the management systems in which maize MON 810 is being grown.

- The management practices (e.g. pesticide applications and timing, crop rotation) associated with the comparator field should be fully described in order to put into context any observed changes in management systems for maize MON 810.

- The raw data, programs, logs and output files related to the statistical analysis of the farmer questionnaires should be provided (see EFSA, 2011a). Confidence intervals for the analysis of the monitoring characteristics should be included in the statistical report.

- Statistical procedures should be followed based on the use of the appropriate statistical distribution for the data.

- Data should be pooled and statistically analysed over years. At the end of the 10 years of GS, the applicant should conduct a statistical analysis with all pooled data.

- A codification for farmers surveyed repeatedly over the years should be set up. These farmers monitored in particular.

- The number of years for which the surveyed farmer has grown maize MON 810 and other GM crops should be indicated.

The EFSA GMO Panel refers to its scientific opinion on the 2009 MON 810 report (EFSA, 2011b) for further improvement of the farmer questionnaire. Additional indicators and parameters (e.g. the occurrence of regionally important lepidopteran pests other than ECB and MCB, occurrence of damaged maize MON 810 plants) should be measured using the farmer questionnaire.

Furthermore, in order to improve the sampling frame of the farmers survey, the EFSA GMO Panel reiterates a previous recommendation (EFSA, 2011a) to set up national cultivation registers, as referred to in Article 31.3 (b) of Directive 2001/18/EC (EC, 2001).

The EFSA GMO Panel is of the opinion that the farmer questionnaire is an adequate tool to gather information such as crop performance, cultivation practices, etc. While the EFSA GMO Panel considers appropriate the overall approach followed by the applicant in relation to the farmer questionnaires, it also recognises that the information supplied by farmers is limited to observations that they can make in their areas of experience, which relate mostly to the areas on their farms cultivated with maize. Data on impacts on biota will be limited mostly to biota directly interacting with the crop and its management. Therefore, the EFSA GMO Panel is of the opinion that other monitoring approaches (e.g. existing monitoring networks; see section 4.2.1.3 of EFSA, 2011a) on different scales should be considered by the applicant.

The EFSA GMO Panel also considered that the information package provided by the applicant in relation to the existing monitoring networks was inadequate. The EFSA GMO Panel therefore

recommends that the applicant follows the guidance provided in its scientific opinion on PMEM of GM plants (for further details, see EFSA, 2011a).

The EFSA GMO Panel acknowledges that the literature review submitted by the applicant was balanced and put into the context of the overall ERA of maize MON 810. However, considering the relevant publications identified as missing, the EFSA GMO Panel invites the applicant to improve its search of the literature.

To conclude, no new data from the GS of maize MON 810 grown in 2010 were provided that would invalidate previous evaluations of maize MON 810 (EFSA, 2009, 2011b).

OVERALL CONCLUSIONS AND RECOMMENDATIONS

From the data submitted by the applicant in its 2010 MON 810 report, the EFSA GMO Panel did not identify adverse effects on the environment or human and animal health due to maize MON 810 cultivation during the 2010 growing season. Furthermore, the EFSA GMO Panel is of the opinion that the outcomes of the 2010 MON 810 report do not invalidate the Panel's previous scientific opinions on maize MON 810 and its subsequent recommendations on risk management.

However, during its evaluation of the 2010 MON 810 report, the EFSA GMO Panel identified a number of shortcomings in the methodology for CSM and GS of maize MON 810. Hence, the EFSA GMO Panel reiterates the general recommendations given in its scientific opinion providing guidance on PMEM of GM plants (EFSA, 2011a) and the additional specific recommendations for the improvement of the PMEM of maize MON 810 in its 2011 scientific opinion on the 2009 MON 810 report (for further details, see sections 2.3 and 3.3 of EFSA, 2011b).

Furthermore, in line with its recent Statement on the similar Cry1Ab-expressing GM maize Bt11 (for further details, see EFSA, 2011c), the EFSA GMO Panel recommends that appropriate IRM strategies for maize MON 810, which should be integrated with those of other Cry1Ab-expressing maize events currently grown commercially in the EU, are implemented in order to delay the possible evolution of resistance to the Cry1Ab protein in target pests.

The EFSA GMO Panel also reiterates its 2009 recommendation (EFSA, 2009) that, especially in areas of abundance of non-target lepidopteran populations, the adoption of the cultivation of maize MON 810 be accompanied by appropriate management measures in order to mitigate the possible exposure of these species to maize MON 810 pollen. The implications of these management measures should be considered in the PMEM plan. Further details on the framework to implement appropriate and proportionate risk mitigation measures and their efficacy are given in the recent Statement on the similar Cry1Ab-expressing GM maize Bt11 (EFSA, 2011c).

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the European Commission, dated 7 November 2011, to the EFSA Executive Director requesting the assessment of MON 810 monitoring report for the 2010 cultivation season provided by Monsanto.

