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 MON810 in 2009[1]

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

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This scientific opinion, published on 13th January 2012, replaces the earlier version published on 25th October 2011.

ABSTRACT

Following a 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 2009 cultivation season of maize MON810 provided by Monsanto Europe S.A. The EFSA GMO Panel 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 MON810 in 2009. 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. From the data submitted by the applicant in its 2009 MON810 report, the EFSA GMO Panel did not identify adverse effects on the environment, human and animal health due to maize MON810 cultivation during the 2009 growing season. The outcomes of the 2009 MON810 report do not invalidate the previous risk assessment conclusions on maize MON810. However, the EFSA GMO Panel notes a number of shortcomings in the methodology for CSM and GS. Hence, this scientific opinion gives specific recommendations for improvement of the strategy, methodology and reporting for the post-market environmental monitoring of maize MON810. The applicant should take into account the guidance on Post-Market Environmental Monitoring (PMEM) of genetically modified plants as outlined in the recent scientific opinion of the EFSA GMO Panel. The recommendations of the EFSA GMO Panel in this opinion supplement the previous recommendations on PMEM of maize

[1] On request from European Commission, Question No EFSA-Q-2010-01254, adopted on 7 September 2011.

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[3] Acknowledgement: The Panel wishes to thank the members of the Standing Working Group on annual PMEM reports: Salvatore Arpaia, Marc Delos, Jozsef Kiss, Antoine Messéan, Joe Perry, Lucia Roda, Jeremy Sweet for the preparation of this opinion, and EFSA staff: Saghir Bashir, Sylvie Mestdagh and Jane Richardson for the support provided to this scientific opinion.

Suggested citation: EFSA Panel on Genetically Modified Organisms (GMO); Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON810 in 2009. EFSA Journal 2011;9(10):2376. [66 pp.] doi:10.2903/j.efsa.2011.2376. Available online: www.efsa.europa.eu/efsajournal

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MON810 in the 2009 scientific opinion for the renewal of the authorisation for continued marketing of maize MON810.

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KEY WORDS

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

SUMMARY

Following a 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 2009 cultivation season of maize MON810 provided by Monsanto Europe S.A.

The EFSA GMO Panel assessed, in close collaboration with the EFSA Unit for Scientific Assessment Support, the methodology applied by the applicant for the Case-Specific Monitoring (CSM) and General Surveillance (GS) of maize MON810 in 2009. Concerning the Case-Specific monitoring, 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, the EFSA GMO Panel paid particular attention to the design and analysis of the farmer questionnaires.

From the data submitted by the applicant in its 2009 MON810 report, the EFSA GMO Panel did not identify adverse effects on the environment, human and animal health due to maize MON810 cultivation during the 2009 growing season. The outcomes of the 2009 MON810 report do not invalidate the previous risk assessment conclusions on maize MON810.

However, the EFSA GMO Panel notes a number of shortcomings in the methodology for CSM and GS. Hence, this scientific opinion gives specific recommendations for improvement of the strategy, methodology and reporting for the post-market environmental monitoring (PMEM) of maize MON810. The applicant should take into account the guidance on Post-Market Environmental Monitoring of genetically modified plants as outlined in the recent scientific opinion of the EFSA GMO Panel. The recommendations of the EFSA GMO Panel in this opinion supplement the previous recommendations on PMEM of maize MON810 in the 2009 scientific opinion for the renewal of the authorisation for continued marketing of maize MON810.

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION AND EFSA

Genetically Modified (GM) maize MON810 (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 MON810 on the market, the EFSA GMO Panel adopted a scientific opinion on the renewal under Regulation (EC) No 1829/2003 of maize MON810 for import, processing for food & feed uses and cultivation in June 2009 (EFSA, 2009a). The EFSA GMO Panel concluded that « *maize MON810 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 MON810 be accompanied by management measures in order to mitigate the possible exposure of these species to maize MON810 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. In addition, the EFSA GMO Panel agreed with the overall approach and methodology proposed by the applicant for General Surveillance (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 MON810 are having unanticipated adverse environmental effects.

From 2005 onwards, the applicant submitted to the European Commission PMEM reports on maize MON810 according to legal requirements in terms of Post-Market Environmental Monitoring (PMEM) laid down in Directive 2001/18/EC (EC, 2001).

On 4 November 2010, the EFSA GMO Panel received a request from the European Commission to assess the PMEM report submitted by Monsanto on the cultivation of maize MON810 in 2009 (hereafter referred to as '2009 MON810 report'). EFSA therefore established a new 'Standing Working Group on the annual PMEM reports' in order to assess the 2009 MON810 report and all forthcoming PMEM reports. The EFSA GMO Panel acknowledged that the 2009 monitoring scheme for maize MON810 could not fully implement the PMEM recommendations of its 2009 scientific opinion as it was issued during the 2009 growing season.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION AND EFSA

On 4 November 2010, the EFSA GMO Panel received a request from the European Commission to assess the maize MON810 monitoring report for the 2009 cultivation season provided by Monsanto. This assessment should be reported through the adoption of an opinion on each yearly monitoring report related to each cultivated GM plant in the EU. This opinion 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 March 2011.

Aiming at a comprehensive assessment of the monitoring report, EFSA asked the European Commission and the applicant to provide respectively, missing information (such as the comments raised by Member States on the report) and clarifications on the methodology. The EFSA GMO Panel committed to complete its evaluation of the report within a period of five months, starting from the reception date of the missing information.

ASSESSMENT

1. INTRODUCTION

Maize MON810 was developed by the applicant, Monsanto Europe S.A., to express the Cry1Ab protein, derived from *Bacillus thuringiensis* subsp. *kurstaki*, which confers protection against the lepidopteran target pests European corn borer (ECB, *Ostrinia nubilalis* Hübner) and Mediterranean corn borer (MCB, *Sesamia nonagrioides* Lefebvre). Maize MON810 is currently cultivated in the EU across different countries such as Czech Republic, Poland, Spain, Portugal, Romania and Slovakia. The applicant reported to the European Commission and Member States on an annual basis the results of its monitoring activities of the cultivation of maize MON810 in the EU.

The EFSA GMO Panel was asked by the European Commission to assess the annual Post-Market Environmental Monitoring (PMEM) report submitted by the applicant on the cultivation of maize MON810 in 2009 (hereafter referred to as '2009 MON810 report'). For the 2009 growing season of maize MON810, the applicant[4]:

(1) reported the results of its Insect Resistance Management (IRM) plan, including data on the non-Bt *refugia* implementation, the evolution of the target pests' resistance, as well as information on farmer education;

(2) reported the results of its general surveillance (GS) monitoring program, including the analysis of the questionnaires answered by selected farmers in the EU Member States where maize MON810 was cultivated in 2009;

(3) submitted a review of peer-reviewed publications on the safety of maize MON810 and the Cry1Ab protein.

In preparing the present scientific opinion, the dedicated EFSA Standing Working Group on annual PMEM reports (PMEM reports WG) took into consideration various sources of information such as comments from Member States on the 2009 MON810 report, most recent scientific data and relevant peer-reviewed publications.

In response to the mandate of the European Commission, the PMEM reports WG, in close collaboration with the EFSA Unit for Scientific Assessment Support (SAS Unit), assessed the appropriateness of the methodology (e.g., statistical analysis of the farmer questionnaires).

During its assessment of the 2009 MON810 report, the PMEM reports WG identified shortcomings of the report, including the lack of relevant information (e.g., raw data, software programme). In order to better understand the details of the methodology, the applicant and its contractor were invited to a meeting of this WG. A representative of the European Commission attended the meeting as an observer. Upon requests of this WG, the applicant also provided further clarifications in writing on 2 March and 26 April 2011.

In the present scientific opinion, the EFSA GMO Panel describes the assessment of the 2009 MON810 report (see chapters 2 and 3), with particular emphasis on the methodology suggested by the applicant (see Appendix 1). The EFSA GMO Panel considered the relevance and implications of the 2009 PMEM results on the previous safety assessment of maize MON810 (EFSA, 2009a). Finally, based on its evaluation of the 2009 MON810 report, the EFSA GMO Panel made specific recommendations to the applicant on the strategy, methodology and reporting for PMEM (see chapters 2.3 and 3.3) that

[4] The 2009 MON810 report submitted by Monsanto is made 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

supplement the guidance provided in the 2011 scientific opinion of the EFSA GMO Panel on PMEM of GM plants (EFSA, 2011).

2. CASE-SPECIFIC MONITORING (CSM)

2.1. Summary of the information provided by the applicant

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 MON810 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 (*O. nubilalis* and *S. nonagrioides)* to maize MON810,

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

(3) the communication with and education of farmers (e.g., technical user guide[6]) and a proactive education programme[7] of farmers on compliance with *refugia* implementation (e.g., letters, interviews, leaflets).

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

(1) The applicant[8] asked the farmers planting more than 5 ha of maize MON810 to plant a refuge area with maize that does not express Cry1Ab protein within a distance of 750 meters from the maize MON810 field and that corresponds to at least 20% of the surface planted with maize MON810[9]. 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 this 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[10] sponsored by ANTAMA (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, bioassays should be performed on F1 progeny whenever possible and 200 to 300 insects should be collected from each sampling location. The methodology used in these assays should follow the methods described in the 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 (supplied by the Working Group) in a diet-overlay format. Estimates for each concentration should be based on no less 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 (for further details, see chapter 2.2.2). The applicant claimed that, in order to be effective, resistance monitoring focused on areas of high selection pressure for resistance,

[5] MON810 2009 PMEM report, Appendix 1
[6] MON810 2009 PMEM report, Appendices 2.1 to 2.6
[7] MON810 2009 PMEM report, Section 3.2.1.3
[8] MON810 2009 PMEM report, Appendix 1
[9] MON810 2009 PMEM report, Appendices 2.1 to 2.6
[10] MON810 2009 PMEM report, Section 3.2.1.1

including those with the highest uptake of Bt maize. The applicant stated that the monitoring plan should be able to detect if the frequency of the resistance allele remains below 5%.

2.2. Assessment by the EFSA GMO Panel

2.2.1. High dose/refuge strategy

2.2.1.1. High dose

The EFSA GMO Panel agrees with the applicant that appropriate IRM strategies are capable of delaying possible evolution of resistance under field conditions (Alstad and Andow, 1995; Andow, 2008; Tabashnik *et al.*, 2008, 2009). Resistance management strategies, relying on a 'high dose/refuge strategy', have been endorsed for several Cry-expressing crops in several countries (Bates *et al.*, 2005; Andow, 2008; MacIntosh, 2010; Gaspers, 2009; Huang *et al.*, 2011). The 'high dose/refuge strategy' proscribes planting Bt-maize that produces a very high concentration of the insecticidal Cry protein (25 times the amount needed to kill > 99 % of susceptible individuals) (EPA, 1998), so that nearly all individuals of target insects that are heterozygous for resistance do not survive. In addition, a nearby refuge area of non-Bt-maize[11] is required where the target insect pests are not exposed to lepidopteran-active Cry proteins (Ives and Andow, 2002). Under these conditions, most of the rare resistant individuals surviving on Bt-maize will mate with abundant susceptible individuals emerging from nearby *refugia* to produce heterozygous progeny that is phenotypically susceptible. If inheritance of resistance is recessive, the hybrid progeny from such matings will die on Bt-maize.

The EFSA GMO Panel is not aware of new information on Cry1Ab expression levels in maize MON810 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 high toxicity dose for some of these lepidopteran pest species, 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 MON810 for those species (see chapter 2.2.2 and EFSA, 2009a).

2.2.1.2. Implementation of non-Bt *refugia*

The EFSA GMO Panel analysed the survey by ANTAMA addressing the implementation of the IRM plan. It concluded that 19% of the farmers growing more than 5 ha of maize MON810 did not plant a refuge area in Spain in 2009. The reasons given by the farmers for not planting a refuge area were: (1) corn borers (*Ostrinia nubilalis*) cause significant economic losses, (2) the sowing is easier (with Bt-maize), (3) they want to try Bt-maize on the whole surface they have for this crop, or (4) they consider their farms as small farms (i.e. less than 5 ha and therefore no refuge required).

The EFSA GMO Panel notes there is an inconsistency as some farmers growing more than 5 ha of maize MON810 declared they did not implement refuge because they considered their farms as small. From the report, it was not clear whether these small farms of less than 5 ha could constitute aggregated MON810 cropping areas greater than 5 ha that would then require a refuge, as requested in the 2009 scientific opinion on the renewal of maize MON810 for cultivation (EFSA, 2009a). Furthermore, it appears that the total aggregated areas of small fields of maize MON810 had not been considered during the development of the *refugia* strategy.

[11] In the present document, 'refuge area of non-Bt maize' is intended to mean a refuge area with maize that does not express Cry proteins which are active against Lepidoptera.

The 2009 MON810 report shows that a certain percentage of farmers growing maize MON810 in 2009 did not comply with the implementation of non-Bt *refugia*. This partial non-compliance with the implementation of non-Bt *refugia* in Spain was further confirmed by the farmer questionnaires (see chapter 3.2.1).

The 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*., 2011). 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 prevent resistance evolution in target pests. The current IRM plan does not necessarily meet this requirement as clusters of small MON810 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 in its 2009 scientific opinion on the renewal of maize MON810 for cultivation (EFSA, 2009a): '*In the case of a cluster*[12] *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.'*

The EFSA GMO Panel assessed to what extent the 5 ha threshold used by the applicant to trigger the implementation of *refugia* was adequate. A 5 ha area corresponds to a cultivation area of approximately 130 meters radius. As for ECB, Hunt *et al*. (1998, 2007) reported that majority of recaptured ECB adults were within 1500 feet[13] from the release site. Showers *et al*. (2001) reported that most male ECBs were trapped (pheromone) at 200 meters from the release site but significant numbers were trapped at 800 meters and greater from the release site. As for MCB, based on field capture/recapture data from Spain (Eizaguirre *et al*., 2004, 2006), the authors concluded that there are important inter-field dispersal flights by MCB adults. Specifically male MCBs may fly at least up to 400 meters from the place of origin during the first two generations. The EFSA GMO Panel therefore considers the 5 ha threshold a reasonable and rather conservative value that should ensure the efficiency of the 'high/dose refuge strategy'.

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 changes in susceptibility indicating possible resistance evolution of target pests. The applicant focused the sampling in areas with high uptake of maize MON810 but no detailed description of possible hotspots could be retrieved from the 2009 MON810 report. The EFSA GMO Panel is of the opinion that such information should be provided. This information could be used to assist risk managers to identify regions where sampling could be focused and where non-compliance with *refugia* might pose a greater risk in relation to resistance evolution (see below).

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 2009 MON810 report did not refer to other pests. However, in its 2009 scientific opinion (EFSA, 2009a), 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 MON810 (see Bergé and Ricroch, 2011). The EFSA GMO Panel reiterates its 2009 recommendation to the applicant that, in areas where lepidopteran pests other than the ECB and MCB are important pests of maize, these species should also be considered in the context of both CSM

[12] In the present document, a '*cluster of fields'* is defined by a group of adjacent MON810 fields that can be from different farms.
[13] The international foot is defined as exactly 0.3048 metres.
[14] In the present document, '*hotspot area'* is defined by an area of high adoption rate of maize MON810 and the presence of multivoltine types of target pests.

for IRM strategy (Alcalde *et al.,* 2007) and GS through farmer questionnaires (Tinland *et al*., 2007; Schmidt *et al*., 2008; EFSA, 2009a).

The EFSA GMO Panel assessed the overall approach for IRM and paid particular attention to key aspects of the IRM plan like (1) the ECB and MCB sampling plan and (2) the monitoring protocol designed for early detection of resistance evolution.

The objective of the resistance monitoring is to assess to what extent resistance of target pests may evolve and reach levels which would reduce the efficacy of maize MON810 for controlling these pests. The Environmental Risk Assessment (ERA) concludes that the 'high dose/refuge strategy' should significantly reduce the likelihood that resistance will evolve. The rationale of CSM is to monitor the resistance levels of target pest populations over time in order to check the assumptions made during the ERA. Two issues should be considered:

- Cry1Ab resistance is most likely to evolve in those situations where there are high levels of selection pressure ('hotspot areas') and more than one generation of the target pests per year, so that the focus of monitoring should be in such areas. As resistance evolution is unlikely in maize areas with a low adoption rate of maize MON810, sampling in these areas could be limited to establish susceptibility baselines;

- the natural bio-geographical variability of baseline susceptibility (Gonzalez-Nuñez *et al.*, 2000; Farinós *et al.*, 2004) might affect the detection of resistance evolution over time. In other words, it might be advisable to monitor the same areas over time to reduce the geographical variation and make it easier to detect early changes in susceptibility.

(1) Natural variation in ECB/MCB populations susceptibility

The EFSA GMO Panel considered existing data on natural variations in ECB and MCB susceptibility, including datasets from the applicant and relevant publications. Baseline susceptibility[15] to the Cry1Ab protein has been investigated and established for MCB and ECB in 2004 and 2005 in Spain (Gonzalez-Nuñez *et al.*, 2000; Farinós *et al.*, 2004), as well as for ECB from 2005 to 2009 for 15 populations according to their geographic locations in the EU (Chaufaux *et al*., 2001). Susceptibility of ECB to Cry1Ab protein was assessed by the applicant for laboratory colony and for samples collected in maize fields in Czech Republic, France, Germany, Italy, Hungary, Slovakia, Poland, Portugal, Romania, and Spain. The 2009 MON810 report describes[16] that "*ECB larvae were exposed to artificial diet treated with increasing Cry1Ab concentrations, and mortality and growth inhibition were evaluated after 7 days. Variation in Cry1Ab susceptibility of samples was up to 13.2-fold. A smaller variability was found for populations pooled according to geographic and climatic conditions (up to 6.6-fold). The results indicate that the observed population variation in susceptibility reflects natural variation in Bt susceptibility among ECB populations.*

For any particular lepidopteran species, estimates of the susceptibility of larvae to Bt-protein vary (Monnerat *et al*., 2006; Saeglitz *et al*., 2006; Schuphan, 2006; Gaspers *et al*., 2010) depending on different factors. The EFSA GMO Panel recognises that there is intra- and inter-population variation in the susceptibility of target pest populations (Gaspers *et al.,* 2010). According to the studies by Gaspers (2009), there is a low genetic differentiation of *O. nubilalis* populations in Europe and no geographic clusters of populations from the same country appeared, which suggested that there was no geographic differentiation which is likely to be the consequence of high rates of gene flow between the populations. This was also confirmed by analysis conducted with ECB in Europe by Saeglitz (2006) and in USA by Kim *et al*. (2009). Despite this low genetic differentiation, baseline susceptibility of ECB populations to Cry1Ab, e.g., the LC50 (µg per ml of diet) values measured using

[15] MON810 2009 PMEM report, Appendices 4 and 5
[16] MON810 2009 PMEM report, Appendix 4

the diet incorporation method varied up to five fold among samples from France, Germany, Greece and Italy (Schuphan, 2006). Similar fluctuations of baseline susceptibility of ECB to the Cry1F protein was reported based on lethal concentrations (LC_{50} or LC_{90} values) among populations in Europe or among samples tested without showing consistency (Gaspers *et al.*, 2010). Baseline susceptibility to Cry1Ab of MCB Spanish field populations analysed in 2003-2005 was very low, with LC50 values fluctuating between 12 and 30 ng Cry1Ab/cm^2, regardless of the region of origin, the type of maize (Bt or non-Bt) and the year. Furthermore, no significant differences were found when comparisons were made with a laboratory population or with field populations from Greece (in Bt-free areas) (Farinós *et al*., 2011).

