

## SCIENTIFIC OPINION

# Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2011<sup>1</sup>

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### ABSTRACT

Following the request from the European Commission, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) assessed the monitoring report for the 2011 growing season of maize MON 810 provided by Monsanto Europe S.A. The EFSA GMO Panel already assessed the 2009 and 2010 monitoring reports and followed the same approach as for the assessment of the methodology applied by the applicant for monitoring maize MON 810 in 2011. The EFSA GMO Panel considered the plan for insect-resistant management and addressed the comments raised by the applicant on its previous recommendations for improving the methodology of the resistance monitoring of target pests. The EFSA GMO Panel also paid particular attention to the design and analysis of the farmer questionnaires. The EFSA GMO Panel notes similar shortcomings in the overall methodology for the post-market environmental monitoring of maize MON 810 as in the previous monitoring reports. Hence, while the EFSA GMO Panel reiterates its previous recommendations for the improvement of the methodology, it also clarifies and elaborates on those related to the monitoring of resistance evolution in target pests. However, from the data submitted by the applicant, the EFSA GMO Panel does not identify adverse effects on the environment, human and animal health due to maize MON 810 cultivation during the 2011 growing season. The outcomes of the 2011 monitoring report do not invalidate the previous EFSA GMO Panel's scientific opinions on maize MON 810.

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### KEY WORDS

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

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## SUMMARY

Upon request from the European Commission, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) adopted a scientific opinion on the Post-Market Environmental Monitoring (PMEM) report of maize MON 810 for the 2009 and 2010 growing seasons provided by Monsanto Europe S.A., on 7 September 2011 and 7 March 2012 respectively. During its assessment of the 2009 and 2010 PMEM reports, the EFSA GMO Panel identified shortcomings in the methodology for both Case-Specific Monitoring (CSM) and General Surveillance (GS) of maize MON 810 and hence provided recommendations for improvement of the PMEM of maize MON 810.

In response to a similar request from the European Commission to assess the PMEM report of maize MON 810 for the 2011 growing season, the EFSA GMO Panel firstly noted that the applicant followed the same methodological approach as in its previous reports. Therefore, the EFSA GMO Panel mainly assessed the datasets specific to the 2011 growing season, i.e. observations from farmer questionnaires, data from a survey in Spain on refugia compliance and outcomes of the literature search. In addition, the EFSA GMO Panel addressed the points raised by the applicant regarding its recommendations to improve the methodology for the monitoring of resistance evolution in target pests.

From its assessment of the methodology applied by the applicant for the monitoring of maize MON 810 in 2011, the EFSA GMO Panel notes similar shortcomings as in the previous reports. Hence, the EFSA GMO Panel reiterates the same recommendations for improvement of the methodology as in its scientific opinions on the 2009 and 2010 PMEM reports. Moreover, in light of the points raised by the applicant, the EFSA GMO Panel clarified and elaborated on some of its previous recommendations on the methodology supporting the monitoring for resistance evolution of target pests.

However, from the data submitted by the applicant, the EFSA GMO Panel does not identify adverse effects on the environment, human and animal health due to maize MON 810 cultivation during the 2011 growing season. The outcomes of this 2011 PMEM report do not invalidate the previous EFSA GMO Panel's scientific opinions on maize MON 810.

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## BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION AND EFSA

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

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

From 2005 onwards, the applicant submitted to the European Commission PMEM reports on the cultivation of maize MON 810 according to the provisions of Directive 2001/18/EC (EC, 2001).

From 2010 onwards, the EFSA GMO Panel received the requests from the European Commission to assess the PMEM reports submitted by Monsanto on the cultivation of maize MON 810 in 2009 and 2010. The EFSA GMO Panel therefore adopted a scientific opinion on these PMEM reports on 7 September 2011 and 7 March 2012, respectively (EFSA GMO Panel, 2011b, 2012a). The EFSA GMO Panel noted shortcomings in the methodology and hence made recommendations for improvement of the PMEM of maize MON 810. However, from the data submitted by the applicant, the EFSA GMO Panel did not identify adverse effects on the environment, human and animal health due to maize MON 810 cultivation in 2009 and 2010.