2. Acknowledgement letter, dated 16 December 2011, from the EFSA Executive Director to the European Commission.

3. Letter from EFSA to the applicant, dated 21 December 2011, requesting additional information.

4. Letter from the applicant to EFSA, dated 23 January 2012, providing the additional information requested by EFSA.

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APPENDIX 1

SAS technical report on the evaluation of farmer questionnaires submitted in the annual monitoring report of MON 810 in 2010

BACKGROUND

This Appendix 1 was prepared by the EFSA SAS Unit to support the EFSA GMO Panel in its evaluation of the monitoring report on maize MON 810 for the 2010 growing season, specifically to provide methodological guidance on evaluation of the farmer questionnaires submitted as part of the GS programme, which aimed to identify adverse affects of the GM maize or its use on human and animal health or the environment that had not been anticipated in the ERA.

METHOD

Evaluation criteria were developed based on the principles of design for cross-sectional studies, and in particular surveys. The evaluation grid can be applied to surveys used for GS of GM plants. These criteria were previously applied in the assessment of the 2009 MON 810 monitoring report (EFSA, 2011b). In July 2011, the EFSA GMO Panel updated its guidance on the PMEM of GM plants (EFSA, 2011a). The criteria have been updated to reflect the recommendations in this guidance document resulting in changes to the instrument design and validity criteria.

Study design principle	Criteria
Sampling frame	1) The sampling frame used is specified 2) The total population included in the sampling frame is specified 3) The characteristics of the population included in the sampling frame are described, including region, agricultural practices, GM crop cultivation 4) The sampling frame coverage is appropriate for GM crop cultivation in the EU
Sampling method (sample bias)	1) The sampling method to select sample units from the sampling frame is described 2) The sampling method ensures that sample units from representative environments, reflecting the range and distribution of plant production systems and environments exposed to the GM crop and its cultivation, are sampled 3) A list of sample units selected from the sampling frame is provided 4) The sampling method minimises selection bias
Sample size (sample precision)	1) The size of the adverse effect to be measured is specified and scientifically justified and is within an acceptable limit of change 2) The significance level is specified and the chosen level is scientifically justified (type I error rate) 3) The power is specified and the chosen level is scientifically justified (type II error rate) 4) A literature reference for the sample size method is provided 5) The sample size calculation method is appropriate for a proportion in a cross-sectional study 6) The sample size is sufficient to detect an adverse effect related to GM crop cultivation
Survey response rate (non-response bias)	1) The follow-up method for non-responders is described and appropriate

	2) The response rate is specified 3) Details of losses in sampling are described 4) The number of partial responses and reasons for non-completion are specified 5) Comparison is made between characteristics of responder group and non-responder group 6) Comparison is made between characteristics of responder group and independent sources of information about the target population 7) The effects of non response bias have been minimised
Instrument design	1) The study design includes considerations to avoid interviewer bias 2) Where interviewers are used the interviewer training is described 3) The selection of open and closed questions is appropriate for the question type 4) The questions are clearly phrased and not open to misinterpretation 5) The questions encourage independent and objective responses 6) The comparator used in the study is described and appropriate for general surveillance[25] 7) The instrument has been previously tested and validated
Instrument validity	1) Content validity – the survey includes questions relevant to assess: • Background data Identifier of location of monitoring site and comparator site, surrounding landscape, type of field margins, proximity to conservation areas, cultivation and management of the GM crop field including recent history and previous cropping, soil (type, structure, quality), nutrient status, fertilisation, irrigation. • Data informing on possible changes in behaviour and performance of the GM crop Other GM crops cultivated, number of years of cultivation of GM crop, cultivation and tillage from the removal of the previous crop to seed sowing, crop husbandry including sowing/planting date, post-planting management, crop emergence, growth (vigour, height), pest, disease and weed management, flowering, standing ability, harvesting date and methods, yield, post-harvest management and subsequent cropping of the site, post-harvest storage, handling, processing and feeding • Data informing on possible ecological/environmental impacts of the GM crop on the protection goals and measurement Weed and pest populations, observations of other flora and fauna such as insects, birds and mammals, pollination and presence of pollinators, health of humans and performance of livestock • Implementation of specific management requirements

[25] New criterion added to the list of criteria used for the evaluation of the 2009 PMEM report on maize MON 810 (EFSA, 2011b)

	Implementation of risk management measures, coexistence segregation measures, stewardship recommendations and specific management due to regional environmental requirements 2) Criterion validity – agronomy parameters reported in the survey are compared with field trial data to test for concurrency 3) External consistency – results from the survey are compared with and conform to independent external data sources (for example pest/weed occurrence reports, soil characteristics from geological surveys, authorisations and use reports for plant protection products) 4) Plausibility of responses – results for cultivation methods, agronomy parameters and weed/pest management practices reported in the survey conform to European agricultural practices 5) Construct validity – consistency and agreement among outcome variables is examined
Data validation	1) The data validation procedure is documented 2) Results excluded from the statistical analysis during validation are reported 3) Missing values are reported
Longitudinal aspects	Comparison with survey results from previous years 1) The survey is applied to the sample unit for multiple years in order to assess residual effects
Statistical analysis	1) Objective and hypotheses for analysis are clearly stated 2) A statistical analysis plan is provided 3) Statistical analysis includes analysis of pre-defined subgroups according to PMEM guidance, e.g. country 4) Statistical analysis is appropriate for the data types 5) The results are clearly and consistently presented 6) The report should include descriptive statistics for the outcome variables 7) The issue of multiplicity is addressed 8) The method for handling missing values are described 9) Where appropriate, confidence intervals are provided 10) The results of post-hoc analysis should be identifiable
Report conclusions	1) The report conclusions are clearly stated 2) The study design is appropriate to assess the conclusions 3) The data presented support the conclusions presented in the report

RESULTS

Sampling frame

1) Sampling frame specification

Appendix 1 of the 2010 MON 810 report specifies that the sampling frame for the survey was customer lists obtained from companies selling seeds.