(2) Monitoring of resistance evolution in target pests

The applicant reported that maize fields (*refugia* or adjacent ones to maize MON810 fields) were sampled for MCB populations in two maize growing areas of the Iberian Peninsula: Northeast Iberia (Ebro Valley) and Southwest Iberia (Extremadura in Spain and South of Portugal) to detect changes in susceptibility to the Cry1Ab toxin. Similar sampling was conducted to collect ECB populations from four countries and included two Iberian areas: Central Iberia (Albacete) and Northeast Iberia (Ebro Valley) to detect changes in susceptibility to maize MON810 of these populations. Two different methods were used: mortality assessed to determine the lethal concentrations (LC) and growth inhibition assessed for the molting inhibition concentrations (MIC). These methods were used to compare susceptibility data to the Cry1Ab protein. Mortality and growth inhibition results for laboratory and field populations in three different sampling years (biannually between 2004 and 2009) were compared where field infestation levels allowed. For MCB, growth inhibition data (MIC values) were more precise and therefore appropriate to reflect changes in susceptibility to Cry1Ab protein. For ECB, both mortality (LC) and MIC values could be used for the same purposes. However, variability of data (higher for LC and lower for MIC data) between years and regions was evident for both target pests. 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 2009 MON810 report or the additional information provided by the applicant do not specify whether the same ECB/MCB populations were monitored over time. The EFSA GMO Panel therefore makes specific recommendations on the sampling of target pests in chapter 2.3.

The EFSA GMO Panel acknowledges that the available dataset does not show evidence of insect resistance evolution. Indeed, the variability between regions was higher than the changes observed over time. Insect resistance evolution was not detected at this early stage by the sampling plan (e.g., limited number of sampled sites), while some changes in susceptibility were detected. Hence the EFSA GMO Panel evaluated to what extent the monitoring protocol 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'*.

The EFSA GMO Panel considered the following issues:

a) Is the monitoring scheme adequate to detect levels of resistance which would result in control failures in the field early enough?

b) If not, how could the sampling scheme be improved to provide earlier detection of insect resistance evolution?

c) Should Bt-maize fields also be surveyed to detect survival of target pests within MON810 fields and which might indicate resistance evolution?

d) Should the F2-screen method be recommended as it allows a more precise estimation of resistance allele frequency?

a) Relevance of the resistance allele frequency detection threshold

The applicant submitted an IRM plan largely based on standards adopted in the USA for maize MON810 aiming at detecting a resistance allele frequency ranging from 1 to 5%. However, agricultural landscape and cropping systems in the EU are sometimes quite different from the USA. Furthermore, the specific characteristics (e.g., reproduction, survival) of the two target pests, namely ECB and MCB, in the EU need to be considered.

In order to develop an optimal sampling frame for ECB and MCB under European conditions (see Appendix 2), the EFSA GMO Panel made use of a theoretical model by Alstad and Andow (1995). The simulation exercise was carried out with varying values of different parameters (e.g., adoption rate of maize MON810, initial frequency of resistance allele, survival). Considering that at least one year is needed for an adaptative response to the detection of insect resistance (Andow and Ives, 2002), the simulation exercise indicated that the monitoring protocol as currently proposed by the applicant includes a rather large range of frequency alleles to be detected which is only sufficient for the timely detection of increasing resistance for univoltine ECB populations. In the case of bivoltine ECB or MCB strains, the remaining time span may not be sufficient to implement additional management measures in a timely manner. Conventionally, a population is considered resistant to a certain toxin when the resistance allele reaches a relative frequency of 0.5. At field level, resistance is defined as a 'repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species' (Insecticide Resistance Action Committee, 2010). Considering that risk managers might need a minimum of two years in order to put in place appropriate management measures before pest populations become resistant (cf. Andow and Ives, 2002), simulation shows that a monitoring protocol should aim at detecting resistance allele frequencies clearly below 5% (see Appendix 2 for further details).

In the case of multivoltine populations, a frequency between 1% and 3% would usually be appropriate to implement management measures in time. This could be done by increasing the number of larvae collected (10 000 larvae for 1% and 1000 larvae for 3% (cf. Andow and Ives, 2002)) or through F2 screening.

b) Sampling scheme

In addition to the size of samples, sampling sites should be considered. The natural biogeographical variation of ECB/MCB susceptibilities across regions might make it difficult to detect evolution over time. From the information provided, the EFSA GMO Panel could not estimate the local selection pressure on the ECB/MCB populations (acreage of maize, uptake of Bt-maize, level of Bt-maize the years(s) before within the same local area). It is therefore impossible to assess to what extent monitored areas were representative of high selection pressure for resistance, including those with the highest intensity of Bt-maize, as indicated in the applicant's plan.

The EFSA GMO Panel therefore considers that recording the exact sampling sites and the local exposure level (percentage of maize in the AUA[17], uptake of Bt-maize) is required. When Bt-maize has been grown in the same area in previous years, the local exposure level should be estimated for these years to help identify 'hotspot areas' that should be included in the sampling scheme and monitored over time.

In its recent scientific opinion providing guidance on PMEM of GM plants (EFSA, 2011), the EFSA GMO Panel reiterated the importance to set up national cultivation registers referred to in Article 31. 3 (b) of Directive 2001/18/EC (EC, 2001).

c) Data generation from maize MON810 fields

[17] AUA = Agricultural Unit of Account

Considering the constant selection pressure to the Cry1Ab protein, resistance evolution in target pests is likely to first appear in maize MON810 fields. The EFSA GMO Panel therefore recommends that maize MON810 fields be surveyed for the occurrence of possible resistant lepidopteran target pests. This could be done:

- by surveying maize MON810 fields for which farmers have reported unusual presence of damaged maize plants and of surviving target pests (see chapter 3.2.1),

- by sampling within maize MON810 fields, together to refugia or conventional maize fields, for collecting larvae within the CSM IRM scheme.

One should note that the detection of plants damaged by target pests does not necessarily mean resistance evolution. A certain percentage maize plants in maize MON810 fields might be plants with reduced or no Cry1Ab protein expression. In addition, surviving target pests larvae may move to maizeMON810 plants from these plants and from adjacent *refugia* plants.

The EFSA GMO Panel recommends sampling target pest larvae in maize MON810 fields as late as possible in the growing season in order to increase the probability of finding potentially resistant (heterozygote) individuals.

d) Potential use of F2-screen method

Susceptibility tests conducted by the applicant are based on bioassays performed with a discriminating dose (e.g., Marçon *et al*., 2000) with F1 progeny larvae obtained by field collected individuals[18].

However, when resistance alleles have not been identified and are believed to be rare and recessive, Andow and Alstad (1998, 1999) and Andow and Ives (2002) suggested that the most efficient method is the F2 screen, which allows to estimate the resistance allele frequency with a small number of samples.

Mated females are considered to be the preferred stage for initiating an F2 screen, but many variant methods have been proposed (Bentur *et al*., 2000; Zhao *et al*., 2002; Stodola and Andow, 2004; Stodola *et al.*, 2006). From the F1 progeny of these field-collected adults, single female lines are reared and sib-mating within each of these lines will produce F2 offspring on which bioassays are conducted.

Experience in Europe (Engels *et al*., 2010) demonstrated the cost effectiveness of this method for detecting resistance alleles in multivoltine strains of *O. nubilalis* Hubn. However, this technique can hardly be applied to univoltine populations of the pest, since dormancy occurs in laboratory reared populations of such strains and therefore it is very difficult to reach an adequate number of replications.

Therefore, the EFSA GMO Panel agrees with the applicant that assays performed on F1 progeny are acceptable for ECB in many areas. However, in 'hotspot areas' where target pests are more likely to be multivoltine and where a resistance allele frequency between 1% and 3% should be detected (see chapter 2.2.2.2), the EFSA GMO Panel recommends to increase the number of larvae collected or to use a F2 screening (Engels *et al*., 2010).

2.2.3. Communication with and education of farmers

Two third of surveyed farmers in Spain considered they were well informed about *refugia* implementation and about 30% considered that the implementation is *little easy/not easy*[19].

[18] MON810 2009 PMEM report, Appendix 1

The EFSA GMO Panel considers that special attention should be paid to *refugia* implementation in those areas where the likelihood of resistance evolution is higher. In these situations, considering the non-compliance of farmers to implement non-Bt *refugia*, the EFSA GMO Panel recommends further education and training of farmers on their obligations to inhibit the evolution of insect resistance (see chapter 2.3). When implementing rules to ensure compliance with non-Bt *refugia*, it is advisable that risk managers and farmers pay particular attention to those situations where pest resistance is most likely to evolve. In particular, applicants need to inform farmers that, where adoption of Bt-maize is high, then there is a need to consider the total areas of Bt-maize cultivation independent of farm and field sizes, and to adopt *refugia* accordingly.

2.3. Conclusions & Recommendations on CSM

The EFSA GMO Panel assessed the 2009 results of the implementation of the CSM plan and particularly the IRM plan, as provided by the applicant, on maize MON810 and concludes that there is no evidence of resistance evolution in target pests based on the available information.

However, in light of the shortcomings identified during the evaluation of the methodology, the EFSA GMO Panel advises the applicant to reconsider its IRM plan taking into consideration the following points:

I. related to the implementation of non-Bt *refugia* and farmers education:

- to consider non-Bt *refugia* for all clusters of fields with an aggregate area greater than 5 ha of Bt-maize, 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 MON810 adoption rate and number of target pests generations at a geographical scale which is relevant to the IRM in order to identify 'hotspot areas';

- to further educate farmers on the need to comply with refugia implementation and to inform them about the situations which increase the probability that resistance to the Cry1Ab protein may evolve in the target pests and other regionally important lepidopteran pests, and thus threaten the efficacy of maize MON810.

II. related to resistance monitoring of target pests:

- to focus the sampling of target lepidopteran pests in 'hotspot areas' over time (e.g., high adoption rate and frequency of maize MON810 and multivoltine populations) to increase the likelihood of detecting resistance evolution. Sampling in areas with lower adoption rate of maize MON810 is also required but at a lower frequency in order to establish susceptibility baselines;

- to include in the samplings surviving target lepidopteran pests within maize MON810 fields in order to detect potentially resistant individuals. The sampling should be mainly done as late as possible within 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 MON810 in the context of CSM for IRM strategy (EFSA, 2009a) and, where appropriate, adjust the design and implementation of the IRM plan accordingly;

[19] MON810 2009 PMEM report, Section 3.2.1.1

- in 'hotspot areas' (i.e., regions with high uptake of maize MON810 and multivoltine populations), to revise the monitoring protocol aiming at a detecting resistance allele frequency between 1% and 3%. The EFSA GMO Panel recommends to increase the number of larvae collected or to use a F2 screening.

According to the 2011 scientific opinion on PMEM of GM plants (EFSA, 2011), the applicant should provide to Member States and European Commission the raw data from CSM.

Furthermore, in order to better target the sampling frame, the EFSA GMO Panel reiterates the recommendation in its recent scientific opinion providing guidance on PMEM of GM plants (EFSA, 2011) to set up national cultivation registers referred to in Article 31. 3 (b) of Directive 2001/18/EC (EC, 2001).

3. GENERAL SURVEILLANCE (GS)

3.1. Summary of the information provided by the applicant

For the 2009 growing season of maize MON810, the applicant reported the results of its GS plan, mainly by analysing results of questionnaires answered by selected farmers in the EU Member States where maize MON810 was cultivated in 2009. The 2009 plan for GS[20] of maize MON810 consists in four elements: (1) a survey of 240 farmers conducted by interviewers following a written questionnaire, (2) the data gathered from publications related to maize MON810, (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 hereunder:

(1) Farmers planting maize MON810 in 2009 were asked to record and report their observations and assessment in and around maize MON810 fields in comparison to a baseline, being their historical local knowledge and experience[21]. Initially, the applicant had defined a total sample size of 2500 questionnaires for the overall duration of the consent, namely ten years. Therefore, the applicant planned to collect approximately 250 questionnaires per year. In 2009, a total of 240 questionnaires were received from farmers in six European countries (49 in Czech Republic, 3 in Poland, 100 in Spain, 42 in Portugal, 40 in Romania and 6 in Slovakia). According to the applicant, the farmers/fields were randomly selected between the countries depending on the maize MON810 market penetration. The farmer surveys were carried out by third parties having experience in agricultural surveys, except in Poland where the applicant interviewed the farmers. In this respect, the 2009 MON810 report states that the interviewers were trained to understand the background of questions and were also provided with a 'user manual' to assist them in filling the questionnaires with the farmers. The questionnaires were completed between November 2009 and January 2010. 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[22]. All data were entered and checked for quality and plausibility before being considered for statistical analysis. In its report, the applicant concluded that the 2009 statistical analysis[23] of the 240 questionnaires did not reveal any unanticipated adverse effects that could be associated to maize MON810. The applicant also concluded that the frequency patterns of farmers answers in 2009 are similar to those of the previous years;

[20] The 2009 MON810 report submitted by Monsanto is made 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
[21] MON810 2009 PMEM report, Section 3.1.2.1.
[22] MON810 2009 PMEM report, Appendix 7
[23] MON810 2009 PMEM report, Appendix 7

(2) A list of peer-reviewed publications on the safety of maize MON810 and the Cry1Ab protein published between June 2009 and June 2010 was submitted. The applicant used specific key words and searched throughout the ISI Web of Knowledge. The first set of papers from ISI Web of Knowledge was screened for relevance to the ERA of maize MON810. The applicant reported ten publications on molecular & food/feed aspects and 22 publications related to the ERA of maize MON810. The applicant concluded that the peer-reviewed literature did not raise safety concern for maize MON810.

The applicant did not provide details on existing monitoring networks likely to be of use for GS of maize MON810. 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[24].

3.2. Assessment by the EFSA GMO Panel

3.2.1. Farmer questionnaires

The EFSA GMO Panel is of the opinion that questionnaires, directed at farms or production systems where GM plants are grown, are considered a useful method for collecting first hand data on the performance and impacts of a GM plant and its cultivation and for comparison with conventional plant cultivation. A major purpose of farmer questionnaire is to detect changes in management practices of GM fields. The EFSA GMO Panel is of the opinion that farmer questionnaire can be used as an early-warning tool which would trigger additional studies, should unanticipated changes occur which might lead to adverse environmental effects. However, it is recognised that the information supplied by farmers will be limited to observations they can make within their areas of experience, related mostly to the areas on their farms cultivated with the GM and non-GM crop and their historical experience (EFSA, 2011). In its recent scientific opinion on PMEM (EFSA, 2011), the EFSA GMO Panel provides guidance to applicants on how to supplement and analyse the farmer questionnaires for an optimised monitoring of the GM plant and of its cultivation sites.

According to the terms of reference of the mandate from the European Commission, the EFSA GMO Panel also assessed the methodology followed by the applicant to analyse the farmer questionnaires. The EFSA GMO Panel was assisted by the EFSA Unit for Scientific Assessment Support (EFSA SAS Unit) which provides a methodological guidance for a systematic evaluation of the farmer questionnaires (see Appendix 1). Appendix 1 sets 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 MON810, its design, it use and analysis are given in Appendix 1.

During its evaluation of the analysis of the farmer questionnaires, the EFSA GMO Panel and the EFSA SAS Unit identified shortcomings of the biometrical analysis[25] and felt the need to further substantiate the results. The raw data from farmer questionnaires, provided upon request by the EFSA GMO Panel, were reanalysed (see Appendix 1 for further details). The outcome of the statistical reanalysis does not change the results reported in the 2009 MON810 report, however the use of confidence intervals facilitates the interpretation of the results and allows the effect of the selection of alternative threshold values other than the arbitrarily selected 10% to be explored.

[24] MON810 2009 PMEM report, Section 3.1.2.3
[25] MON810 2009 PMEM report, Appendix 7

From the 2009 analysis of the farmer questionnaires on maize MON810, the EFSA GMO Panel concludes that no unanticipated adverse effect was identified based on the available data. However, the EFSA GMO Panel, assisted by the EFSA SAS Unit, identified weaknesses in the methodology and gives recommendations to the applicant (see chapter 3.3.).

3.2.2. Existing Monitoring Networks

While the EFSA GMO Panel recommends the applicants to use farmer questionnaires for monitoring the GM plant and its cultivation sites, the EFSA GMO Panel is of the opinion that monitoring at a larger scale (than in and near GM fields) should also be conducted. The EFSA GMO Panel is of the opinion that 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 to the implementation of the plan (see EFSA, 2011 for further guidance).