On 14 May 2013, the EFSA GMO Panel received from the European Commission a request to assess the PMEM report submitted by Monsanto on the cultivation of maize MON 810 in 2011 taking into consideration comments from Member States on the report as well as points raised by the applicant on its previous assessment of the methodology for case-specific monitoring.

## TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION AND EFSA

On 21 May 2013, the EFSA GMO Panel received a request from the European Commission (EC): (1) to evaluate the findings of the monitoring activities reported in the 2011 PMEM report on maize MON 810, taking into consideration the comments from Member States; (2) to assess the appropriateness of the methodology if this is found to differ compared to the previous season; and (3) to address the points raised by the applicant.<sup>4</sup>

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<sup>4</sup> See Section 'Documentation provided to EFSA'

## ASSESSMENT

### 1. Introduction

Maize MON 810 was developed by the applicant, Monsanto Europe S.A., to express the Cry1Ab protein, derived from *Bacillus thuringiensis* subsp. *kurstaki* (Bt), which confers protection against the lepidopteran target pests European corn borer (ECB; *Ostrinia nubilalis* Hübner) and Mediterranean corn borer (MCB; *Sesamia nonagrioides* Lefebvre). In 2011, maize MON 810 was cultivated in the EU, i.e. in the Czech Republic, Poland, Spain, Portugal, Romania and Slovakia.

The applicant reports to the European Commission and Member States on an annual basis the results of its monitoring activities on the cultivation of maize MON 810 in the EU. In preparing the present scientific opinion, the EFSA GMO Panel made the best use of its previous assessments of the 2009 and 2010 reports (EFSA GMO Panel, 2011b, 2012a) and considered, for example, relevant peer-reviewed publications and comments from Member States on the 2011 report.

Considering the timeline, the EFSA GMO Panel acknowledges that the applicant could not have fully implemented the Panel's recommendations on PMEM, as referred to in its scientific opinion on the 2010 report, in the 2011 monitoring scheme for maize MON 810 (EFSA GMO Panel, 2012a). Therefore, because the applicant had used the same methodological approach as in its previous reports, the EFSA GMO Panel mainly assessed the novel datasets specific to the 2011 growing season, i.e. questionnaires answered by selected farmers in the EU countries where maize MON 810 was cultivated in 2011, data from a specific survey on refugia compliance by Spanish farmers and outcomes of the search or peer-reviewed publications on the safety of maize MON 810 and the Cry1Ab protein. In addition, the EFSA GMO Panel addressed the points raised by the applicant regarding its recommendations to improve the methodology for resistance monitoring of target pests.

The EFSA GMO Panel, in close collaboration with the EFSA Unit for Scientific Assessment Support (EFSA SAS Unit), assessed the appropriateness of the methodology for the farmer survey as part of the general surveillance of maize MON 810 (see Appendix B).

### 2. Case-Specific Monitoring (CSM)

#### 2.1. Summary of the information provided by the applicant

As in its previous reports, the applicant submitted an insect resistance management (IRM) plan developed from the approach<sup>5</sup> described by the industry-based EU Working Group on Insect Resistance Management. The IRM plan for maize MON 810 therefore consists of:

- (1) a strategy based on a high dose of Cry protein accompanied by non-Bt refugia in order to delay the potential evolution of resistance of the target pests (ECB and MCB) to maize MON 810. Moreover, farmers' satisfaction and compliance with refugia implementation were assessed through a specific survey<sup>6</sup> in Spain;
- (2) studies to establish the baseline susceptibility for target pest population(s) and the subsequent proposed strategy for monitoring their possible resistance evolution;
- (3) communication with farmers (e.g. a technical user guide<sup>7</sup>) and a proactive education programme for farmers on compliance with implementation of refugia (e.g. letters, interviews and press articles, leaflets);
- (4) a remedial action plan in the event of any confirmed evolution of pest resistance.