2) Population included the sampling frame

Appendix 1 of the 2010 MON 810 report did not include information on the number of farmers in the sampling frame.

3) Characteristics of the population included in the sampling frame

Appendix 1 of the 2010 MON 810 report did not include information on the characteristics of the farmers included in the sampling frame. Information on the number of farmers in the sampling frame according to country, region, size of farm/number of fields and previous cultivation of GM crops is important.

4) Sampling frame coverage

Information on the sampling frame was not provided in Appendix 1 of the 2010 MON 810 report, and therefore this is difficult to assess. Table 3.2 indicates that farmers from all the countries growing maize MON 810 were included in the survey. In Table 3.2, it can also be seen that all farms growing maize MON 810 in Romania were covered by the survey. However, in Poland 7.9 % and in Spain 4.7 % of maize MON 810-planted surfaces were surveyed. For Europe as a whole, 13.3 % of maize MON 810-planted surfaces were surveyed – this is a slight increase from 12.5 % in 2009. Full details on the source of the sampling frame, the number of farmers and the major characteristics of the farmers should be included in the survey report. The national registers set by Member States on the cultivation of GM crops would be the optimum sampling frame, if available.

Sampling method

1) Selection of sample units

Appendix 1 of the 2010 MON 810 report states "The farmers are selected from customer lists of the seed selling companies". Survey design methodology requires the sampling frame to be representative for the target population, in this case European farmers growing MON 810, and that the random selection process is applied to the sample units in the sampling frame prior to proceeding with the interviews. A description of the method to ensure that units are randomly selected from the sampling frame should be included in the report, including where relevant the statistical software and/or the program code used for this procedure.

2) Sampling of units from representative environments

Appendix 1 of the 2010 MON 810 report states "For selecting farmers in countries with higher market penetration a procedure is applied to select: at least 10 % of farmers and 10 % GM area per region and at least 20 % of new farmers each year." If this sampling protocol procedure is implemented correctly, the largest proportion of farmers surveyed would be those that had previously cultivated maize MON 810 and come from regions with a high uptake of maize MON 810.

3) Proportion of sample units selected

The monitoring report indicates that 10 % of farmers and farms covering 10 % of the MON 810 planted area per region, including 20 % of new farmers, are selected. The number of farmers surveyed in each country is provided, but no indication of the total number of farmers in each country and region included in the sampling frame is given. Table 3.2 describes the proportion of maize MON 810-planted area covered in the survey (and for two countries this value is below 10 %), but no information is provided on the numbers of new farmers per country included in the survey. This information should be provided as evidence that the sampling method has been successfully implemented.

4) Selection bias

For countries in which all farmers were selected from the sampling frame, there is no selection bias if the sampling frame is comprehensive for all farmers growing maize MON 810, e.g. Romania. The report provides limited information on the sampling methodology. The grouping of sample units according to the strata and random selection of sample units from within the strata should be performed using the specified sampling frame prior to conducting the interviews. A description of the method to ensure that units are randomly selected from the sampling frame should be included in the report, including where relevant the statistical software and/or the program code used for this procedure. The proportion of new farmers and farmers with previous experience of maize MON 810 selected from the sampling frame for each region should be presented in the report to provide evidence that the sampling method ensures that areas of intensive maize MON 810 cultivation are appropriately covered in the survey.

Sample size

1) Size of the adverse effect

Appendix 1 of the 2010 MON 810 report states that the null hypothesis is that the proportion of responses that are not "as usual" is above 10 %. Therefore, this is a non-inferiority test (i.e. the MON 810 field is no more adverse than the conventional comparator field; EMEA, 2000), and the threshold for adverse effects, or non-inferiority margin, is 10 %. No specific reference from the scientific literature was provided to support the selection of 10 %; however, for this type of study 10 % represents an acceptable limit of change. A 10 % effect size has also been selected in a framework proposal for post-release monitoring of second-generation crops with novel traits in Canada (Beckie *et al.*, 2010).

2) Type I error rate

The type I error rate is $\alpha = 0.01$ in Appendix 1 of the 2010 MON 810 report. This denotes that there is a 1 % probability of rejecting the null hypothesis that there is an effect when it is true, i.e. failure to detect a true adverse effect. A type I error rate of 1 % is conservative and acceptable.

3) Type II error rate

The type II error rate is $\beta = 0.01$ in Appendix 1. This denotes that there is a 1 % probability of rejecting the null hypothesis that there is an effect when it is false, i.e. falsely detecting an adverse effect. The selection of 0.01 will result in a large sample size.

4) Reference for the sample size method

The sample size calculation was performed using the methodology described in Rasch *et al.* (2007).