3.2.3. Literature review

The EFSA GMO Panel is of the opinion that the literature review provided by the applicant is limited, too selective and that not all relevant published information is provided. The applicant used one single searching tool. The EFSA GMO Panel advises the applicant to perform a more comprehensive review by considering the EFSA Guidance Document on systematic literature review methodology (EFSA, 2010) to select relevant papers likely to have an impact on the previous risk assessments of maize MON810. An explanation of the criteria used to select the relevant papers should be provided and finally a discussion of the publications (e.g., assessment endpoints, exposure, effects). Moreover, the EFSA GMO Panel expected the selected papers to be put into context and considered in the light of the overall ERA of maize MON810 (EFSA, 2011).

As outlined in the recent scientific opinion on PMEM of GM plants (EFSA, 2011), there is considerable research and development studies ongoing around the world on the management, cultivation and impacts of GM plants. These studies include experimental research, developmental and advisory studies on crop cultivation, variety registration and variety performance trials. The applicant should show an awareness of these activities particularly on GM plants with similar traits or characteristics. The results of these studies should be reviewed and put into the context of the original ERA by relating each study to the respective area of risk to be addressed in the ERA; finally, the implications of the results should be considered (EFSA, 2011).

3.3. Conclusions & Recommendations on GS

From the data provided in the 2009 survey for the farmer questionnaire to monitor adverse effects associated with the cultivation of maize MON810, no adverse effect can be identified. However, a number of improvements to the survey design and reporting have been identified and are listed in the recommendations below.

In addition to its general recommendations on the farmer questionnaire set in the 2011 scientific opinion on PMEM (EFSA, 2011), the EFSA GMO Panel advises the applicant also to take into account the following points:

- the sampling frame should be comprehensive and a stratification should be applied consistently in each country. Adequate sampling should be carried out from the previous stratification exercise;

- the cultivation areas, with high uptake of maize MON810 and where maize MON810 has been continuously grown in previous years, should be over-represented in the sampling scheme;

- the number of farmers not participating in the survey and the reasons thereof should be documented;

- the comparator should be clearly identified. If no comparators are being grown spatially or temporally close to the GM plant, then the rationale for selecting another comparator (e.g., historical data) should be fully described (see EFSA, 2011);

- impartial and standardised interviews should be carried out by independent parties and effective quality and auditing procedures should be considered;

- additional questions to the farmer questionnaire should be considered to better describe the cultivation of Bt-maize in the local area and/or the previous years, the receiving environments and the management systems in which maize MON810 is being grown;

- relevant data as from other sources of information (e.g., official statistics on crop management practices) should/could be considered for validity check of the questionnaires (e.g., consistency, representativeness);

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

- appropriate statistical procedures should be used based on using a distribution for appropriate outcomes;

- the use of a standard default effect size of 5% is not relevant for all assessment endpoints and, where scientifically justified, different default effect sizes should be considered for some assessment endpoints;

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

- a codification for farmers repeatedly surveyed over years should be set up. These farmers should be particularly monitored;

- the number of years the surveyed farmer has grown maize MON810 and other GM crops should be indicated.

Further details are provided in Appendix 1.

In order to further improve the farmer questionnaire and in addition to the provisions of its 2011 scientific opinion on PMEM of GM plants (EFSA, 2011), the EFSA GMO Panel advises the applicant to include the following indicators and parameters to be measured via the farmer questionnaire (see chapter 4.2.2.1(2) of EFSA, 2011):

- the occurrence of regionally important lepidopteran pests other than ECB and MCB (see chapter 2.2.1.1) in maize MON810 fields and surrounding areas;
- in addition to the questions on pest and disease incidence, the farmer questionnaire should specifically request information on the occurrence of damaged maize MON810 plants which might be associated with corn borers as this information will complement the monitoring of resistance evolution in target pests (see chapter 2.2.2 (2) c),
- detailed information on the proportion of non-Bt maize compared with MON810 on the farm, the distance between the refuge area and the monitored maize MON810 field and the differences in the pest management practices of the non-Bt crop refuge areas.

Furthermore, in order to improve the sampling frame of the farmers survey, the EFSA GMO Panel reiterates the recommendation in its recent scientific opinion providing guidance on PMEM of GM plants (EFSA, 2011) to set up national cultivation registers 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 are limited to observations they can make on their areas of experience, which relate mostly to the areas on their farms cultivated with maize MON810. The 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., from existing monitoring networks, see chapter 4.2.1.3. of EFSA, 2011) at 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 and that the literature review needed considerable improvement. The EFSA GMO Panel therefore recommends the applicant to follow the guidance provided in its recent scientific opinion on PMEM of GM plants (for further details, see EFSA, 2011).

OVERALL CONCLUSIONS AND RECOMMENDATIONS

From the data submitted by the applicant in its 2009 MON810 report, the EFSA GMO Panel did not identify adverse effects on the environment, human and animal health due to maize MON810 cultivation during the 2009 growing season. Furthermore, the EFSA GMO Panel is of the opinion that the outcomes of the 2009 MON810 report do not invalidate the previous risk assessment of maize MON810 and the subsequent recommendations on risk management. In this respect, the EFSA GMO Panel reiterates its 2009 recommendation that, especially in areas of abundance of non-target Lepidoptera populations, the adoption of the cultivation of maize MON810 be accompanied by management measures in order to mitigate the possible exposure of these species to maize MON810 pollen. The implications of these management measures should be considered in the PMEM plan.

However, during its evaluation of the 2009 MON810 report, the EFSA GMO Panel identified a certain number of shortcomings in the methodology for CSM and GS of maize MON810. Hence, the EFSA GMO Panel recalls the general recommendations given in its scientific opinion providing guidance on PMEM of GM plants (EFSA, 2011). The EFSA GMO Panel also makes additional specific recommendations for the improvement of the PMEM of maize MON810 in chapters 2.3 and 3.3 of the present scientific opinion.

The recommendations of the EFSA GMO Panel in this opinion supplement the previous recommendations on PMEM of maize MON810 in the 2009 scientific opinion for the renewal of the authorisation for continued marketing of maize MON810 (EFSA, 2009a).

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the European Commission, dated 4 November 2010, to the EFSA Executive Director requesting the assessment of MON810 monitoring report for the 2009 cultivation season provided by Monsanto.

2. Acknowledgement letter, dated 23 November 2010, from the EFSA Executive Director to the European Commission.

3. E-mail from EFSA to the applicant, dated 6 December 2010, requesting additional information.

4. Letter from the applicant to EFSA, dated 6 January 2011, providing the additional information requested by EFSA.

5. E-mail from the European Commission, dated 21 January 2011, to EFSA including the comments from Member States on the 2009 PMEM report on maize MON810.

6. E-mail from EFSA to the applicant, dated 17 February 2011, requesting additional information.

7. Letter from the applicant to EFSA, dated 2 March 2011, providing the additional information requested by EFSA.

8. Letter from EFSA to the applicant, dated 28 March 2011, requesting additional information.

9. Letter from the applicant to EFSA, dated 20 April 2011, providing the additional information requested by EFSA.

REFERENCES

Alcalde E, Amijee F, Blache G, Bremer C, Fernandez S, Garcia-Alonso M, Holt K, Legris G, Novillo C, Schlotter P, Storer N, Tinland B, 2007. Insect resistance monitoring for Bt maize cultivation in the EU: proposal from the industry IRM working group. *Journal of Consumer Protection and Food Safety*, 2(S1), 47-49.

Alstad DA and Andow DA, 1995. Managing the evolution of insect resistance to transgenic plants. Science, 268: 1894-1896.

Andow DA, 2008. The risk of resistance evolution in insects to transgenic insecticidal crops. Collection of Biosafety Reviews 4, 142-199.

Andow DA and Alstad DN, 1998. F2 Screen for rare resistance alleles. Journal of Economic Entomology 91, 572-578.

Andow DA and Alstad DN, 1999. Credibility interval for rare resistance allele frequencies. Journal of Economic Entomology 94, 755-758.

Andow DA and Ives AR, 2002. Monitoring and adaptive resistance management. Ecological Applications 12, 1378-1390.

Andreadis SS, Álvarez-Alfageme F, Sánchez-Ramos I, Stodola TJ, Andow DA, Milonas PG, Savopoulou-Soultani M, and Castánera P, 2007. Frequency of Resistance to Bacillus thuringiensis Toxin Cry1Ab in Greek and Spanish Population of Sesamia nonagrioides (Lepidoptera: Noctuidae), Journal of Economic Entomology 100(1):195-201.

Armitage P, Berry G, Matthews JNS, 2002. Statistical Methods in Medical Research. 4th ed. Blackwell, Oxford, 817pp.

Bates SL, Zhao JZ, Roush RT, Shelton AM, 2005. Insect resistance management in GM crops: past, present and future. Nature Biotechnology 25, 57-62.

Beckie HJ, Hall LM, Simard MJ, Leeson JY, and Willenborg CJ, 2010. "A framework for postrelease environmental monitoring of second-generation crops with novel traits." Crop Science, 50(5), pp. 1587-1604. doi: 10.2135/cropsci2009.12.0743

Bentur JS, Andow DA, Cohen MB, Romena AM, Gould F, 2000. Frequency of alleles conferring resistance to a *Bacillus thuringiensis* toxin in a Philippine population of *Scirpophaga incertulas* (Lepidoptera: Pyralidae). Journal of Economic Entomology 93, 1515-1521.

Bergé JB and Ricroch A, 2011. La gestion de la durabilité des PGM résistantes à certains insectes. In Biotechnologies végétales: environnement, alimentation, santé; Troisième partie: Agriculture et environnement. Vuibert editions, 272 p.

CABI Crop Protection Compendium http://www.cabi.org/cpc/

Carringer RD, Marcinkiewicz BR, Schottel BS, 2004. Collection of plant tissue and grain samples from test corn hybrids containing M810xMON863 and MON863xNK603, parental controls and references in 2002-2003 Argentina field production. Report No. MSL-18567

Chafaux J, Seguin M, Swanson JJ, Bourguet D, Siegfried BD, 2001. Chronic exposure of the European Corn Borer (Lepidoptera: Crambidae) to Cry1Ab *Bacillus thuringiensis* Toxin. Journal of Economic Entomology 94, 1564-1570.

Comins HN, 1977. The development of insecticide resistance in the presence of migration. Journal of Theoretical Biology 64:177–197.

EC, DG SANCO EU Pesticides database http://ec.europa.eu/sanco_pesticides/public/index.cfm?event=activesubstance.selection

EC, 1990. Council Directive 90/220/EEC of 23 April 1990 on the deliberate release into the environment of genetically modified organisms. Official Journal of the European Communities, L117, 15-27.

EC, 1998. Commission Decision of 22 April 1998 concerning the placing on the market of genetically modified maize (*Zea mays* L. line MON810), pursuant to Council Directive 90/220/EEC (98/294/EC). *Official Journal of the European Communities*, L131: 32- 33.

EC, 2001. Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Official Journal L106**,** 1-39.

EFSA, 2005. Opinion of the Scientific Panel on Genetically Modified Organisms on an application (reference EFSA-GMO-BE-2004-07) for the placing on the market of insect-protected glyphosate-tolerant genetically modified maize MON863 x MON810 x NK603, for food and feed use, import and processing under Regulation (EC) No 1829/2003 from Monsanto, The EFSA Journal (2005) 256, 1-25.

EFSA, 2009a. Scientific Opinion of the Panel on Genetically Modified Organisms on applications (EFSA-GMO-RX-MON810) for the renewal of authorisation for the continued marketing of (1) existing food and food ingredients produced from genetically modified insect resistant maize MON810; (2) feed consisting of and/or containing maize MON810, and maize MON810 for feed use (including cultivation); and of (3) food additives and feed materials produced from maize MON810, all under Regulation (EC) No 1829/2003 from Monsanto. *The EFSA Journal,* 1149: 1-84.

EFSA, 2009b. Standard Operating Procedure EFSA/SOP/00–16 - Procedure for conducting a survey.

EFSA, 2010. Application of systematic review methodology to food and feed safety assessments to support decision making. The EFSA Journal 1637, 1-90.

EFSA, 2011. Scientific Opinion on guidance on the Post-Market Environmental Monitoring (PMEM) of genetically modified plants. The EFSA Journal 2316, 1-43.

Eizaguirre M, López C, Albajes R, 2004. Dispersal capacity in the Mediterranean corn borer, *Sesamia nonagrioides*. Entomologia Experimentalis et Applicata 113, 25–34.

Eizaguirre M, Albajes R, López C, Eras J, Lumbieres B, Pons X, 2006. Six years after the commercial introduction of Bt maize in Spain: field evaluation, impact and future prospects. Transgenic Research 15, 1-12.

EMEA (European Agency for the Evaluation of Medicinal Products – Committee for Proprietary Medicinal Products), 2000. Points to consider on switching between superiority and non-inferiority. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003658.pdf

Engels H, Bourguet D, Cagáň L, Manachini B, Schuphan I, Stodola TJ, Micoud A, Brazier C, Mottet C, Andow DA, 2010. Evaluating Resistance to Bt Toxin Cry1Ab by F_2 Screen in European Populations of Ostrinia nubilalis (Lepidoptera: Crambidae). Journal of Economic Entomology 103 (5), 1803-1809.

EPA, 1998. The Environmental Protection Agency's White Paper on Bt Plant-Pesticide Resistance Management (Environmental Protection Agency, Washington, D.C., U.S.A.).

EPPO European and Mediterranean Plant Protection Organisation http://www.eppo.org/

EUROSTAT Farm Structure http://epp.eurostat.ec.europa.eu/statistics_explained/index.php/Farm_structure

Fantinou AA, Perdikis DC, Zota K, 2004. Reproductive responses to photoperiod and temperature by iapausing and non-diapausing populations of Sesamia nonagrioides Lef. (Lepidoptera – Noctuidae). Physiological Entomology, 29, 169–175.

Farinós GP, de la Poza M, Hernández-Crespo P, Ortego F, Castañera P, 2004. Resistance monitoring of field populations of the corn borers *Sesamia nonagrioides* and *Ostrinia nubilalis* after 5 years of Bt maize cultivation in Spain. Entomologia Experimentalis et Applicata 110, 23-30.

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, doi:10.1016/j.cropro.2011.03.011

Gail and Benichou, 2000. Encyclopedia of Epidemiologic Methods. John Wiley & Sons, Chichester.

Gaspers C, 2009. The European corn borer (Ostrinia nubilalis, Hbn.), its susceptibility to the Bt-toxin Cry1F, its pheromone races and its gene flow in Europe in view of an Insect Resistance Management. Unpublished PhD thesis: Rheinisch-Westfälischen Technischen Hochschule Aachen, Germany.

Gaspers C, Siegfried BD, Spencer T, Alves AP, Storer NP, Schuphan I, Eber S, 2010. Susceptibility of European and North American populations of the European corn borer to the Cry1F insecticidal protein. Journal of Applied Entomology, doi:10.1111/j.1439-0418.2010.01541.x.

Gillyboeuf N, Anglade P, Lavenseau L, Peypelut L, 1994. Cold hardiness and overwintering strategy of the pink maize stalk borer, Sesamia nonagrioides Lef (lepidoptera, noctuidae) Oecologia, Volume 99, Numbers 3-4, 366-373, DOI: 10.1007/BF00627750

Gonzalez-Nuñez M, Ortego F, Castañera P, 2000. Susceptibility of Spanish Populations of the Corn Borers *Sesamia nonagrioides* (Lepidoptera: Noctuidae) and *Ostrinia nubilalis* (Lepidoptera: Crambidae) to a Bacillus thuringiensis Endotoxin. Journal of Economic Entomology 93(2), 459 – 463.

Hollis JM, Jones RJA, Marshall CJ, Holden A, Van de Veen JR, Montanarella L, 2006. SPADE-2: The soil profile analytical database for Europe, version 1.0. European Soil Bureau Research Report No.19, EUR 22127 EN, 38pp. Office for Official Publications of the European Communities, Luxembourg.

Huang FN, Andow DA, 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. doi: 10.1111/j.1570-7458.2011.01138.x

Hunt TE, Kamble ST, Danielson SD, 1998. Pesticide use on irrigated and non-irrigated corn in Nebraska—1994: Final Report. National Agriculture Library, Beltsville, MD.

Hunt TE, Buschman LL, Sloderbeck PE, 2007. Insecticide use in Bt and non-Bt field corn in the western corn belt: as reported by crop consultants in a mail survey. American Entomologist 53, 86-93.

Insecticide Resistance Action Committee, 2010. Resistance Management for Sustainable Agriculture and Improved Public Health, 2nd edn. Available at: http://www.irac-online.org/wp-content/uploads/2009/09/VM-Layout-v2.6_LR.pdf.

Ives AR and Andow DA, 2002. Evolution of resistance to *Bt* crops: directional selection in structured environments. Ecology Letters 5, 792-801.

Kelley K, Clark B, Brown V, Sitzia J. Good practice in the conduct and reporting of survey research. Int J Qual Health Care 2003; 15(3):261-266.

Kim KS, Bagley MJ, Coates BS, Hellmich RL, Sappington TW, 2009. Spatial and Temporal Genetic Analyses Show High Gene Flow Among European Corn Borer (Lepidoptera: Crambidae) Populations Across the Central U.S. Corn Belt. Environmental Entomology 38(4), 1312-1323.

Kirkwood BR and Sterne JAC, 2003. Essential medical statistics (seconded.). Malden, Mass. USA: Blackwell Publishing Company. Shavelson RJ, &Webb NM (1991).

Kruger M, Van Rensburg JBJ, Van den Berg J, 2011. Transgenic Bt maize: farmers' perceptions, refuge compliance and reports of stem borer resistance in South Africa. Journal of Applied Entomology, doi: 10.1111/j.1439-0418.2011.01616.x

Legg CJ and Nagy L, 2006. Why most conservation monitoring is, but need not be, a waste of time. Journal of Environmental Management 78, 194-199.

MacIntosh SC, 2010. Managing the risk of insect resistance to transgenic insect control traits: practical approaches in local environments. Pest Management Science 66, 100-106.

Marçon PCRG, Taylor DB, Mason CE, Hellmich RL, Siegfried BD, 1999. Genetic similarity among pheromone and voltinism races of *Ostrinia nubilalis* Hübner (Lepidoptera: Cramidae). Insect Molecular Biology 8, 213-221.

Marçon PCRG, Siegfried BD, Spencer T, Hutchison WD, 2000. Development of diagnostic concentrations for monitoring *Bacillus thuringiensis* resistance in European corn borer (Lepidoptera: Crambidae). Journal of Economic Entomology, 93:925–930. BioOne, PubMed, CSA

Monnerat R, Martins E, Queiroz P, Orduz S, Jaramillo G, Benintende G, Cozzi J, Real MD, Martinez-Ramirez A, Rausell C, Ceron J, Ibarra JE, Del Rincon-Castro MC, Espinoza AM, Meza-Basso L, Cabrera L, Sanchez J, Soberon M, Bravo A, 2006. Genetic Variability of Spodoptera frugiperda Smith (Lepidoptera: Noctuidae) Populations from Latin America Is Associated with Variations in Susceptibility to Bacillus thuringiensis Cry Toxins. Applied and Environmental Microbiology 72, 7029–7035.

Perry JN, Rothery P, Clark SJ, Heard MS, Hawes C, 2003. Design, analysis and power of the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. Journal of Applied Ecology, 40, 17-31.

Rasch D, Herrendörfer G, Bock J, Victor N, Guiard V, 1996. 3.62.1 Binomialtest. Pages 159-162 in Verfahrensbibliothek Versuchsplanung und auswertung. Volume 1. Rasch D, Herrendörfer G, Bock J *et al*. (eds.), Oldenbourg, München.

Saeglitz C, Bartsch D, Eber S, Gathmann A, Priesnitz KU, Schuphan I, 2006. Monitoring the Cry1Ab susceptibility of European corn borer in Germany. Journal of Economic Entomology, 99, 1768-1773.

Schmidt K, Wilhelm R, Schmidtke J, Beißner L, Mönkemeyer W, Böttinger P, Sweet J, Schiemann J, 2008. Farm questionnaires for monitoring genetically modified crops: a case study using GM maize. Environmental Biosafety Research 7, 163-179.

Schuphan I, 2006. Protecting the benefits of Bt-toxins from insect resistance development by monitoring and management. RWTH Aachen, http://www.bio5.rwth-aachen.de/german/downloads/EU-Review.pdf

Showers WB, Hellmich RL, Derrick-Robinson ME, Hendrix III WH, 2001. Aggregation and dispersal behaviour ofmarked and released European corn borer (Lepidoptera: Crambidae) adults. Environmental Entomology 30, 700– 710.

Stodola TJ and Andow DA, 2004. F2 screen variations and associated statistics. Journal of Economic Entomology 97, 1756-1764.

Stodola TJ, Andow DA, Hyden AR, Hinton JL, Roark JJ, Buschman LL, Porter P, Cronholm GB, 2006. Frequency of resistance to Bacillus thuringiensis toxin Cry1Ab in southern U.S. Corn Belt population of European corn borer (Lepidoptera: Crambidae). Journal of Economic Entomology 99, 502-507.

Tabashnik BE, Gassmann AJ, Crowder DW, Carrière Y, 2008. Insect resistance to *Bt* crops: evidence versus theory? Nature Biotechnology, 26: 199-202.

Tabashnik BE, Van Rensburg JBJ, Carriere Y, 2009. Field-evolved insect resistance to Bt crops: definition, theory, and data. Journal of Economic Entomology 102, 2011-2025.

Tinland B, Delzenne P, Pleysier A, 2007. Implementation of a post-market monitoring for insect-protected maize MON 810 in the EU. Journal of *Consumer Protection and Food Safety*, 2(S1), 7-10.

Wilhelm R, Beißner L, Schmidt K, Schmidtke J, Schiemann J, 2004. Monitoring des Anbaus gentechnisch veränderter Pflanzen - Fragebögen zur Datenerhebung bei Landwirten. Nachrichtenbl. Deut. Pflanzenschutzd 56 (8), 184-188.

Yates F, 1981. Sampling Methods for Censuses and Surveys. Charles Griffin & Company LTD.

Zhao JZ, Li YX, Collins HL, Shelton AM, 2002. Examination of the F2 screen for rare resistance alleles to Bacillus thuringiensis toxins in the diamondback moth (Lepidoptera: Plutellidae). Journal of Economic Entomology 95, 14-21.

APPENDIX 1

SAS technical report on the evaluation of farmer questionnaires submitted in the annual monitoring report of MON810 in 2009

BACKGROUND

This SAS internal technical report has been written to support the EFSA GMO Panel in its evaluation of the monitoring report on maize MON810 for the 2009 cultivation season; and specifically to provide methodological guidance in the evaluation of the farmer questionnaires submitted as part of the general surveillance program aimed at identifying the occurrence of adverse affects of the GMO or its use on human or animal health or the environment, which were not anticipated in the environmental risk assessment.

METHOD

Evaluation criteria were developed based on the principles of design for cross sectional studies, and in particular surveys (Armitage *et al.,* 2002 ; EFSA, 2009b ; Gail & Benichou, 2000 ; Kelley *et al*., 2003 ; Kirkwood & Sterne, 2003 ; Legg and Nagy, 2006 ; Perry *et al.*, 2003). The evaluation grid can be applied to surveys used for general surveillance of GM plants.

Study design principle	Criteria
Sampling frame	1) The sampling frame used is specified 2) The total population included the sampling frame is specified 3) The characteristics of the population included in the sampling frame are described, including region, agricultural practices, GM cultivation 4) The sampling frame coverage is appropriate for GM 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 sampling units from representative environments, reflecting the range and distribution of plant production systems and environments exposed to the GM plants and its cultivation are sampled 3) A list of sample units selected from the sample 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 cultivation
	1) Follow-up method for non-responders is described and

Survey response rate (non response bias)	appropriate 2) 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 instrument has been previously tested and validated
Instrument validity	1) Content validity – the survey includes questions relevant to assess • geographical location • cultivation methods • agronomy parameters • weed/pest management practices • unforeseen weediness and invasiveness • changes in biodiversity of fauna, including non-target arthropods, beneficial organisms and protected species • changes in biodiversity of flora, including seedbank, wild species, weeds and protected species • soil quality and functionality • agro-ecosystems sustainability, including pollinator populations • effects on human health resulting from handling the GM plant • compliance with good agricultural practice 2) Criterion validity – agronomy parameters reported in the survey are compared with field trial data to test for concurrency 3) External consistency - results from survey are compared to and conform with 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 between outcome variables is examined

Data validation	1) Data validation procedure are 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 sub-groups according to PMEM guidance e.g country 4) Statistical analysis is appropriate for the data types 5) 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) Method for handing missing values are described 9) Where appropriate confidence intervals should be 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 supports the conclusions presented in the report

RESULTS

Sampling frame

1) Sampling frame specification

The sampling frame used to select farmers for the survey is not specified in Appendix 7 of the 2009 MON810 report. In the written response from Monsanto on the 2 March 2011 it was indicated that the sampling frame was developed from lists held by the companies selling the seeds in each country and compiled by either the survey organisation or by Monsanto.

2) Population included in the sampling frame

Appendix 7 did not include information on the number of farmers in the sampling frame.

3) Characteristics of the population included in the sampling frame

Appendix 7 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 crop is of importance.

4) Sampling frame coverage

Information on the sampling frame was not provided in Appendix 7 and therefore this is difficult to assess. Table 3.2 indicates farmers from all the countries growing MON810 were included in the survey. The response from Monsanto states that "In countries with low market penetration almost all MON810 cultivating farmers are interviewed", however in Table 3.2 for the Czech Republic, Poland, Romania and Slovakia the percentage area under GM cultivation surveyed is less than 100% and for Poland the farmers surveyed represented only 1% of the area under GM cultivation. This indicates that the sampling frame is not comprehensive for all MON810 farmers and that for Poland the sampling frame coverage is clearly insufficient. 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 member state National registers for the cultivation of GM crops would be a suitable sampling frame.

Sampling method

1) Selection of sample units

Appendix 7 states "The farmers/fields were randomly selected between the countries depending on the grade of market maturity; theoretically within each country each field of MON810 cultivation had the same chance to be surveyed." This indicates random selection is used however there is no description of the mechanism by which farmers are randomly selected from the sampling frame. The response from Monsanto explains that in Spain "interviewers go to each municipality and ask to a randomized sample of farmers, "Have you grown MON810 this year?" If the answer is "yes", then they asked if they are willing to answer the questionnaire". Survey design methodology requires the sampling frame to be representative for the target population, in this case European farmers growing maize MON810, and that the random selection process is applied to the sample units in the sampling frame prior to proceeding with the interviews.

2) Sampling of units from representative environments

Appendix 7 states that stratification by country based on the number of hectares of maize MON810 under cultivation is included in the sampling methodology. The response from Monsanto indicates that within country stratification was used in Spain and Portugal. In Spain representative regions were chosen (3 according to the written response or 4 in Appendix 7) and the number of farmers selected was proportional to acreage of maize grown in each region. In Portugal within country stratification based on region, production systems and farmers new to GM cultivation was applied. The types of production systems sampled in Portugal were not described. Stratification to account for the multi-level structures of the population can ensure the sample is representative (e.g., region, farm size, proportion of farm under maize cultivation, number of years growing MON810). The report should clearly specify where stratification was used and which characteristics were selected and the rational for including the characteristic. Moreover since the results of the survey are combined at European level the within country stratification should be applied consistently in all countries, with the exception of those countries where all sample units in the sampling frame are surveyed.

3) Proportion of sample units selected

The number of farmers surveyed in each country is provided, however no indication of the total number of farmers in each country included in the sampling frame is given. In the written response from Monsanto it was indicated that for some countries with a low level of MON810 cultivation all farmers in the sampling frame were included in the survey. It is essential to know the proportion of farmers selected from the sampling frame according to the characteristics used for stratification.

4) Selection bias

For countries where all farmers were selected from the sampling frame there is no selection bias if the sampling frame is comprehensive for all farmers growing MON810. For countries where all units in the sampling frame are not surveyed a sampling method using stratification and the random selection of farmers from the sampling frame within the strata can minimise selection bias and improve the accuracy of the results (Yates, 1981). The report provides limited information on the sampling methodology and in the written response the methodology described is ambiguous and not applied consistently in those countries mentioned. Stratification should be based on a scientific rational to ensure a representative sample is chosen. 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 full description of the sampling methodology and randomisation techniques should be included in the 2009 MON810 report.

Sample size

1) Size of the adverse effect

Appendix 7 of the 2009 MON810 report states that the null hypothesis is that the proportion of responses not “as usual” is above 10%. Therefore this is a non inferiority test (i.e. that the MON810 field is no more adverse than the conventional comparator field (EMEA, 2000)) and the threshold for adverse effects or non inferiority margin is 10%. In the response from Monsanto it was explained that the threshold of 10% is based on practical experience with plant protection products. No specific reference in 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 □ = 0.01 in Appendix 7. 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 □ = 0.01 in Appendix 7. 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. This represents the "producer's risk" and 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 CADEMO light. The help file for this product indicated the sample size calculation was based on the formula from Rasch, Herrendorfer, Bock, Victor and Guiard (1996). This was confirmed in the written response from Monsanto. A reference for the sample size calculation methodology should be included in the 2009 MON810 report.

5) Sample size calculation

The help file of CADEMO light indicates sample size calculation was performed using the formula in Figure 1 but details of the parameters used in the calculation were not available. Since the responses are categorised into three classes "As unusal", "Minus" and "Plus" a trinomial distribution should be used for the sample size calculation.

Mathematical Background

- ***Formula***

The sample size will be determined approximately for p <= 1/2 by

$$n = \frac{\left[u_p\sqrt{p_0(1-p_0)} + u_{1-\beta}\sqrt{(p_0+d)(1-p_0-d)}\right]^2}{d^2}$$

and for p > ½ by

$$n = \frac{\left[u_{1-\beta}\sqrt{(p_0-d)(1-p_0+d)} + u_p\sqrt{p_0(1-p_0)}\right]^2}{d^2}$$

Figure 1: Sample size calculation extracted from CADEMO light help file.

6) Sample size

The sample size is calculated assuming difference testing and not non-inferiority testing. The difference between the null hypothesis and the baseline proportions (the minimal difference (d) in Figure 1) was set at 3.5% for the sample size calculation, this is inconsistent with Figure 2.2 in Appendix 7 of the 2009 MON810 report which set the effect size at 5%, but the selection of 3.5% is more conservative and results in a larger sample size. Additionally the □ and □ values used for

the sample size calculations are also conservative and consequently the sample size is large. Nonetheless it is likely that the same farmer may be surveyed in different years and therefore each sample unit 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 only be achieved when the sample size of 2500 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 to postal surveys. In the written response Monsanto explained that "As the interviewers explain clearly the purpose of the interview to the selected farmers and as it is not connected with any marketing/sales purpose, the farmers are usually willing to give their inputs." It appears that the response rate in the survey has not required the development of follow-up techniques.

2) Response rate

Appendix 7 of the 2009 MON810 report indicates that 240 surveys were completed from the 250 planned. The written response from Monsanto provided the following additional information "In 2009, there were six farmers (out of 49) in Czech Republic and 18 farmers (out of 118) who refused to answer the questionnaire because the survey is not mandatory for them and also because they are already under heavy administrative obligations linked to MON810 specific requirements.". Therefore the response rate for the survey in the Czech Republic is 89% and for all six countries is 90%. In the EU farm structure survey of 2007 the reported response rates were Czech Republic 61% (overcoverage error 39%), Poland no information, Portugal 99%, Romania 96%, Slovakia 99.6% and Spain 90.8% (EUROSTAT Farm Survey). The MON810 survey response rate is at the lower end of these figures although it should be acknowledged that this is a voluntary survey.

3) Losses in sampling

No details of losses in sampling are included in the 2009 MON810 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 2009 MON810 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 2009 MON810 report. The response from Monsanto indicates that administrative burden is stated as a reason for not participating in the survey. It would be of particular concern if farmers with multiple years of experience with GM cultivation were no longer participating in the survey due to the administrative burden since this would prevent the detection of residual effects. It is important to know if a specific sub-group of farmers are not participating in the survey and therefore are not represented in the survey findings, consequently this comparison should be presented in the report.

6) Characteristics of responder group compared to the target population

In the response from Monsanto it was indicated that for Spain data was obtained from the Ministry of Agriculture / Environment for maize acreage per municipality. It would be of value to compare the maize area figures reported by the Spanish farmers in the survey with the Ministry of Agriculture / Environment figures and present this comparison in the report. The presentation of this information could provide evidence that the farmers surveyed are representative for GM farmers in Spain (although it is acknowledged that there may be differences between GM and non GM farmers). In cases where the national registers for the cultivation of GM crops have not been used as the sampling frame, comparison with the characteristics of the farmers surveyed in terms of geographical location and farming practices with those of the national register could ensure that the farmers surveyed are representative for 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. The use of interviewers has resulted in a reasonable response rate for the survey (90%) however it is important to know if a specific sub-group of farmers are not participating in the survey and therefore are not represented in the survey findings.

Instrument design

1) Interviewer bias

Appendix 7 of the 2009 MON810 report indicates that the study uses third parties to perform the interviews, however in 2009 for Poland Monsanto field representatives assisted the farmers in filling in the questionnaire. The use of third party interviewers can prevent interviewer bias. The response from Monsanto also explains that in order to ensure reliable information is obtained from the farmers during the interview process, the interviewer's cross-check the responses by inspecting the farm records/notebooks.

2) Interviewer training

The response from Monsanto explains that Biomath and/or Monsanto organise annual workshops to train interviewers. A part of the training focuses on ensuring the questionnaire is completed correctly, in particular that the opened ended questions are completed when required by a closed question response. In addition, a "User's manual" is provided to the interviewers (Appendix 9 of the 2009 MON810 report); this document comprises instructions for completing the questionnaire and provides guidance on the choice of a representative MON810 field and the choice of a representative conventional maize field prior to answering the questions requiring a comparison between the GM crop and the conventional crop.

3) Question type

The questionnaire contains 25 closed questions which require a comparison between the representative GM field and the representative conventional maize field. For these questions, the response options are either "same or different/changed" or "as usual or worse or better". It is these questions which 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 area. The combination of open and closed questions allows quantitative analysis of the comparisons between GM field and conventional maize field; 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 relies on a comparison between a representative GM 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. At the hearing with Monsanto it was explained that currently all farmers surveyed are growing a mix of GM and conventional maize. In situations where no conventional maize is grown the questionnaire is unlikely to be suitable.

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 later more fully.

6) Validation of the instrument

The questionnaire was developed by the German Federal Biological Research Centre and Forestry, 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 has been used in annual PMEM reports 2006-2009. Improvements have been made based on experience with the survey and comments from the European Commission. Any changes to questions should be made with caution. During the annual data analysis the amendment of a question will not have a serious effect, but to achieve the statistical power of the survey the results from 10 years must be pooled and the question needs to be consistent for all 10 years to allow an effective analysis of the effect type it measures. An example of this problem can be seen for the question on the occurrence of wildlife. In 2007 and 2008 the null hypothesis that less wildlife was observed in the GM field could not be rejected. The question was split in 2009 into the occurrence of insects, birds and mammals making the pooled analysis complex, consequently it may not be possible to determine if the occurrence of less wildlife is statistically significant or not when the pooled analysis is performed. If the question had been amended as shown in the example below this problem could have been avoided.

General impression of the occurrence of wild life (mammals, birds and insects) in MON810 compared to conventional maize fields

As usual	More	Less

If the above answer is different from “As usual” please specify the difference below

Mammals	More	Less
Birds	More	Less
Insects	More	Less
Other:------	More	Less

Any future question amendments should be made with consideration to the pooled analysis of the results from 10 years.

Instrument validity

1) Content validity

- geographical location

The questionnaire records the country and county where the farm is located. It would be of value to take longitude and latitude measurements of the representative GMO field, information of this nature would facilitate linkage with other spatial monitoring datasets.

- cultivation methods

Section 2 of the questionnaire collects general information on irrigation, crop rotation, tillage, planting and weed and pest control practices, use of fertilizers and sowing and harvest times. Section 3.1 assesses changes in agricultural practices associated with the cultivation of MON810, for the following characteristics crop rotation, sowing time, tillage and planting techniques, plant protection products application, use of fertilizer, irrigation and harvest time. It is noted that information on plant protection products applied to the GM field are collected but not for the conventional field. There are no questions in this section which assess the cultivation of GM crops on the farm other than maize in the year of the survey, (e.g., How many years has GM maize been grown on the farm? Are there GM crops other than maize in cultivation on the farm?).