5) Sample size calculation

Sample size calculation is as reported in the 2009 monitoring report. The sample size is calculated assuming difference testing and not non-inferiority testing.

6) Sample size

As concluded for the 2009 monitoring report, the selection of parameters for the sample size calculation is conservative, and consequently the resulting sample size is large. In 2010, 271 farmers were sampled – this is greater than the planned 250 farmers per year. Nonetheless, it is

likely that the same farmer may be surveyed in different years and therefore sample units may not be independent from each other. Consideration of this factor should be included in the sample size calculation. Most importantly, the power of the study will be achieved only when the sample size of 2 500 farmers/fields surveyed is achieved after 10 years.

Survey response rate

1) Follow-up for non-responders

The survey uses telephone and face-to-face interviews, thereby reducing the number of non-responders in comparison with postal surveys. No information is provided in the report on the follow-up for non-responders.

2) Response rate

No information on the response rate is provided in Appendix 1 of the report.

3) Losses in sampling

No details of losses in sampling are included in the report. The number of farmers selected from the sampling frame but not contacted by the interviewers and the number of farmers refusing to participate should be stated in the report.

4) Partial responses and reasons for non-completion

This information was not presented in the report; however, the use of trained interviewers may have resulted in no cases of partial completion of the survey.

5) Characteristics of responder group and non-responder group

This information was not included in the report. It is important to know if a specific subgroup of farmers is not participating in the survey and therefore is not represented in the survey findings; consequently, this comparison should be presented in the report.

6) Characteristics of responder group compared with the target population

No comparison between the responder group and the target population is provided in the report. Where available, national registers for the cultivation of GM crops should be compared with the characteristics of the farmers surveyed in terms of geographical location and farming practices to ensure that the farmers surveyed are representative of the target population.

7) Non response bias

The losses to sampling should be fully documented in the report to provide evidence that there is no non-response bias. It is important to know if a specific subgroup of farmers is not participating in the survey and therefore is not represented in the survey findings.

Instrument design

1) Interviewer bias

Appendix 1 of the report indicates that the study used third parties to perform the interviews, with the exception of Poland, where Monsanto field representatives assisted the farmers to fill in the questionnaire. The use of third-party interviewers can prevent interviewer bias.

2) Interviewer training

Appendix 1 states that "all interviewers have been trained to understand the background of the questions", and mentions that the interviewers also draw on previous experience in administering the questionnaire to ensure that the questions are completed correctly. In addition a "user's manual" is provided to the interviewers.

3) Question type

The questionnaire contains 27 closed questions, which require a comparison between the representative GM maize field and the representative conventional maize field. For these questions the response options are " 'the same' or 'different/changed' " or " 'as usual' or 'worse' or 'better' ". It is these questions that are primarily analysed in the report. Where the response is not "same/as usual" there is an option to provide more details as free text. There is also a mix of closed and open questions to gather additional information about the farming practices on the farm and five closed questions to gather information about good agricultural practice and implementation of a refuge. The combination of open and closed questions allows quantitative analysis of the comparisons between the GM maize field and the conventional maize field, and, where differences occur between the two field types, explanatory analysis can be performed using the information from the free text questions.

4) Phrasing of questions

The questionnaire uses questions based on farm records and should be understood by a grower.

5) Independent and objective responses

Overall, the questionnaire seeks to obtain an objective set of responses to summarise the results and experiences during the growing season for maize. Nevertheless, the questionnaire could be improved by adjusting the balance between crop performance questions and questions on the general farm environment by addressing the latter more fully.

6) Comparator

The questionnaire relies on a comparison between a representative GM maize field and a representative conventional field to in order to detect unanticipated adverse effects. Consequently, the choice of representative fields and the recollection of similarities and differences is crucial to the success of the survey. The report provides no indication about the comparator fields selected by the farmer for comparison in the survey. In the Excel file of survey results "PMEM_MON 810_raw_2010.xls" provided by the applicant, 16 farmers in Spain, two in Romania and one in Portugal did not report any conventional maize varieties planted in the 2010 cultivation season. It is recommended that the questionnaire contain questions to record whether the comparator field is growing on the same farm in the same growing season and the variety of the comparator. If no comparators are being grown spatially or temporally close to the GM crop, then the rationale for selecting another comparator (e.g. maize grown in previous years) should be fully described. The comparators selected by the farmers for the survey should be summarised in the monitoring report.

7) Validation of the instrument

The questionnaire was developed by the German Federal Biological Research Centre for Agriculture and Forestry and maize breeders and statisticians in Germany, and the results of the pilot of this questionnaire were published in 2004 (Wilhelm *et al.*, 2004). The questionnaire was

used in annual monitoring reports in the period 2006–2010. Any future amendments to the questions should be made giving consideration to pooled analysis of the results over 10 years.

Instrument validity

1) Content validity

- Background data

Background data relating to geographical location at country and county level, surrounding environment, soil type, crop rotations in the previous 2 years and fertiliser treatments and irrigation is collected by the questionnaire. It would be of value to take longitude and latitude measurements of the representative GM maize field; information of this nature would facilitate linkage with other spatial monitoring datasets. In addition, the questionnaire should record for how many years the farmer has been growing MON 810 on the farm, and the question on crop rotation should also record, for rotations in which maize was grown, whether this was GM or conventional maize.