- agronomy parameters

The questions in Section 3.2 make a comparison between the GM field and the conventional field for the following agronomy parameters, germination vigour, time to emergence, time to male flowering, plant growth and development, incidence of stalk/root lodging, time to maturity and yield.

- weed/pest management practices

Question 1.6 requests information on local pressure (for diseases, pests and weeds) on the farm. Where farmers respond that the local pressure is high it would be useful to record the pest, disease or weed which has elicited this response.

In addition to the questions on the use of plant protection products in Section 3 information on the susceptibility of the GM maize compared to conventional maize to diseases and pests is sought in section 3.3-3.5. Section 3.6 compares the weed pressure between the GM field and the conventional field and requires the identification of the three most abundant weeds in the GM field, information on the three most abundant weeds in the conventional field is not requested.

- unforeseen weediness and invasiveness

In section 3.2, the farmers are requested to report whether the occurrence of volunteers from the previous year in the GM field compared to the conventional field is “same / more / less”. Obviously to assess this parameter the farmer must have grown MON810 in the previous year, consequently it is important that a proportion of the farmers selected for the survey have previously grown maize MON810.

- changes in biodiversity of fauna, including non-target arthropods, beneficial organisms and protected species

Section 3.7 contains three questions which attempt to capture information relevant to assess this parameter, comparing the occurrence in the GM field and the conventional field of insects, birds and mammals. For these closed questions 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. It

may be relevant to include a question on the occurrence of beneficial predator insects in the GM crop in this section.

- changes in biodiversity of flora, including seedbank, wild species, weeds and protected species

Beyond the question in section 3.6 to assess weed pressure within the maize field there are no other questions relevant to assess the biodiversity of flora. A question assessing biodiversity in field margins may be of relevance (e.g., The plants found in the field margin of the GM crop compared to the field margin of the conventional crop are same / different, because: ---).

- soil quality and functionality

Section 1.5 requests information of soil characteristics of the maize grown area, however in contrast to the other questions in the survey no comparison is made between the GM field and the conventional field. It would be of value to collect information to allow a comparison of organic matter content between the GM field and the conventional field, although it can be seen from the survey that only a limited number of farmers have access to this information (34%). Interestingly all Portuguese farmers were able to report this information. It would be useful to know how Portuguese farmers obtain this information and whether there could be a mechanism to assist other GM farmers in getting access to this information.

- agro-ecosystems sustainability, including pollinator populations

There are no questions in the survey that specifically assess pollinator populations beyond the question on the occurrence of insects. Questions designed to investigate the efficiency of pollination on the farm could be used to assess this, however they would only be relevant for farmers growing insect pollinated crops (e.g. Do you grow insect pollinated crops on the farm (for example orchards, soft or cane fruit, cucurbiteae family)? Yes / No - If yes was the yield? as usual / more / less)

- effects on human health resulting from handling the GM plant

There are no questions to assess this factor included in the survey. 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 to discriminate 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. In this section details of the variety grown in the refuge and the dimensions of the refuge would be of value for assessing the compliance to good agricultural practice.

2) Criterion validity

The original field trial data from the notification in 1995 with the agronomy parameters was not available in EFSA. The scientific opinion on the renewal of the authorisation for MON810 (EFSA, 2009a) states that "*The information available in the renewal applications gives no reason to change the opinion that maize MON810 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 MON863 x MON810 x NK603 (EFSA, 2005) states

"*Plants of the same field trials as for compositional analysis, except for a difference in glyphosate treatment (see 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.*" The report MSL-18567 (Carringer *et al*., 2004) includes data on the agronomic parameters assessed in the above opinion. For seedling vigour both MON810 and the reference varieties had "Excellent" vigour with the exception of one site where one reference variety was classed as poor and one average. Stalk lodging in plants near harvest was observed more frequently in the reference varieties and at one site root lodging in plants near harvest was observed more frequently in the reference varieties. For the other agronomy parameters there was no particular deviation between MON810 and the reference varieties. Appendix 7 of the 2009 MON810 report assessing the characteristics of MON810 reported "germinates 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 farmer's responses. It appears that there may be differences between field trial data and the questionnaire, there are a number of possible explanations including that 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 in 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.

Since the questionnaire collected information of plant protection products this information can be checked against the national authorisations for pesticide usage available from the DG SANCO EU Pesitcides database (DG SANCO EU pesticides database). The results are shown below (Table 1-3). There is agreement between authorised use of active substances and the plant protection products reported to be used in the survey. Currently there is no harmonised database for plant protection product authorisations at crop level, when this data becomes available this would be a good source of information to test for external consistency.

Table 1: Insecticides applied to maize MON 810 field and authorisation status

Active Substance *Insecticides*	*Authorisation*
Abamectin	All countries
Beta-Cyfluthrin	All countries
Chlorpirifos	All countries

Clothianidin	All countries
Cypermethrin	All countries
Deltamethrin	All countries
Imidachloprid	All countries
Lambda-Cyhalothrin	All countries
Methiocarb	All countries
Propargite	Authorisations are to be withdrawn by 2011
Thiacloprid	All countries
Thiametoxam	All countries
Zeta-Cipermetrin	Not in PT – Reported use in RO

Table 2: Herbicides applied to maize MON 810 field and authorisation status

Active substance Herbicides	*Authorisation*
2.4 D	All countries
Acetochlor	Authorisations are to be withdrawn by 2011 not authorised for use in PT
Aclonifen	Not in CZ, PT, PL, RO, SK – Reported use in ES
Alachlor	Not authorised – Reported use in ES
Atrazine	Not authorised – Reported in mixed formulations used in ES, CZ, PT
Bentazone	Not in CZ – Reported use in PT
Bromoxynil	All countries
Clopyralid	All countries
Dicamba	All countries
Dichlormid	No information
Dimethenamid-P	Not in PL – Reported use in PL and RO
Florasulam	Not in PT – Reported use in CZ, SK, PL, RO
Flufenacet	Not in ES, RO – Reported use in PT
Fluroxypyr	All countries
Foramsulfuron	All countries
Flusilazole	All countries
Glyphosate	All countries
Iodosulfuron	All countries
Isoxadifen	No information
Isoxaflutole	Not in PT – Reported use in CZ, RO, SK, ES
Linuron	All countries
Mesotrione	All countries
Nicosulfuron	All countries
Niferol	Substance unknown
Prosulfuron	Not in CZ, PL, PT – Reported use in RO
Rimsulfuron	All countries
S-Metolachlor	All countries
Sulcotrione	Not in CZ, SK – Reported use in PT
Tembotrione	Not in ES, PL – Reported use in PT
Terbuthylazine	All countries, authorisations are to be withdrawn by 2011
Thifensulfuron-methyl	Not in PT – Reported use in CZ
Tritrosulfron	Not in PT, ES –Reported use in CZ, SK

Table 3: Fungicides applied to maize MON 810 field and authorisation status

Active substance in fungicide	*Authorisation*
Carbendazim	All countries
Metalaxyl-M	All countries
Thiram	All countries
Fludioxonil	All countries

The information on soil quality offers the opportunity to compare with the information held in The Soil Profile Analytical Database for Europe (SPADE-2) (Hollis *et al*., 2006). Figure 2 shows the information on top soil organic carbon contained in this database. The maize MON810 survey reports organic carbon content values between 0.7 and 5.0 with a mean of 2.3. 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.

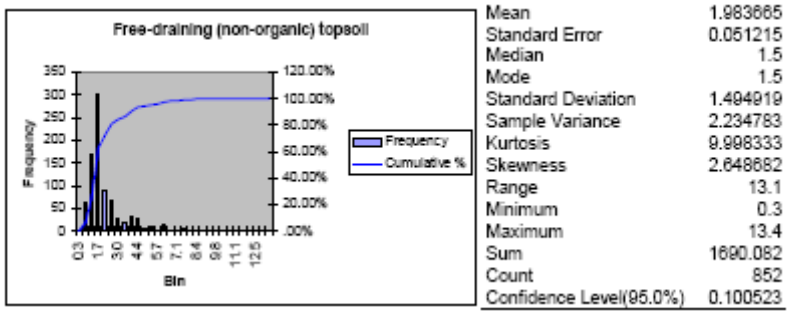

Figure 2: Distribution and descriptive statistics of topsoil organic carbon contents in SPADE-2 for free draining non organic soils

The report of pests to which the GM maize was more or less susceptible provides another opportunity to check survey responses for external consistency. In Table 4, the reported pests are compared with the known distribution of these pests in Europe as reported in either Crop Protection Compendium (CABI) or European and Mediterranean Plant Protection Organisation (EPPO) websites. For the reported pests in the survey, there is a correspondence between the country of the pest report and the known distribution of the pests according to external data sources.

Table 4: Reported pest susceptibility and known distribution

Pest with susceptibility report	*Reported in*	*Known Distribution*	*Source*
Agriotes spp	CZ	A. Lineatus, A. obscurus widespread in CZ	EPPO
Agrotis spp	PT	A. segetum present in Europe	EPPO
Heliothis	PT, ES	Quarantine pests, Helicoverpa armigera widespread in PT and ES	CABI
Spodoptera spp	PT	Quarantine pests, S. littoralis in South PT	EPPO
Teranychus spp	PT	Present in Europe	CABI
Diabrotica virgifera	CZ	Quarantine pest, Present in CZ	EPPO
Mythimna spp	ES	M. loreyi present in Europe	CABI

Overall there is good agreement between the farmers responses in the survey and information from external data sources for plant protection product use, organic carbon content in soil and pests and this provides evidence for external consistency for the maize MON810 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 10 March 2009 to 10 July 2009 and the harvest time from 10 August to 15 December 2009.

5) Construct validity

The questionnaire is able to detect changes in characteristics of the GM field compared with the conventional field which could be predicted when the nature of the genetic event in MON810 is considered. Maize MON810 expresses the insecticidally-active Cry1Ab protein active against certain lepidopteran pests (i.e. corn borers). The responses to the survey indicated that for maize MON810 field insecticide application and corn borer control practices were different due 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 European corn borer and Pink borer in maize MON810 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 between outcome variables.

Data validation

1) Validation procedures

Section 2.7 of Appendix 7 describes the data management and quality control procedures. In the response Monsanto it was explained that *"For any missing or implausible data, the interviewers are asked through a written query from BioMath to contact the farmers again to complete or provide clarification on the response"*. The number of questionnaires which 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 (240) were included in the analysis.

3) Missing values

In the analysis of each of the monitoring characteristics the number of responses for each value is shown in the table including the missing values where they occur.

Longitudinal aspects

1) Sampling over multiple years

Each completed questionnaire is assigned a unique identifier, in the response from Monsanto it was stated that *"The coding system does not allow identifying if same farmers have been sampled in consecutive years and there is no reason to do so as the famers are selected randomly."* Consequently the analysis is only applied the farms/fields surveyed in a single year. This issue of study design is important, it is clear that in some countries the same farmers are sampled on consecutive years plus for certain aspects, for example weediness/invasiveness, it is important to have information from the same sample unit on consecutive years. The repeated sampling of a sampling unit also 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.

Statistical analysis

1) Objective and hypotheses

Appendix 7 states *"The aim of the survey is to identify potential adverse effects that might be related to MON810 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 7 describes the statistical test procedure. The effect is specified as an 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. Additional 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 is less than 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 sub-groups

The analysis was performed for all fields surveyed in 2009. There was no analysis of country level data. Given the number of farmers surveyed in some countries analyses of country level sub group may not have been statistically valid, however consideration should be given to the fact that Member States may require country level -results. In addition analysis according the number of years of maize MON810 cultivation could assist in detecting residual effects, but this would require a different statistical analysis plan and the information on number of years of cultivation of maize MON810 is not currently collected in the questionnaire.

4) Statistical analysis

From the response from Monsanto it is unclear as to the type of statistical test that is used although it appears to be an exact binomial test. References for the statistical methodology used should be included in the report. 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 is provided with percent and "valid percentages" (the proportion of answers excluding missing values) plus a bar chart of the frequency of responses. The valid percentages are used in the binomial test.

6) Descriptive statistics

Descriptive statistics are 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 is provided as frequency tables.

7) Multiplicity

Significance level of 0.01 is used but the issue of multiplicity of testing is not addressed. Another major problem is related to the fact that the analysis needs to pooled after 10 years to achieve the statistical power described in the sample size calculations. Each annual PMEM 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 are presented the "Percent" which included missing values and the "Valid percentages" where 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 are not included in Appendix 7. In the table summarising the analysis of the monitoring characteristics (e.g., Table 3.1 in Appendix 7) the confidence intervals should be included. The inclusion of confidence intervals would allow an understanding the sensitivity of the analysis to the choice threshold.

10) Post-hoc analysis

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

Report conclusions

1) Report conclusions

Appendix 7 contains the following conclusions:

2009 data indicates that in comparison to conventional maize plants, MON810 plants

- *received less insecticides caused by their inherent protection against certain lepidopteran pests,*
- *were harvested later caused by increased flexibility (cropping system, logistics, channelling and coexistence) and the status of the plant (development, health, maturity, water content),*

- *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 MON810 performed slightly different compared to those fed with conventional maize. MON810 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 MON810 characteristics. The observed significant effects are not adverse. They mostly relate to the increased fitness of MON810 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 MON810 cultivating farmers.

2) Study design

The study design is appropriate for the assessment of the plant performance characteristics in the current year of the survey, specific questions to assess unanticipated adverse effects on human health or the environment are limited (occurrence of volunteers, mammals, insects and birds and an assessment of weed pressure). Farmer questionnaires should only focus on changes that would be recognised by the farmer during the daily management of the farm, however additional questions could be included with a focus on environmental protection goals. Certain effects may only reach a sufficient magnitude for detection with repeated cultivation of a GM plant, study design and analysis plan amendments should be considered in order to assess the effect of multiple years of GM cultivation. Table 4.1 in Appendix 7 presents the results from the previous three years and the 2009 results the inclusion of the pooled results would be of interest.

3) Substantiation of results

Forty four farmers (18.3%) indicated that that they had changed the application procedure of insecticides in the maize MON810 field with the exception of 1 farmer these were the farmers which usually used insecticides specifically to control corn borers.

Thirty-five farmers (14.6%) indicated that the germination of maize MON810 was more vigorous than conventional maize. Seventy-six farmers (31.9%) reported a reduction in stalk and root lodging in maize MON810 field compared to the conventional field. Increased germination vigour and reduction in stalk and root lodging was also observable in the field trail studies. Thirty-five farmers (14.6%) reported delayed maturity.

Twenty-one farmers (10.8%) reported a reduction in volunteers in the maize MON810 field compared to the conventional field. It should be noted that only 195 (81%) farmers responded to this question as cultivation of maize MON810 in the previous year is required in order to make an assessment. This result is of interest when considering adverse effects in the environment. The agronomy parameters above suggest better fitness in the maize MON810 plant, increased fitness could lead to invasiveness if the GM plant has a competitive advantage over native flora and this in turn may result in changes in the biodiversity of flora. However a reduction in volunteers is an indicator of a reduced risk of weediness/invasiveness.

Seventy farmers (29.3%) reported that maize MON810 field was less susceptible to diseases, 224 farmers (93.7%) and 141 farmers (99.3%) reported that maize MON810 provided "very good" control of European corn borer and Pink borer respectively and 41 farmers (17.2%) reported maize MON810 to be less susceptible to pests other than the borers. These results are to be predicted since the genetic modification provides protection from corn borers and therefore should result in a healthier crop. An increased yield was reported by 136 farmers (56.9%) since maize MON810 crop has reduced 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. The interpretation of the results should be viewed with caution since the conclusions are drawn on the basis of assumption of a binomial distribution for monitoring characteristics with three possible outcomes the selection of a multinomial statistical test would have been more appropriate.

The data was reanalysed using a multinomial method to estimate confidence intervals for each of the monitoring characteristics. The SAS (SAS Enterprise guide software, Version 4.2 of the SAS System Copyright © 2006 SAS Institute Inc. Cary, NC, USA.) LOGISTIC function (see Addendum 2) using the generalised logit function and profile likelihood function was used to calculate 95% confidence intervals. The results of the analysis are shown in Figures 4 and 5 and the values are reported in Addendum 1.

Figure 3 provides an illustration of the interpretation of the results. If the upper confidence interval is less than the non-inferiority margin then non-inferiority is shown, the proportion of not "as usual" responses indicates the monitoring characteristic is no more adverse in the maize MON810 field than the in conventional comparator field (for example time to male flowering, time of planting). If the upper confidence interval is greater than the non-inferiority margin then non-inferiority cannot be proven, the proportion of not "as usual" responses indicates the monitoring characteristic can not be considered to be no more adverse in the maize MON810 field than the in conventional comparator field (for example less occurrence of volunteers). If the lower confidence interval is greater than non-inferiority margin then superiority is indicated, the proportion of not "as usual" responses indicates there is a difference in the monitoring characteristic between the maize MON810 field and the conventional comparator field (for example higher yield, less susceptible to pests).

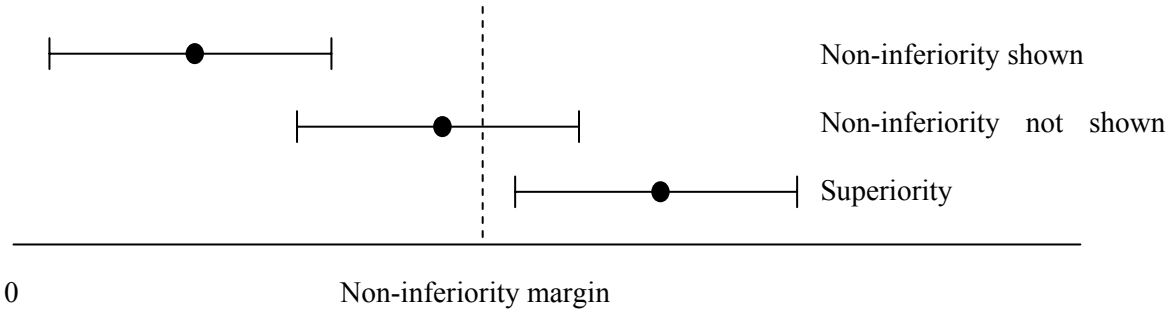

Figure 3: Illustration of possible outcomes

The outcome of the statistical reanalysis does not change the results reported in the 2009 annual report, however the use of confidence intervals facilitates the interpretation of the results and allows the effect of the selection of alternative threshold values other than the arbitrarily selected 10% to be explored.