- Data informing on possible change in behaviour and performance of the GM crop

The following characteristics were monitored to obtain data on any change in the behaviour and performance of maize MON 810: crop rotation, time of planting, tillage and planting technique, insect control practices, weed control practices, fungal control practices, fertiliser application, irrigation practice, time of harvest, germination vigour, time to emergence, time to male flowering, plant growth and development, incidence of stalk/root lodging, time to maturity, and yield. It is noted that information on plant protection products applied to the GM maize field was collected, but the same information was not supplied for the conventional field. In order to fully explain changes in plant protection product use, the products applied to the conventional field should also be recorded, and the quantities applied over the season to the GM maize field and the comparator field should be recorded.

- Data informing on possible ecological/environmental impacts of the GM crop on the protection goals and measurement

The following characteristics were monitored to obtain information on possible ecological/environmental impacts of maize MON 810 on protection goals: occurrence of MON 810 volunteers, disease susceptibility, insect pest control (*O. nubilalis*), insect pest control (*Sesamia* spp.), pest susceptibility, weed pressure, occurrence of insects, occurrence of birds, occurrence of mammals. For the closed questions on occurrence of insects, birds and mammals the option “Do not know” is included; however, it has been excluded in other closed analysis questions, forcing the farmer to make a clear assessment. Allergenicity in people handling the GM crop during production and harvesting could be an adverse effect: a question to assess this should be included in the questionnaire. It is important that the question is phrased in such a way that it discriminates between allergenicity to the GM crop and background levels of hay fever-type symptoms.

- Compliance with good agricultural practice

Section 4 requests information on compliance with good agricultural practice, and in this case the planting of a refuge.

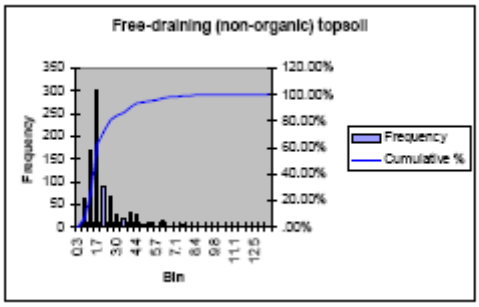
2) Criterion validity

The scientific opinion on the renewal of the authorisation for MON 810 (EFSA, 2009) states that "The information available in the renewal applications gives no reason to change the opinion that maize MON 810 is agronomically and phenotypically equivalent to currently grown non-GM maize varieties, with exception of the insect resistance conferred by the Cry1Ab protein." The 2005 opinion for MON 863 × MON 810 × NK603 (EFSA, 2005) states "Plants of the same field trials as for compositional analysis, except for a difference in glyphosate treatment (see section 3.2.2) were compared for their agronomic and phenotypic characteristics. These characteristics included seedling vigour, crop growth stages (for example, the stage at which silking and pollination occurred), height of the plant and ear (attachment containing the cob and kernels), root lodging (plants leaning to the surface), stalk lodging (plants with stalks broken below the ear), dropped ears, final stand count, stay-green and kernel yield. The plants tested showed no particular deviations in any of these parameters. In addition, plant damage due to insect feeding in two locations and due to weather in one location appeared to occur preferentially in plots planted with reference lines." Report MSL-18567 (Carringer *et al*., 2004) includes data on the agronomic parameters assessed in the above opinion. In the case of seedling vigour, both maize MON 810 and the reference varieties had "excellent" vigour, with the exception of one site where one reference variety was classed as poor and one as average. Stalk lodging in plants near harvest was observed more frequently in the reference varieties, and at one site root lodging in plants close to harvest was observed more frequently in the reference varieties. In the case of the other agronomy parameters, there was no particular deviation between MON 810 and the reference varieties. Appendix 1 of the 2010 MON 810 report assessing the characteristics of maize MON 810 reported "MON 810 plants germinate more vigorously, grew and developed slightly faster, less incidence of stalk/root lodging, had a longer time to maturity". Comparing the field trial data with the farmer survey data provides an opportunity to check the validity of the farmers' responses. It appears that there may be differences between field trial data and the questionnaire: there are a number of possible explanations for this, e.g. the conventional crops grown on the farms differ from the comparator variety used in the field trials, the information provided by the farmers is biased or erroneous or the GM crop is performing differently on farm-scale cultivation (possibly performing better when the cultivation conditions are less than optimal). It is of value to select parameters measured using a "gold standard" methodology and to contrast these with the responses in the survey to ensure the validity of the reported responses.

3) External consistency

Comparison of the data reported in the survey with information from independent data sources provides a further opportunity to test the validity of the responses.

The information on soil quality offers the opportunity to compare it with the information held in the Soil Profile Analytical Database for Europe (SPADE-2) (Hollis *et al.*, 2006). Figure 1 shows the information on top soil organic carbon contained in this database. The MON 810 survey reports organic carbon content values between 0.75 and 5.5 with a mean of 2.1. It can be seen that this range falls within that of the SPADE-2 range for organic carbon content. It should be noted that the SPADE-2 database provides a useful dataset for European soil properties but that the values are based on a limited set of soil samples for each EU country.