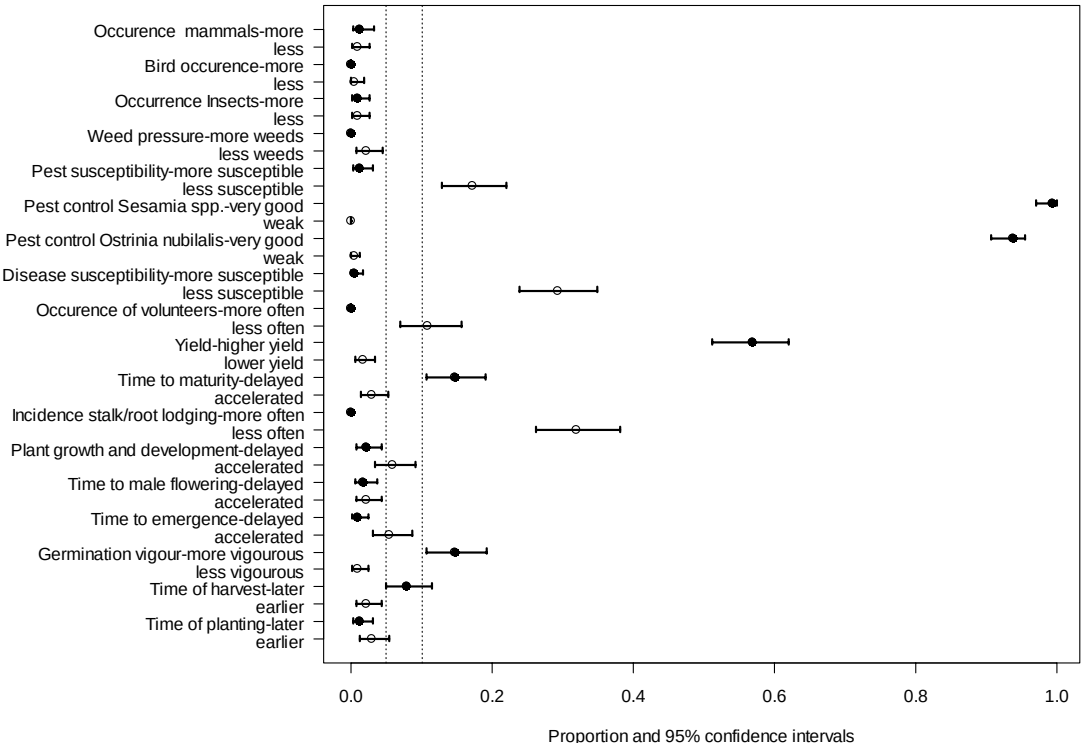

Figure 4: Monitoring characteristics 2009 MON810 report: trinomial responses proportion and 95% confidence intervals

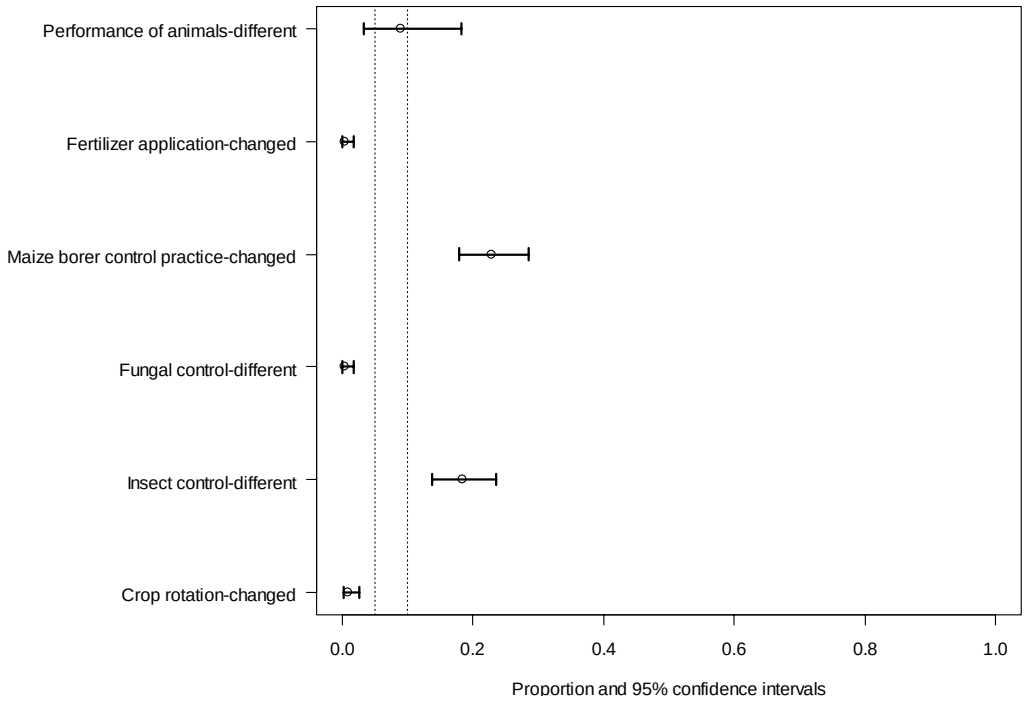

Figure 5: Monitoring characteristics 2009 MON810 report: binomial responses proportion and 95% confidence intervals

RECOMMENDATIONS AND CONCLUSIONS

From the data provided in the 2009 survey for the farmer questionnaire to monitor adverse effects associated with the cultivation of maize MON810, no adverse effect can be identified. However a number of improvements to the survey design and reporting have been 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 of the farmers should be included in the survey report. The member state National registers for the cultivation of GM crops would be a suitable sampling frame if available.

For countries where only a proportion of the farmers are surveyed stratification should be used to account for the multi-level structures of the population and ensure farmers are selected from representative environments. 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 full description of the sampling methodology and randomisation techniques should be included in the 2009 MON810 report.

Losses in sampling should be fully described in the 2009 MON810 report in order to document that non response bias has been avoided. It is important to know if a specific sub-group of farmers are not participating in the survey and therefore are not represented in the survey findings.

It is recommended to use independent trained interviewers to reduce interviewer bias.

Farmer questionnaires should only focus 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 farming environment in which the GM crop is grown and potentially the

monitoring characteristics measured could be expanded with a focus on environmental protection goals.

The questions to assess agricultural practices and agronomy parameters are comprehensive and well structured, however currently limited information is requested to assess effects on the environmental protections goals in terms of the ecosystem services; flora biodiversity, soil formation, nutrient recycling, pest/disease regulation and pollination. Additional questions appropriate for the agricultural environment should be developed to improve the assessment of adverse environmental effects.

Where accessible data sources exist the responses in the survey should be compared with alternative data sources to check the validity of the farmer's responses. The results of criterion validity and external consistency checks should be included in the 2009 MON810 report.

Confidence intervals for the analysis of the monitoring characteristics should be included in the statistical report. This would allow an understanding of the significance of the results and is standard practice for non inferiority tests. 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 characters according to the length of GM cultivation in order to assess residual effects. Since the statistical power of the study is only achieved after 10 years this will require a pooled analysis, consequently consideration should be given to the consistency of questions to assess of 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 PMEM reports when conducting the survey.

Addendum 1: Monitoring characteristics 2009 MON810 report: proportions and confidence intervals

Monitoring Characteristics	*n*	*Minus Response*	*N -*	*Minus Proportion*	*Minus Lower CL*	*Minus Upper CL*	*N =*	*As Usual Proportion*	*As Usual Lower CL*	*As Usual Upper CL*	*Plus Response*	*N +*	*Plus Proportion*	*Plus Lower CL*	*Plus Upper CL*
Crop rotation	240						238	99.2%	97.4%	99.9%	changed	2	0.8%	0.1%	2.6%
Time of planting	240	earlier	7	2.9%	1.3%	5.5%	230	95.8%	91.4%	98.4%	later	3	1.3%	0.3%	3.1%
Insect control	240						196	81.7%	76.4%	86.2%	different	44	18.3%	13.8%	23.6%
Fungal control	240						239	99.6%	98.2%	100.0%	different	1	0.4%	0.0%	1.8%
Maize borer control practice	240						185	77.1%	71.5%	82.1%	changed	55	22.9%	17.9%	28.5%
Fertilizer application	240						239	99.6%	98.2%	100.0%	changed	1	0.4%	0.0%	1.8%
Time of harvest	240	earlier	5	2.1%	0.8%	4.2%	216	90.0%	84.2%	94.2%	later	19	7.9%	5.0%	11.5%
Germination vigour	239	less vigourous	2	0.8%	0.1%	2.4%	202	84.5%	78.4%	89.2%	more vigourous	35	14.6%	10.6%	19.2%
Time to emergence	239	accelerated	13	5.4%	3.1%	8.7%	224	93.7%	88.9%	96.8%	delayed	2	0.8%	0.1%	2.5%
Time to male flowering	239	accelerated	5	2.1%	0.8%	4.3%	230	96.2%	91.9%	98.7%	delayed	4	1.7%	0.5%	3.8%
Plant growth and development	239	accelerated	14	5.9%	3.4%	9.1%	220	92.1%	86.6%	95.8%	delayed	5	2.1%	0.8%	4.3%
Incidence stalk/root lodging	238	less often	76	31.9%	26.2%	38.0%	162	68.1%	62.0%	73.8%	more often				
Time to maturity	239	accelerated	7	2.9%	1.3%	5.3%	197	82.4%	75.7%	87.9%	delayed	35	14.6%	10.7%	19.0%
Yield	239	lower yield	4	1.7%	0.6%	3.3%	99	41.4%	34.7%	48.2%	higher yield	136	56.9%	51.2%	61.9%
Occurence of volunteers	195	less often	21	10.8%	6.9%	15.6%	174	89.2%	84.4%	93.1%	more often				
Disease susceptibility	239	less susceptible	70	29.3%	23.9%	34.8%	168	70.3%	63.5%	76.1%	more susceptible	1	0.4%	0.0%	1.7%
Pest control Ostrinia nubilalis	239	weak	1	0.4%	0.0%	1.2%	14	5.9%	3.3%	9.3%	very good	224	93.7%	90.6%	95.5%
Pest control Sesamia spp.	142	weak					1	0.7%	0.0%	3.1%	very good	141	99.3%	96.9%	100.0%
Pest susceptibility	238	less susceptible	41	17.2%	12.9%	21.9%	194	81.5%	75.0%	86.8%	more susceptible	3	1.3%	0.3%	3.0%
Weed pressure	239	less weeds	5	2.1%	0.8%	4.4%	234	97.9%	95.6%	99.2%	more weeds				
Occurrence Insects	231	less	2	0.9%	0.1%	2.6%	227	98.3%	94.8%	99.7%	more	2	0.9%	0.1%	2.6%
Bird occurence	231	less	1	0.4%	0.0%	1.9%	230	99.6%	98.1%	100.0%	more				
Occurence mammals	232	less	2	0.9%	0.1%	2.6%	227	97.8%	94.2%	99.5%	more	3	1.3%	0.3%	3.3%
Performance of animals	56						51	91.1%	81.8%	96.7%	different	5	8.9%	3.3%	18.2%

Addendum 2: SAS Programming code used for statistical analysis

```
/* ---------------------------------------
Code exported from SAS Enterprise Guide
DATE: Wednesday, May 25, 2011     TIME: 9:21:54 AM
PROJECT: MON810Reanalysis
PROJECT PATH: d:\SAS_DATA\SAS\Project\PMEM\MON810Reanalysis.egp
--------------------------------------- */

/* Library assignment for SASApp.MON810 */
Libname MON810 BASE 'd:\SAS_DATA\SAS\Project\PMEM' ;


%global dataset obs;

%MACRO _EG_CHARACT(data, lib, dsn, catobs);

/* ---------------------------------------------------------------------
   Define the variables in the work accumulation data sets
and clear them out so that we can record the statistics for
the current data set.
   --------------------------------------------------------------------- */
DATA WORK.TTAFTempTableAccumFreq;
      LENGTH DataSet $ 41 Variable $32 Label $ 256 Format $ 31 Value $ 32
Count Percent 8;
      LABEL Count='Frequency Count' Percent='Percent of Total Frequency';
      RETAIN DataSet Variable Label Format Value ' ' Count Percent 0;
      STOP;
RUN;

DATA WORK.TTAUTempTableAccumUniv;
      LENGTH DataSet $ 41 Variable $32 Label $ 256 Format $ 31 N NMiss
Total Min Mean Median Max StdMean 8;
      RETAIN DataSet Variable Label Format ' ' N NMiss Total Min Mean
Median Max StdMean 0;
      STOP;
RUN;

/* ---------------------------------------------------------------------
   Get all the variable information for the input data set.
   --------------------------------------------------------------------- */
PROC CONTENTS
      DATA=&data.
      OUT=WORK.TCONTempTableContents
      NOPRINT;
RUN;

/* ---------------------------------------------------------------------
   Get the number of variables in the input data set.
   --------------------------------------------------------------------- */
DATA _NULL_;
      CALL SYMPUT('numobs',PUT(numobs, 12.));
/* ---------------------------------------------------------------------
   There is no need to actually read any observations, we"
are only interested in the observation count.
   --------------------------------------------------------------------- */
      STOP;
      SET WORK.TCONTempTableContents NOBS=numobs;
RUN;

/* ---------------------------------------------------------------------
   Each time the macro is executed the macro variable
type flags have to be initialized. They are used by
the graphing, reporting and output data set generation
```

```
code to determine if data exists to be processed.
   ------------------------------------------------------------------- */
%LET charVarsFlag = 0;
%LET numVarsFlag = 0;
/* ------------------------------------------------------------------
   Loop for each variable in the input data set and
depending on its type (character or numeric) gather
the relevant statistics for its values.
   ------------------------------------------------------------------- */
%DO i=1 %to &numobs.;
/* ------------------------------------------------------------------
   Create macro variables to provide information about
the current variable to subsequent DATA and PROC
steps.
   ------------------------------------------------------------------- */
      DATA _NULL_;
            POINTER=&i.;
            SET WORK.TCONTempTableContents point=pointer;
            CALL SYMPUT('var', QUOTE(name));
            CALL SYMPUT('var_n', QUOTE(name) || "n");
            CALL SYMPUT('type', PUT(type, 1.));
            CALL SYMPUT('label', label);
            CALL SYMPUT('format', format);
            STOP;
      RUN;

/* ------------------------------------------------------------------
   Process the variable if it is numeric.
   ------------------------------------------------------------------- */
      %IF &type.=1 %THEN %DO;
/* ------------------------------------------------------------------
   Set the macro variable flag to indicate that the
input data set contains at least one numeric
variable.
   ------------------------------------------------------------------- */
            %LET numVarsFlag = 1;
/* ------------------------------------------------------------------
   Get the statistics for the numeric variable.
   ------------------------------------------------------------------- */
            PROC UNIVARIATE DATA=&data. NOPRINT;
                  VAR &var_n.;
                  OUTPUT
                        OUT=WORK.TPUNTempTableUnivariate2
                        N=N
                        NMISS=NMiss
                        MEAN=Mean
                        MIN=Min
                        MAX=Max
                        MEDIAN=Median
                        STDMEAN=StdMean
                        SUM=Total;
            RUN;

/* ------------------------------------------------------------------
   Append the statistics for the numeric variable
to the data set used to accumulate information
about numeric variables in the current data
set.
   ------------------------------------------------------------------- */
            DATA WORK.TTAUTempTableAccumUniv;
                  SET WORK.TTAUTempTableAccumUniv
WORK.TPUNTempTableUnivariate2(IN=intemp);

                  IF intemp = 1 THEN DO;
```

```
                        Variable=&var.;
                        Label="%nrbquote(&label.)";
                        DataSet="&lib..&dsn.";
                        Format="&FORMAT.";
                  END;
            RUN;
      %END;

/* ------------------------------------------------------------------
   Process the variable if it is character.
   ------------------------------------------------------------------ */
      %ELSE %DO;
/* ------------------------------------------------------------------
   Set the macro variable flag to indicate that the
input data set contains at least one
character variable.
   ------------------------------------------------------------------ */
            %LET charVarsFlag = 1;
/* ------------------------------------------------------------------
   Get the frequency statistics for the values
within the character variable.
   ------------------------------------------------------------------ */
            PROC FREQ DATA=&data. NOPRINT;
                  TABLES &var_n./MISSING
OUT=WORK.TPFRTempTableFrequencies2;
            RUN;

/* ------------------------------------------------------------------
   Append the value frequency counts for the
character variable to the data set used to
accumulate information about all the character
variables in the current data set.
   ------------------------------------------------------------------ */
            DATA WORK.TTAFTempTableAccumFreq;
                  DROP InVar;
                  LENGTH Value $ 32;
                  SET WORK.TTAFTempTableAccumFreq
                      WORK.TPFRTempTableFrequencies2(IN=intemp
RENAME=(&var_n.=InVar));

                  IF intemp = 1THEN DO;
                        Value=InVar;
                        Variable=&var.;
                        Label="%nrbquote(&label.)";
                        DataSet="&lib..&dsn.";
                        Format="&FORMAT.";
                  END;
            RUN;
      %END;
%END;

/* ------------------------------------------------------------------
   Character data requires some additional
processing.
   ------------------------------------------------------------------ */
%IF &charVarsFlag = 1 %THEN
  %DO;
/* ------------------------------------------------------------------
   Sort the accumulated character variable
information by name and value frequency count.
   ------------------------------------------------------------------ */
PROC SORT DATA=WORK.TTAFTempTableAccumFreq;
      WHERE dataset NE ' ';
      BY variable label descending count;
```

```
RUN;

/* ---------------------------------------------------------------------
   Provide a label for missing values and if
the number of categorical values reported
needs to be limited, then all categorical
values' frequencies are accumulated into an
additional 'all others' item.
   --------------------------------------------------------------------- */
DATA WORK.TTAFTempTableAccumFreq;
      DROP i newcount newperc;
      RETAIN i newcount newperc 0;
      SET WORK.TTAFTempTableAccumFreq;
      BY variable;
      IF value=' ' THEN
            value='***Missing***';
  %IF %EVAL(&catobs.) NE -1 %THEN
    %DO;
      IF FIRST.variable = 1 THEN
            i=1;
      ELSE
            i=i+1;
      IF i > %EVAL(&catobs.) THEN DO;
            newcount=newcount+count;
            newperc=newperc+percent;
      END;
      IF i > %EVAL(&catobs.) AND LAST.variable = 0 THEN
            DELETE;
      IF LAST.variable & i > %EVAL(&catobs.) THEN DO;
            value='***All other values***';
            count=newcount;
            percent=newperc;
            newcount=0;
            newperc=0;
      END;
    %END;
RUN;
  %END;

/* ---------------------------------------------------------------------
   Create the output data sets.
   --------------------------------------------------------------------- */
%IF &charVarsFlag = 1 %THEN
  %DO;
PROC APPEND BASE=MON810.FREQRESULTS DATA=WORK.TTAFTempTableAccumFreq FORCE;
RUN;
  %END;

%IF &numVarsFlag = 1 %THEN
  %DO;
PROC APPEND BASE=SASUSER.UNIVCharUnivariateForTRINOMIALMO
DATA=WORK.TTAUTempTableAccumUniv FORCE;
RUN;
  %END;

%MEND _EG_CHARACT;

/* ---------------------------------------------------------------------
   Code generated by a SAS task

   Generated on Tuesday, May 10, 2011 at 5:45:09 PM
   By task:     Import Data

   Source file:
```

```
   d:\SAS_DATA\SAS\Project\PMEM\PMEM_MON810_2009rawdata.xls
   Server:      SASApp

   Output data: MON810.PMEM_MON810_2009rawdata
   Server:      SASApp
   ------------------------------------------------------------------- */

/* ---------------------------------------------------------------------
   This DATA step reads the data values from a temporary text file
   created by the Import Data task. The values within the temporary
   text file were extracted from the Excel source file.
   ---------------------------------------------------------------------

Import data from Excel file*/

DATA MON810.PMEM_MON810_2009rawdata;
    LENGTH
        questnr            8
        codeyear           8
        codeeven         $ 6
        codepart         $ 50
        codecoun         $ 50
        codeinte           8
        codefarm           8
        codearea           8
        macountr         $ 50
        macounty         $ 50
        surenv           $ 50
        matotal            8
        mamon810           8
        numbfiel           8
        mamonva1         $ 50
        mamonva2         $ 50
        mamonva3         $ 50
        mamonva4         $ 50
        mamonva5         $ 50
        maconva1         $ 50
        maconva2         $ 50
        maconva3         $ 50
        maconva4         $ 50
        maconva5         $ 15
        othergm          $ 2
        masoilty         $ 50
        masoilqu         $ 50
        maorcaco           8
        mapresdi         $ 8
        maprespe         $ 8
        mapreswe         $ 8
        tapirri          $ 3
        typeirri         $ 50
        taprotpr         $ 50
        taprot2y         $ 50
        taptill          $ 3
        taptitil         $ 50
        tappltec         $ 50
        tapherbi         $ 3
        tapinsec         $ 3
        tapinsmb         $ 3
        tapfungi         $ 3
        tapmechc         $ 3
        tapbioco         $ 3
```

```
tapother        $ 1
tapothsp        $ 1
tapferti        $ 3
tapsowf           8
tapsowt           8
taphargf          8
taphargt          8
tapharff          8
tapharft          8
agchcror        $ 8
agchcro1        $ 50
agpplan         $ 8
agpplar1        $ 250
agptilp         $ 8
agptilpr        $ 1
agpinse1        $ 100
agpinse2        $ 100
agpinse3        $ 100
agpinse4        $ 100
agpherb1        $ 100
agpherb2        $ 100
agpherb3        $ 100
agpherb4        $ 100
agpherb5        $ 1
agpherb6        $ 1
agpherb7        $ 1
agpfung1        $ 100
agpfung2        $ 1
agpfung3        $ 1
agpfung4        $ 1
agpinsc         $ 9
agpinscr        $ 250
agpherc         $ 9
agphercr        $ 1
agpfunc         $ 9
agpfuncr        $ 250
agpmbcp         $ 9
agpmbcpr        $ 250
agpfert         $ 9
agpfertr        $ 250
agpirri         $ 9
agpirrir        $ 1
agpharv         $ 8
agpharvr        $ 250
chagermi        $ 20
chaemerg        $ 20
chaflowe        $ 20
chadevel        $ 20
chainsrl        $ 20
chamatur        $ 20
chayield        $ 12
chavolun        $ 20
chaspec1        $ 250
chaspec2        $ 250
chaspec3        $ 250
chaobse1        $ 250
dissusce        $ 20
disfusar        $ 4
disustil        $ 4
dissphac        $ 4
```

```
        dishelmi        $ 4
        disanthr        $ 1
        dismdmvy        $ 4
        dishonfu        $ 4
        disrhiso        $ 4
        dispucso        $ 4
        disviros        $ 1
        disothet        $ 50
        disother        $ 50
        discomm1        $ 250
        discomm2        $ 250
        inscornb        $ 20
        inssesam        $ 20
        inscomm1        $ 250
        pestsus         $ 20
        pest1t          $ 50
        pest1           $ 4
        pest2t          $ 50
        pest2           $ 4
        pest3t          $ 50
        pest3           $ 4
        pest4t          $ 50
        pest4           $ 4
        pest5t          $ 1
        pest5           $ 1
        pestcom1        $ 250
        pestcom2        $ 250
        weedpres        $ 20
        weed1           $ 50
        weed2           $ 50
        weed3           $ 50
        weedobs1        $ 250
        weedobs2        $ 250
        weedobs3        $ 250
        insectoc        $ 20
        insspec1        $ 250
        insspec2        $ 250
        birdocc         $ 20
        mamocc          $ 20
        mamspec1        $ 250
        mamspec2        $ 250
        feeduse         $ 3
        feedperf        $ 20
        feedspe1        $ 250
        feedspe2        $ 250
        remark1         $ 250
        remark2         $ 250
        remark3         $ 250
        remark4         $ 250
        iminfoap        $ 3
        iminfoev        $ 20
        seedlabl        $ 3
        imseedla        $ 3
        imseedc1        $ 250
        imrefuge        $ 50
        imrefug1        $ 250 ;
    FORMAT
        questnr         BESTX12.
        codeyear        F12.
        codeeven        $CHAR6.
        codepart        $CHAR50.
```

```
codecoun        $CHAR50.
codeinte        BEST12.
codefarm        BEST12.
codearea        BEST12.
macountr        $CHAR50.
macounty        $CHAR50.
surenv          $CHAR50.
matotal         BEST12.
mamon810        BEST12.
numbfiel        BEST12.
mamonva1        $CHAR50.
mamonva2        $CHAR50.
mamonva3        $CHAR50.
mamonva4        $CHAR50.
mamonva5        $CHAR50.
maconva1        $CHAR50.
maconva2        $CHAR50.
maconva3        $CHAR50.
maconva4        $CHAR50.
maconva5        $CHAR50.
othergm         $CHAR2.
masoilty        $CHAR50.
masoilqu        $CHAR50.
maorcaco        BEST12.2
mapresdi        $CHAR8.
maprespe        $CHAR8.
mapreswe        $CHAR8.
tapirri         $CHAR3.
typeirri        $CHAR50.
taprotpr        $CHAR50.
taprot2y        $CHAR50.
taptill         $CHAR3.
taptitil        $CHAR50.
tappltec        $CHAR50.
tapherbi        $CHAR3.
tapinsec        $CHAR3.
tapinsmb        $CHAR3.
tapfungi        $CHAR3.
tapmechc        $CHAR3.
tapbioco        $CHAR3.
tapother        $CHAR1.
tapothsp        $CHAR1.
tapferti        $CHAR3.
tapsowf         DATE9.
tapsowt         DATE9.
taphargf        DATE9.
taphargt        DATE9.
tapharff        DATE9.
tapharft        DATE9.
agchcror        $CHAR8.
agchcro1        $CHAR50.
agpplan         $CHAR8.
agpplar1        $CHAR250.
agptilp         $CHAR8.
agptilpr        $CHAR1.
agpinse1        $CHAR100.
agpinse2        $CHAR100.
agpinse3        $CHAR100.
agpinse4        $CHAR100.
agpherb1        $CHAR100.
agpherb2        $CHAR100.
agpherb3        $CHAR100.
agpherb4        $CHAR100.
agpherb5        $CHAR1.
```

```
agpherb6          $CHAR1.
agpherb7          $CHAR1.
agpfung1          $CHAR100.
agpfung2          $CHAR1.
agpfung3          $CHAR1.
agpfung4          $CHAR1.
agpinsc           $CHAR9.
agpinscr          $CHAR250.
agpherc           $CHAR9.
agphercr          $CHAR1.
agpfunc           $CHAR9.
agpfuncr          $CHAR259.
agpmbcp           $CHAR9.
agpmbcpr          $CHAR250.
agpfert           $CHAR9.
agpfertr          $CHAR250.
agpirri           $CHAR9.
agpirrir          $CHAR1.
agpharv           $CHAR8.
agpharvr          $CHAR250.
chagermi          $CHAR20.
chaemerg          $CHAR20.
chaflowe          $CHAR20.
chadevel          $CHAR20.
chainsrl          $CHAR20.
chamatur          $CHAR20.
chayield          $CHAR12.
chavolun          $CHAR20.
chaspec1          $CHAR250.
chaspec2          $CHAR250.
chaspec3          $CHAR250.
chaobse1          $CHAR250.
dissusce          $CHAR20.
disfusar          $CHAR4.
disustil          $CHAR4.
dissphac          $CHAR4.
dishelmi          $CHAR4.
disanthr          $CHAR1.
dismdmvy          $CHAR4.
dishonfu          $CHAR4.
disrhiso          $CHAR4.
dispucso          $CHAR4.
disviros          $CHAR1.
disothet          $CHAR50.
disother          $CHAR50.
discomm1          $CHAR250.
discomm2          $CHAR250.
inscornb          $CHAR20.
inssesam          $CHAR20.
inscomm1          $CHAR250.
pestsus           $CHAR20.
pest1t            $CHAR50.
pest1             $CHAR4.
pest2t            $CHAR50.
pest2             $CHAR4.
pest3t            $CHAR50.
pest3             $CHAR4.
pest4t            $CHAR50.
pest4             $CHAR4.
pest5t            $CHAR1.
pest5             $CHAR1.
pestcom1          $CHAR250.
pestcom2          $CHAR250.
weedpres          $CHAR20.
```

```
        weed1           $CHAR50.
        weed2           $CHAR50.
        weed3           $CHAR50.
        weedobs1        $CHAR250.
        weedobs2        $CHAR250.
        weedobs3        $CHAR250.
        insectoc        $CHAR20.
        insspec1        $CHAR250.
        insspec2        $CHAR250.
        birdocc         $CHAR20.
        mamocc          $CHAR20.
        mamspec1        $CHAR250.
        mamspec2        $CHAR250.
        feeduse         $CHAR3.
        feedperf        $CHAR20.
        feedspe1        $CHAR250.
        feedspe2        $CHAR250.
        remark1         $CHAR250.
        remark2         $CHAR250.
        remark3         $CHAR250.
        remark4         $CHAR250.
        iminfoap        $CHAR3.
        iminfoev        $CHAR20.
        seedlabl        $CHAR3.
        imseedla        $CHAR3.
        imseedc1        $CHAR250.
        imrefuge        $CHAR50.
        imrefug1        $CHAR250. ;
    INFORMAT
        questnr         BESTX12.
        codeyear        BEST12.
        codeeven        $CHAR6.
        codepart        $CHAR50.
        codecoun        $CHAR50.
        codeinte        BEST12.
        codefarm        BEST12.
        codearea        BEST12.
        macountr        $CHAR50.
        macounty        $CHAR50.
        surenv          $CHAR50.
        matotal         BEST12.
        mamon810        BEST12.
        numbfiel        BEST12.
        mamonva1        $CHAR50.
        mamonva2        $CHAR50.
        mamonva3        $CHAR50.
        mamonva4        $CHAR50.
        mamonva5        $CHAR50.
        maconva1        $CHAR50.
        maconva2        $CHAR50.
        maconva3        $CHAR50.
        maconva4        $CHAR50.
        maconva5        $CHAR50.
        othergm         $CHAR2.
        masoilty        $CHAR50.
        masoilqu        $CHAR50.
        maorcaco        BESTX12.2
        mapresdi        $CHAR8.
        maprespe        $CHAR8.
        mapreswe        $CHAR8.
        tapirri         $CHAR3.
        typeirri        $CHAR50.
        taprotpr        $CHAR50.
        taprot2y        $CHAR50.
```

```
taptill          $CHAR3.
taptitil         $CHAR50.
tappltec         $CHAR50.
tapherbi         $CHAR3.
tapinsec         $CHAR3.
tapinsmb         $CHAR3.
tapfungi         $CHAR3.
tapmechc         $CHAR3.
tapbioco         $CHAR3.
tapother         $CHAR1.
tapothsp         $CHAR1.
tapferti         $CHAR3.
tapsowf          DATE9.
tapsowt          DATE9.
taphargf         DATE9.
taphargt         DATE9.
tapharff         DATE9.
tapharft         DATE9.
agchcror         $CHAR8.
agchcro1         $CHAR50.
agpplan          $CHAR8.
agpplar1         $CHAR250.
agptilp          $CHAR8.
agptilpr         $CHAR1.
agpinse1         $CHAR100.
agpinse2         $CHAR100.
agpinse3         $CHAR100.
agpinse4         $CHAR100.
agpherb1         $CHAR100.
agpherb2         $CHAR100.
agpherb3         $CHAR100.
agpherb4         $CHAR100.
agpherb5         $CHAR1.
agpherb6         $CHAR1.
agpherb7         $CHAR1.
agpfung1         $CHAR100.
agpfung2         $CHAR1.
agpfung3         $CHAR1.
agpfung4         $CHAR1.
agpinsc          $CHAR9.
agpinscr         $CHAR250.
agpherc          $CHAR9.
agphercr         $CHAR1.
agpfunc          $CHAR9.
agpfuncr         $CHAR250.
agpmbcp          $CHAR9.
agpmbcpr         $CHAR250.
agpfert          $CHAR9.
agpfertr         $CHAR250.
agpirri          $CHAR9.
agpirrir         $CHAR1.
agpharv          $CHAR8.
agpharvr         $CHAR250.
chagermi         $CHAR20.
chaemerg         $CHAR20.
chaflowe         $CHAR20.
chadevel         $CHAR20.
chainsrl         $CHAR20.
chamatur         $CHAR20.
chayield         $CHAR12.
chavolun         $CHAR20.
chaspec1         $CHAR250.
chaspec2         $CHAR250.
chaspec3         $CHAR250.
```

```
    chaobse1        $CHAR250.
    dissusce        $CHAR20.
    disfusar        $CHAR4.
    disustil        $CHAR4.
    dissphac        $CHAR4.
    dishelmi        $CHAR4.
    disanthr        $CHAR1.
    dismdmvy        $CHAR4.
    dishonfu        $CHAR4.
    disrhiso        $CHAR4.
    dispucso        $CHAR4.
    disviros        $CHAR1.
    disothet        $CHAR50.
    disother        $CHAR50.
    discomm1        $CHAR250.
    discomm2        $CHAR250.
    inscornb        $CHAR20.
    inssesam        $CHAR20.
    inscomm1        $CHAR250.
    pestsus         $CHAR20.
    pest1t          $CHAR50.
    pest1           $CHAR4.
    pest2t          $CHAR50.
    pest2           $CHAR4.
    pest3t          $CHAR50.
    pest3           $CHAR4.
    pest4t          $CHAR50.
    pest4           $CHAR4.
    pest5t          $CHAR1.
    pest5           $CHAR1.
    pestcom1        $CHAR250.
    pestcom2        $CHAR250.
    weedpres        $CHAR20.
    weed1           $CHAR50.
    weed2           $CHAR50.
    weed3           $CHAR50.
    weedobs1        $CHAR250.
    weedobs2        $CHAR250.
    weedobs3        $CHAR250.
    insectoc        $CHAR20.
    insspec1        $CHAR250.
    insspec2        $CHAR250.
    birdocc         $CHAR20.
    mamocc          $CHAR20.
    mamspec1        $CHAR250.
    mamspec2        $CHAR250.
    feeduse         $CHAR3.
    feedperf        $CHAR20.
    feedspe1        $CHAR250.
    feedspe2        $CHAR250.
    remark1         $CHAR250.
    remark2         $CHAR250.
    remark3         $CHAR250.
    remark4         $CHAR250.
    iminfoap        $CHAR3.
    iminfoev        $CHAR20.
    seedlabl        $CHAR3.
    imseedla        $CHAR3.
    imseedc1        $CHAR250.
    imrefuge        $CHAR50.
    imrefug1        $CHAR250. ;
INFILE 'D:\SAS Temporary Files\_TD132\#LN00058'
    LRECL=2460
    ENCODING="WLATIN1"
```

```
        TERMSTR=CRLF
        DLM='7F'x
        MISSOVER
        DSD ;
    INPUT
        questnr          : BEST32.
        codeyear         : BEST32.
        codeeven         : $CHAR6.
        codepart         : $CHAR50.
        codecoun         : $CHAR50.
        codeinte         : BEST12.
        codefarm         : BEST12.
        codearea         : BEST12.
        macountr         : $CHAR50.
        macounty         : $CHAR50.
        surenv           : $CHAR50.
        matotal          : BEST32.
        mamon810         : BEST32.
        numbfiel         : BEST32.
        mamonva1         : $CHAR50.
        mamonva2         : $CHAR50.
        mamonva3         : $CHAR50.
        mamonva4         : $CHAR50.
        mamonva5         : $CHAR50.
        maconva1         : $CHAR50.
        maconva2         : $CHAR50.
        maconva3         : $CHAR50.
        maconva4         : $CHAR50.
        maconva5         : $CHAR50.
        othergm          : $CHAR2.
        masoilty         : $CHAR50.
        masoilqu         : $CHAR50.
        maorcaco         : BEST12.2
        mapresdi         : $CHAR8.
        maprespe         : $CHAR8.
        mapreswe         : $CHAR8.
        tapirri          : $CHAR3.
        typeirri         : $CHAR50.
        taprotpr         : $CHAR50.
        taprot2y         : $CHAR50.
        taptill          : $CHAR3.
        taptitil         : $CHAR50.
        tappltec         : $CHAR50.
        tapherbi         : $CHAR3.
        tapinsec         : $CHAR3.
        tapinsmb         : $CHAR3.
        tapfungi         : $CHAR3.
        tapmechc         : $CHAR3.
        tapbioco         : $CHAR3.
        tapother         : $CHAR1.
        tapothsp         : $CHAR1.
        tapferti         : $CHAR3.
        tapsowf          : BEST32.
        tapsowt          : BEST32.
        taphargf         : BEST32.
        taphargt         : BEST32.
        tapharff         : BEST32.
        tapharft         : BEST32.
        agchcror         : $CHAR8.
        agchcro1         : $CHAR50.
        agpplan          : $CHAR8.
        agpplar1         : $CHAR250.
        agptilp          : $CHAR8.
        agptilpr         : $CHAR1.
```

```
agpinse1          : $CHAR100.
agpinse2          : $CHAR100.
agpinse3          : $CHAR100.
agpinse4          : $CHAR100.
agpherb1          : $CHAR100.
agpherb2          : $CHAR100.
agpherb3          : $CHAR100.
agpherb4          : $CHAR100.
agpherb5          : $CHAR1.
agpherb6          : $CHAR1.
agpherb7          : $CHAR1.
agpfung1          : $CHAR100.
agpfung2          : $CHAR1.
agpfung3          : $CHAR1.
agpfung4          : $CHAR1.
agpinsc           : $CHAR9.
agpinscr          : $CHAR250.
agpherc           : $CHAR9.
agphercr          : $CHAR1.
agpfunc           : $CHAR9.
agpfuncr          : $CHAR250.
agpmbcp           : $CHAR9.
agpmbcpr          : $CHAR250.
agpfert           : $CHAR9.
agpfertr          : $CHAR250.
agpirri           : $CHAR9.
agpirrir          : $CHAR1.
agpharv           : $CHAR8.
agpharvr          : $CHAR250.
chagermi          : $CHAR20.
chaemerg          : $CHAR20.
chaflowe          : $CHAR20.
chadevel          : $CHAR20.
chainsrl          : $CHAR20.
chamatur          : $CHAR20.
chayield          : $CHAR12.
chavolun          : $CHAR20.
chaspec1          : $CHAR250.
chaspec2          : $CHAR250.
chaspec3          : $CHAR250.
chaobse1          : $CHAR250.
dissusce          : $CHAR20.
disfusar          : $CHAR4.
disustil          : $CHAR4.
dissphac          : $CHAR4.
dishelmi          : $CHAR4.
disanthr          : $CHAR1.
dismdmvy          : $CHAR4.
dishonfu          : $CHAR4.
disrhiso          : $CHAR4.
dispucso          : $CHAR4.
disviros          : $CHAR1.
disothet          : $CHAR50.
disother          : $CHAR50.
discomm1          : $CHAR250.
discomm2          : $CHAR250.
inscornb          : $CHAR20.
inssesam          : $CHAR20.
inscomm1          : $CHAR250.
pestsus           : $CHAR20.
pest1t            : $CHAR50.
pest1             : $CHAR4.
pest2t            : $CHAR50.
pest2             : $CHAR4.
```

```
        pest3t          : $CHAR50.
        pest3           : $CHAR4.
        pest4t          : $CHAR50.
        pest4           : $CHAR4.
        pest5t          : $CHAR1.
        pest5           : $CHAR1.
        pestcom1        : $CHAR250.
        pestcom2        : $CHAR250.
        weedpres        : $CHAR20.
        weed1           : $CHAR50.
        weed2           : $CHAR50.
        weed3           : $CHAR50.
        weedobs1        : $CHAR250.
        weedobs2        : $CHAR250.
        weedobs3        : $CHAR250.
        insectoc        : $CHAR20.
        insspec1        : $CHAR250.
        insspec2        : $CHAR250.
        birdocc         : $CHAR20.
        mamocc          : $CHAR20.
        mamspec1        : $CHAR250.
        mamspec2        : $CHAR250.
        feeduse         : $CHAR3.
        feedperf        : $CHAR20.
        feedspe1        : $CHAR250.
        feedspe2        : $CHAR250.
        remark1         : $CHAR250.
        remark2         : $CHAR250.
        remark3         : $CHAR250.
        remark4         : $CHAR250.
        iminfoap        : $CHAR3.
        iminfoev        : $CHAR20.
        seedlabl        : $CHAR3.
        imseedla        : $CHAR3.
        imseedc1        : $CHAR250.
        imrefuge        : $CHAR50.
        imrefug1        : $CHAR250. ;
RUN;

/*select monitoring characteristics*/
PROC SQL;
   CREATE TABLE MON810.TrinomialMonitoringChars AS
   SELECT t1.questnr,
          t1.agpplan,
          t1.agpharv,
          t1.chagermi,
          t1.chaemerg,
          t1.chaflowe,
          t1.chadevel,
          t1.chainsrl,
          t1.chamatur,
          t1.chayield,
          t1.chavolun,
          t1.dissusce,
          t1.inscornb,
          t1.inssesam,
          t1.pestsus,
          t1.weedpres,
          t1.insectoc,
          t1.birdocc,
          t1.mamocc,
          t1.agchcror,
          t1.agptilp,
          t1.agpinsc,
```

```
          t1.agpherc,
          t1.agpfunc,
          t1.agpmbcp,
          t1.agpfert,
          t1.agpirri,
          t1.feedperf
      FROM MON810.PMEM_MON810_2009RAWDATA AS t1;
QUIT;

/*obtain frequencies for monitoring characteristics*/

DATA MON810.FREQRESULTS(LABEL="Frequency Counts for
MON810.TRINOMIALMONITORINGCHARS");
      LENGTH DataSet $ 41 Variable $32 Label $ 256 Format $ 31 Value $ 32
Count Percent 8;
      LABEL Count='Frequency Count' Percent='Percent of Total Frequency';
      RETAIN DataSet Variable Label Format Value ' ' Count Percent 0;
      STOP;
RUN;

%_EG_CHARACT(MON810.TRINOMIALMONITORINGCHARS, MON810,
TRINOMIALMONITORINGCHARS, 30);


/*recode response variables*/
PROC SQL;
   CREATE TABLE MON810.RESULTSPLUSMINUS AS
   SELECT DISTINCT t1.Variable,
          t1.Value,
          t1.Count,
          t1.Percent,
          /* ResultType_Recode */
            (CASE
               WHEN 'accelerated' = t1.Value THEN 'minus'
               WHEN 'delayed' = t1.Value THEN 'plus'
               WHEN 'different' = t1.Value THEN 'changed'
               WHEN 'earlier' = t1.Value THEN 'minus'
               WHEN 'good' = t1.Value THEN 'as usual'
               WHEN 'higher yield' = t1.Value THEN 'plus'
               WHEN 'later' = t1.Value THEN 'plus'
               WHEN 'less' = t1.Value THEN 'minus'
               WHEN 'less often' = t1.Value THEN 'minus'
               WHEN 'less susceptible' = t1.Value THEN 'minus'
               WHEN 'less vigourous' = t1.Value THEN 'minus'
               WHEN 'less weeds' = t1.Value THEN 'minus'
               WHEN 'lower yield' = t1.Value THEN 'minus'
               WHEN 'more' = t1.Value THEN 'plus'
               WHEN 'more susceptible' = t1.Value THEN 'plus'
               WHEN 'more vigourous' = t1.Value THEN 'plus'
               WHEN 'similar' = t1.Value THEN 'as usual'
               WHEN 'very good' = t1.Value THEN 'plus'
               WHEN 'weak' = t1.Value THEN 'minus'
               ELSE t1.Value
            END) LABEL="ResultType_Recode" AS ResultType_Recode
      FROM MON810.FREQRESULTS AS t1
      WHERE t1.Value NOT = '***Missing***' AND t1.Value NOT = 'do not know'
      ORDER BY t1.Variable, ResultType_Recode;
QUIT;

/*use logistic function to calculate confidence intervals*/
ods listing close;
proc logistic data=MON810.RESULTSPLUSMINUS;
   freq Count;
```

```
   /*model ResultType_Recode (ref=first) = /clparm=wald alpha=0.05
link=glogit;*/
   model ResultType_Recode (ref=first) = /clparm=both alpha=0.05
link=glogit;
   by Variable;
   ods output CLparmPL=CLparmPL CLparmWald=CLparmWald;
run;



PROC SQL;
   CREATE TABLE WORK.RESCLPARMPL AS
   SELECT t1.Variable,
          t1.Parameter,
          t1.Response,
          t1.Estimate,
          t1.LowerCL,
          t1.UpperCL,
          /* CIMeth */
            ("Profile likelihood") AS CIMeth
      FROM WORK.CLPARMPL AS t1;
QUIT;

/*convert log result values*/
PROC SQL;
   CREATE TABLE WORK.EXPCLPARMPL AS
   SELECT t1.CIMeth,
          t1.Variable,
          t1.Parameter,
          t1.Response,
          t1.Estimate,
          t1.LowerCL,
          t1.UpperCL,
          /* ExpEstimate */
            (exp(t1.Estimate)) AS ExpEstimate,
          /* ExpLowerCL */
            (Exp(t1.LowerCL)) AS ExpLowerCL,
          /* ExpUpperCL */
            (Exp(t1.UpperCL)) AS ExpUpperCL
      FROM WORK.RESCLPARMPL AS t1;
QUIT;

/* transform table and recalculate proportions*/
PROC SQL;
   CREATE TABLE WORK.PLUSEXPCLPARMPL AS
   SELECT t1.CIMeth,
          t1.Variable,
          t1.ExpEstimate LABEL="PlusExpEstimate" AS PlusExpEstimate,
          t1.ExpLowerCL AS PlusExpLowerCL,
          t1.ExpUpperCL LABEL="PlusExpUpperCL" AS PlusExpUpperCL
      FROM WORK.EXPCLPARMPL AS t1
      WHERE t1.Response = 'plus' OR t1.Response = 'changed';
QUIT;

PROC SQL;
   CREATE TABLE WORK.MINUSEXPCLPARMPL AS
   SELECT t1.CIMeth,
          t1.Variable,
          t1.ExpEstimate LABEL="MinusExpEstimate" AS MinusExpEstimate,
          t1.ExpLowerCL LABEL="MinusExpLowerCL" AS MinusExpLowerCL,
          t1.ExpUpperCL LABEL="MinusExpUpperCL" AS MinusExpUpperCL
      FROM WORK.EXPCLPARMPL AS t1
      WHERE t1.Response = 'minus';
QUIT;
```

```
PROC SQL;
   CREATE TABLE MON810.RESULTSLOGISTICCONFIDENCEINTS AS
   SELECT /* CIMethod */
            (case when t1.CIMeth = "" then t2.CIMeth else t1.CIMeth end) AS
CIMethod,
          /* MonitoringChar */
            (case when t1.Variable = "" then t2.Variable else t1.Variable
end) AS MonitoringChar,
          t1.PlusExpEstimate,
          t1.PlusExpLowerCL,
          t1.PlusExpUpperCL,
          t2.MinusExpEstimate,
          t2.MinusExpLowerCL,
          t2.MinusExpUpperCL,
          /* PlusProportion */
            (CASE WHEN t2.MinusExpEstimate = . THEN
t1.PlusExpEstimate/(1+t1.PlusExpEstimate) ELSE
             t1.PlusExpEstimate/(1+t1.PlusExpEstimate+t2.MinusExpEstimate)
END) LABEL="Plus Proportion" AS
             PlusProportion,
          /* PlusLowerCL */
            (CASE WHEN t2.MinusExpLowerCL = . THEN
t1.PlusExpLowerCL/(1+t1.PlusExpLowerCL)  ELSE
             t1.PlusExpLowerCL/(1+t1.PlusExpLowerCL+t2.MinusExpLowerCL)
END) AS PlusLowerCL,
          /* PlusUpperCL */
            (CASE WHEN t2.MinusExpUpperCL= . THEN
t1.PlusExpUpperCL/(1+t1.PlusExpUpperCL)  ELSE
             t1.PlusExpUpperCL/(1+t1.PlusExpUpperCL+t2.MinusExpUpperCL)
END) AS PlusUpperCL,
          /* MinusProportion */
            (CASE WHEN t1.PlusExpEstimate = . THEN
t2.MinusExpEstimate/(1+t2.MinusExpEstimate) ELSE
             t2.MinusExpEstimate/(1+t1.PlusExpEstimate+t2.MinusExpEstimate)
END) AS MinusProportion,
          /* MinusLowerCL */
            (CASE WHEN t1.PlusExpLowerCL = . THEN
t2.MinusExpLowerCL/(1+t2.MinusExpLowerCL)   ELSE
             t2.MinusExpLowerCL/(1+t1.PlusExpLowerCL+t2.MinusExpLowerCL)
END) LABEL="MinusLowerCL" AS MinusLowerCL,
          /* MinusUpperCL */
            (CASE WHEN t1.PlusExpUpperCL = . THEN
t2.MinusExpUpperCL/(1+t2.MinusExpUpperCL)  ELSE
             t2.MinusExpUpperCL/(1+t1.PlusExpUpperCL+t2.MinusExpUpperCL)
END) AS MinusUpperCL
      FROM WORK.PLUSEXPCLPARMPL AS t1 FULL JOIN WORK.MINUSEXPCLPARMPL AS t2
ON (t1.Variable = t2.Variable) AND
           (t1.CIMeth = t2.CIMeth);
QUIT;
```