Mean	1.983665
Standard Error	0.051215
Median	1.5
Mode	1.5
Standard Deviation	1.494919
Sample Variance	2.234783
Kurtosis	9.998333
Skewness	2.648682
Range	13.1
Minimum	0.3
Maximum	13.4
Sum	1690.082
Count	852
Confidence Level(95.0%)	0.100523

Figure 1: Distribution and descriptive statistics of top soil organic carbon contents in SPADE-2 for free-draining non-organic soils.

The report of pests to which the GM maize was more or less susceptible provides another opportunity to check the survey responses for external consistency. In Table 1 the reported pests are compared with the known distribution of these pests in Europe, as reported in either the Crop Protection Compendium (CABI) or European and Mediterranean Plant Protection Organization (EPPO) websites. For most pests reported in the survey there is correspondence between the country of the pest report and its known distribution. However, some pests reported by Portuguese farmers represent new reports; this could be because of misidentification of species or an extension of the range of invasive species.

Table 1: Reported pest susceptibility and known distribution

Pest with susceptibility report	**Reported in**	**Known distribution**	**Source**
Agrotis spp.	PT	*A. segetum* present in Europe, widespread in Portugal *A. ipsilon* present in Europe, widespread in Portugal	CABI
Diabrotica spp.	PT	*D. virgifera* subsp. *virgifera* present in areas of Europe, has not been reported in PT	CABI, EPPO
Helicoverpa zea	PT	Has been intercepted in only UK and CH	CABI
Mythimna spp. (Mitima)	SP, PT	*M. unipuncta* present in Europe *M. loreyi* present in Europe	CABI
Spodoptera spp.	PT	*S. littoralis* in south PT, *S. exigua* present in PT	CABI, EPPO
Tetranychus spp.	PT	Present in Europe	CABI
Thysanoptera	PL	Present in Europe	CABI
Oscinella frit	PL	Present in Europe	CABI

Overall there is good agreement between the farmers' responses in the survey and information from external data sources for organic carbon content in soil and pests, and this provides evidence for external consistency for the MON 810 survey. It would be of value to include external consistency checks in the report to provide evidence of the validity of the survey responses.

4) Plausibility of responses

The sowing and harvest times were used to check the plausibility of the responses provided by the farmers: the sowing time ranged from 1 February 2010 to 15 July 2010 and the harvest time from 1 August 2010 to 15 January 2011.

5) Construct validity

The questionnaire is able to detect changes in characteristics of the GM maize field compared with the conventional field that could be predicted when the nature of the genetic event in MON 810 is considered. MON 810 expresses the cry1Ab coding sequence, which encodes an insecticidally active protein, Cry1Ab. The responses to the survey indicated that, for the MON 810 field, insecticide application and corn borer control practices were different: owing to a reduction in insecticides applied to control corn borers, the yield was higher, there was a lower incidence of root and stalk lodging and less susceptibility to diseases and pests. The questionnaire also indicated that the control of ECB and pink borer in MON 810 fields was very good. The report proposes that the change in characteristics is due to the increased protection from corn borer damage. This hypothesis is credible and indicates consistency and agreement among outcome variables.

Data validation

1) Validation procedures

Section 2.7 of Appendix 1 describes the data management and quality control procedures. It states that "For not readable entries in the questionnaires, queries were formulated and the field representatives or farmers were asked for explanation. These entries in the database were corrected". The number of questionnaires that require further clarification with the farmers should be included in the report, including a classification by error types.

2) Exclusion of results

All completed questionnaires (271) were included in the analysis.

3) Missing values

In the analysis of each of the monitoring characteristics, the number of responses for each value was shown in the table, including the missing values where they occur. With the exception of the occurrence of wild fauna questions, there were very few missing values.

Longitudinal aspects

1) Sampling over multiple years

As in some countries nearly all farmers growing MON 810 were sampled, and the sampling method selected for 80 % of the farmers with previous experience of MON 810 cultivation, it is clear that some farmers will have been surveyed in multiple years. The repeated sampling of a sample unit needs to be considered in the sample size calculations and in the statistical analysis of the results. It is important that a mechanism for recording repeated sampling is introduced and the numbers of sample units repeatedly sampled are included in the report. If this information were available, it would allow an analysis considering the intensity of maize MON 810 cultivation and the possible changes in monitoring characteristic assessment as maize MON 810 cultivation is repeated in consecutive years.

Statistical analysis

1) Objective and hypotheses

Appendix 1 states "The aim of the survey is to identify potential adverse effects that might be related to MON 810 plants and their cultivation. For that reason, most questions are formulated to

get ordinary data, i.e. with three possible answers (Plus/As usual/Minus). The Plus- and Minus-answers indicate a deviation from the situation with conventional maize and are provided with a specification to describe the specific effect and its potential cause. High frequency (> 10 %) of Plus or Minus- answers would indicate possible effects."

2) Statistical analysis plan

Section 2.4 of Appendix 1 describes the statistical test procedure. The effect is specified as a 5 % increase from the baseline of 5 %, setting the threshold for responses that are not "as usual" at 10 %. It would be expedient to provide scientific references to support the selection of the 10 % threshold. Additionally, for certain responses, 10 % may be greater than the acceptable limit of change. Alternative statistical analyses allowing the exploration of different effect sizes for certain monitoring characteristics would assist in the interpretation of the results.

The null hypothesis is that the proportion of responses not "as usual" is above 10 %. This is a test of non-inferiority. A significance level of 0.01 % was used in the statistical test. If $p < 0.01$, then the null hypothesis that the minus/plus response is greater than 10 % is rejected and therefore no effect can be identified.

3) Pre-defined subgroups

The analysis was performed for all fields surveyed in 2010. There was no analysis of country level data. Given the number of farmers surveyed in some countries, analyses of country-level subgroup may not have been statistically valid; however, consideration should be given to the fact that Member States may require country-level results. In addition, as the sampling method selected 20 % of new farmers, analysis of the assessment of monitoring characteristics by new farmers compared with farmers with previous experience of cultivation of MON 810 would be of interest. This could assist in detecting residual effects.

4) Statistical analysis

The reports states that plus responses and minus responses were "statistically tested by using the exact binomial test". This test is appropriate for the "same/different" type of question. However, for questions of the "as usual or worse or better" type, where there are three outcomes, an analysis using a multinomial test should be performed (in this case a trinomial test).

5) Results presentation

For each monitoring characteristic measured by the survey, a table of the responses was provided with percentage and "valid percentages" (the proportion of answers excluding missing values) plus a bar chart of the frequency of responses. The valid percentages were used in the binomial test.

The reasoning between the valid percentages in the table of responses and the table of the results of the binomial test for different "treatments/practices" should be further explained by the applicant in order to facilitate interpretation of the results.

6) Descriptive statistics

Descriptive statistics were provided for the continuous outcome values number of fields, maize area in hectares, percentage humus content, sowing date and harvest date. The analysis of the categorical values was provided as frequency tables.

7) Multiplicity

A significance level of 0.01 was used, but the issue of multiplicity of testing was not addressed. Another major problem is related to the fact that the analysis needs to be pooled after 10 years to achieve the statistical power described in the sample size calculations. Each annual report represents an interim analysis, and the statistical analysis plan needs to compensate for these interim analyses.

8) Handing missing values

In the tables two percentages were presented: the "Percent", which included missing values, and the "Valid percentages", in which the missing data or the "Don't know" responses were excluded.

9) Confidence intervals

For a non-inferiority test it is standard practice to use confidence intervals, and these were not included in Appendix 1. In the table summarising the analysis of the monitoring characteristics (e.g. Table 3.1 in Appendix 1) the confidence intervals should be included. The inclusion of confidence intervals would allow an understanding of the sensitivity of the analysis to the choice threshold.

10) Post-hoc analysis

Post-hoc analysis was performed only when an effect was identified and further explanatory analysis was possible using less structured information, e.g. free text collected in the questionnaire.

Report conclusions

1) Report conclusions

Appendix 1 contains the following conclusions:

2010 data indicates that in comparison to conventional maize plants, MON 810 plants

• received less insecticides caused by their inherent protection against certain lepidopteran pests,

• were harvested later caused by the used variety, the status of the plant (development, health, maturity, water content), increased flexibility (cropping system, logistics, channeling and coexistence) and weather,

• germinated more vigorously caused by the high quality germplasm,

• grew and developed slightly faster caused by better fitness of the plant and the high quality germplasm,

• had less incidence of stalk/root lodging caused by the inherent protection against certain lepidopteran pests,

• had a longer time to maturity caused by the absence of pest pressure of certain lepidopteran pests,

• gave a higher yield caused by the better fitness of the plant,

• were observed less as volunteers from previous year's planting caused by a more effective previous year's harvest,

• were less susceptible to diseases caused by hardly any insect feeding damage,

• controlled corn borers very well caused by the inherent protection against certain lepidopteran pests, and

• were less susceptible to pests, other than corn borers, especially lepidopteran pests caused by the inherent protection against certain lepidopteran pests and the resulting better fitness of the plants.

Moreover the animals fed with MON 810 performed slightly different compared to those fed with conventional maize. MON 810 fed animals were healthier resulting from a lower incidence of mycotoxins in the feed (due to lower ECB feeding damage on the plant).

The identified deviations have been expected, due to the knowledge of the MON 810 characteristics. The observed significant effects are not adverse. They mostly relate to the increased fitness of MON 810 plants resulting from the inherent protection against certain lepidopteran pests. Overall, the monitoring results substantiate the results from scientific research.

In this year of data collection no adverse effects have been identified by MON 810 cultivating farmers.

2) Study design

The study design was appropriate to evaluate whether a set of monitoring characteristics relating to plant performance and management practices for MON 810 cultivation in the current year of the survey differed from a comparator variety by a threshold of 10 %. Nonetheless, the result of this assessment was very much dependent on the selection of an appropriate comparator. In addition, certain effects may reach a sufficient magnitude for detection only with repeated cultivation of a GM crop, and so amendments to study design and the analysis plan should be considered in order to assess the effect of multiple years of GM crop cultivation. Table 4.1 in Appendix 1 presents the results from the previous four years and the 2010 results. The inclusion of the pooled results would be of interest.