APPENDIX 2

Simulation exercise to optimize Case-Specific Monitoring for Insect Resistance Management

Case-Specific Monitoring (CSM) plans, aimed at early detections of possible onset of resistance in target pests are usually undertaken by applicants in post-commercial phases in most countries where Bt-crops are cultivated. In fact, the rationale of the 'high dose/refuge strategy' to delay resistance is based on the well known "Hardy-Weimberg" law that is the base for models which estimate allele frequency changes over time in a given population.

Several features linked to the biology of the target insect and the receiving environment where the Bt-crop is to be released are the major drivers for such allele frequency trends in specific conditions.

The applicant prepared a CSM plan largely based on standards adopted in the USA for MON810 maize; their goal is detection of an allele frequency ranging from 1 to 5%.

However, European conditions are sometimes quite different from the USA and more importantly, two different target pests need to be considered in European cultivations of Bt-maize: the ECB, also present in the USA, and the MCB.

In order to find an optimal sampling plan for European conditions, the EFSA GMO Panel ran some simulations using the model by Alstad and Andow (1995) using the shareware software Populus[26].

In particular, the model was run using the following parameter values:

Parameter	ECB	Ref.	MCB	Ref.
Initial allele frequency	0,0003 (F-D) 0,006 (I, SK)	Engels *et al.*, 2010	0,0086 – 0,0094 (G, E)	Andreadis *et al.*, 2007
Adoption rate of Bt maize	50%	High adoption rate	50%	High adoption rate
Fecundity	200		300	Fantinou *et al.*, 2004
Dominance	0,01	Almost fully recessive	0,01	Almost fully recessive
Preference for Bt maize in 2nd generation	120%		120%	
Overwinter survival	0,01		0,05	Gillyboeuf *et al.*, 1994
Survival of susceptible homozygotes on Bt	0.001		0,001	

The starting population is supposed to be equally abundant in Bt stands and refugia in the first year of Bt-maize release. All other parameters were set as default in the software.

The EFSA GMO Panel ran simulations in order to anticipate the speed of possible adaptation of either one of the insect pests to Bt-maize once their resistance allele frequency had reached 1, 3 or 5%.

[26] Populus, Vers. 5.4. Copyright © 2007 D. N. Alstad, University of Minnesota, http://wwumnw.cbs..edu/populus

Results

In the following table an overview of the results is presented. Particularly, the estimated number of generations before resistance in the population is reached is indicated.

Target species	Initial resistance allele frequency	No. of generations before resistance	No. of generations if the detected frequency is 1%	No. of generations if the detected frequency is 3%	No. of generations if the detected frequency[27] is 5%
O. nubilalis (F-D)	0,0003	32	10	6	5
O. nubilalis (I-SK)	0,006	12	10	6	5
S. nonagrioides	0,009	11	11	7	6

The level of an allele frequency of 0.5 is normally considered in the literature as a record of a resistant population.

Considering that a minimum of 1 year-delay from detection of resistance and taking an adaptive response is required (Andow and Ives, 2002), the simulations indicate that the current proposed strategy is only sufficient to a timely detection of increasing resistance for univoltine populations of ECB. In the case of bivoltine strains of ECB and of MCB, the remaining time span does not seem sufficient to enable an adaptive response in due time.

This prediction is based on a hypothetical sampling done in the refuge areas (as currently conducted by the applicant), while the increase in allele frequencies in Bt stands, should this appear, is expected to be faster. For instance, in the case of MCB surviving in Bt stands, the detection of 3% will only leave two more generations before resistance is achieved.

Discussion

The early detection of an increased allele frequency in the population of the target pest is the main goal of a CSM plan. The CSM is a proactive measure necessary to ensure the effectiveness of this measure in preventing a possible adaptation (see EFSA, 2011). The agronomic consequence of the onset of resistance in a pest population is assumed to be a population level of 70-80% of pre-control densities one year after resistance allele frequency reaches 0.5 (Comins, 1977; Alstad and Andow, 1995).

Based on our simulation we estimate that a level of detection of an allele frequency of 0.05 does not allow the necessary time for taking any adaptive response either for polivoltine strains of ECB, or for MCB.

These results are in agreement with Andow and Ives (2002) who considered the case of ECB in the USA.

Therefore, to reach the required susceptibility a monitoring plan should aim at detecting allele frequencies clearly below 5%.

[27] The level of an allele frequency of 0.5 is normally considered in the literature as a record of a resistant population.