3) Substantiation of results

Forty-three farmers (16 %) indicated that that they had changed the application procedure for insecticides in the MON 810 field; with the exception of one, these were farmers who usually used insecticides specifically to control corn borers.

Forty-four farmers (16 %) indicated that the germination of MON 810 was more vigorous than conventional maize. Ninety-five farmers (35 %) reported a reduction in stalk and root lodging in the MON 810 field compared with the conventional field. Increased germination vigour and reduction in stalk and root lodging was also observed in the field trial studies. Forty-four farmers (16 %) reported delayed maturity.

Sixty-nine farmers (25 %) reported that the MON 810 field was less susceptible to diseases, with associated reports of reduced susceptibility to *Fusarium* spp. (57 farmers) and *Ustilago maydis* (39 farmers). The reports of reduced susceptibility to fungal infections were substantiated with similar findings from the scientific literature. Two hundred and thirty-two farmers (86 %) and 164 farmers (valid percentage 84 %) reported that MON 810 provided "very good" control of

ECB and pink borer, respectively, and 50 farmers (18 %) reported MON 810 to be less susceptible to pests other than the borers. These results are to be expected, as the genetic modification provides protection from corn borers and therefore should result in a healthier crop. An increased yield was reported by 135 farmers (50 %); as the MON 810 crop has less insect damage, an increased yield is not unexpected.

For the monitoring characteristics above, the report states that the effect was greater than 10 %, and the null hypothesis that an effect was evident could not be rejected. For the other monitoring characteristics the effect was below 10 %, but in some cases the null hypothesis could not be rejected. Interpretation of the results should be viewed with caution as the conclusions are drawn on the basis of an assumed binomial distribution for monitoring characteristics with three possible outcomes, for which the selection of a multinomial statistical test would have been more appropriate. Presenting the results with confidence intervals would have facilitated the interpretation of the results and allowed the effect of the selection of alternative threshold values other than the arbitrarily selected 10 % to be explored.

The monitoring characteristics that were not "as usual" described above were also observed in the 2009 monitoring report. In addition, in 2009 a lower occurrence of volunteers (10.8 %) was reported by the farmers surveyed. The consistency of the results in each year of survey indicates the stability of the observed effects; however, it is important to monitor for changes in monitoring characteristics over time, and in particular in areas of intensive MON 810 cultivation.

RECOMMENDATIONS AND CONCLUSIONS

From the data provided in the 2010 survey from the farmer questionnaire to monitor adverse effects associated with the cultivation of maize MON 810, no adverse effect can be identified. However, a number of improvements to the survey design and reporting were identified and are listed in the recommendations below.

- Full details on the source of the sampling frame, the number of farmers and the major characteristics (e.g. previous cultivation of maize MON 810) of the farmers should be included in the survey report. The national registers set by Member States for the cultivation of GM crops would be the optimum sampling frame, if available.

- A description of the method to ensure that units are randomly selected from the sampling frame should be included in the report, including where relevant the statistical software and/or the program code used for this procedure. The proportion of new farmers and farmers with previous experience of maize MON 810 selected from the sampling frame for each region should be presented in the monitoring report to provide evidence that the sampling method ensures that areas of intensive maize MON 810 cultivation are appropriately covered by the survey.

- The losses to sampling should be fully documented in the report to provide evidence that there is no non-response bias. It is important to know if a specific subgroup of farmers is not participating in the survey and therefore is not represented in the survey findings;

- It is recommended that independent trained interviewers are used to reduce interviewer bias.

- It is recommended that the farmer questionnaire contain questions to record whether the comparator field is growing on the same farm in the same growing season and the variety of the comparator. If no comparators are being grown spatially or temporally close to the GM crop, then the rationale for selecting another comparator (e.g. maize grown in previous years)

should be fully described. The comparators selected by the farmers for the survey should be summarised in the monitoring report.

- Farmer questionnaires should focus only on changes that would be recognised by the farmer during the daily management of the farm. However, additional questions could be included to gain a better understanding of the intensity of GM maize cultivation on the farm (number of years of MON 810 cultivation and frequency of MON 810 crop rotations), and further information on plant protection product usage (in particular, in the comparator field) should be obtained to facilitate a full understanding of any observed changes.

- Confidence intervals for the analysis of the monitoring characteristics should be included in the statistical report. This is standard practice for non-inferiority tests. Presenting the results with confidence intervals would have facilitated their interpretation and allowed the effect of the selection of alternative threshold values other than the arbitrarily selected 10 % to be explored. The choice of statistical test should be based on the number of possible outcomes: a binomial test for two outcomes and a trinomial test for three outcomes.

- The statistical analysis should be planned to allow an analysis of the monitoring characteristics according to the length of GM crop cultivation in order to assess residual effects. As the statistical power of the study will be achieved only after 10 years, this will require a pooled analysis. Consequently, when conducting the survey, consideration should be given to the consistency of questions to assess monitoring characteristics, the inclusion of the same farmers in consecutive years in the survey (and the enumeration of these farmers in the report) and the interim analyses performed for the annual reports.

- The presentation of the results reported in Appendix 1 of the report should be improved in order to facilitate their interpretation.