<sup>5</sup> MON 810 2011 PMEM report, Appendix 6.

<sup>6</sup> MON 810 2011 PMEM report, section 3.2.1.1.

<sup>7</sup> MON 810 2011 PMEM report, Appendices 3.1 to 3.6

For further details, please consult the 2011 report published at [http://ec.europa.eu/food/plant/gmo/reports\\_studies/report\\_2011\\_mon\\_810\\_en.htm](http://ec.europa.eu/food/plant/gmo/reports_studies/report_2011_mon_810_en.htm).

Moreover, the applicant<sup>8</sup> made several points regarding previous recommendations of the EFSA GMO Panel for the improvement of the methodology for resistance monitoring of target pests (see section 2.3 of EFSA GMO Panel, 2012a). The applicant confirmed the relevance and appropriateness of the proposed 1-5 % range of resistance allele frequency aiming at early detection of resistance, sought clarifications of the concept of ‘hotspot’ as defined in previous opinions from the EFSA GMO Panel; and claimed that scouting pest larvae on Bt-plants is not cost-effective. Moreover, the applicant questioned the need for improved training of growers on non-Bt refugia compliance as well as the need to monitor regionally occurring pests other than ECB and MCB.

## 2.2. Assessment by the EFSA GMO Panel

In general, the EFSA GMO Panel evaluated to what extent the monitoring protocols designed by the applicant ‘allow for early detection of potential pest resistance before field failures occur and therefore enable additional management measures to be effectively implemented in a timely manner’ (see harmonized IRM plan<sup>9</sup> for cultivation of Bt-maize in the EU).

Given the similarities of the methodology applied for maize MON 810 monitoring in 2009 and 2010, the EFSA GMO Panel mostly refers to the conclusions and recommendations in its previous scientific opinions (EFSA GMO Panel, 2011b, 2012a). Nevertheless, in light of applicant’s concerns, the EFSA GMO Panel clarified and provided additional supporting information for some of its previous recommendations for improvement of the methodology for the monitoring of resistance evolution.

### 2.2.1. High-dose/refuge strategy

#### 2.2.1.1. High dose

As in its scientific opinions on the previous maize MON 810 reports (EFSA GMO Panel, 2011b, 2012a), the EFSA GMO Panel agrees with the applicant that appropriate IRM strategies are capable of delaying the evolution of resistance under field conditions (Alstad and Andow, 1995; Andow, 2008; Tabashnik et al., 2008, 2009, 2013). Furthermore, the EFSA GMO Panel is not aware of new information on Cry1Ab expression levels in maize MON 810 that would invalidate the efficiency of the ‘high-dose/refuge strategy’ for the two major European target pests, namely *O. nubilalis* and *S. nonagrioides*.

The EFSA GMO Panel also considers that, as the larvae of other regionally occurring non-target lepidopteran pests (e.g. *Helicoverpa armigera*, *Agrotis*, *Mythimna* spp.) will be exposed to lepidopteran active Bt-toxin(s) through their feeding on maize plants, they have the potential to evolve resistance to these toxins (EFSA GMO Panel, 2012a).

In addition, the EFSA GMO Panel wishes to stress that it is very difficult to assess the likely efficacy of maize MON 810 high-dose/refuge strategy (as developed for the target pests) for regionally occurring non-target lepidopteran pests. For an assessment of whether this refuge strategy will work on these species, additional information on all underlying assumptions of the strategy are important. It is possible that exploitation of Bt-maize to control/reduce the density of these regionally occurring non-target lepidopteran pests may happen in some regions. The EFSA GMO Panel reiterates its previous recommendation to ‘consider regionally important lepidopteran pests (other than ECB and MCB) of maize MON 810 in the context of CSM for IRM strategy’. However, accounting for the aspects concerning host-plant spectrum, biology, migratory behaviour, and the sporadic occurrence of

<sup>8</sup> See Section ‘Documentation provided to EFSA’

<sup>9</sup> MON 810 2011 PMEM report, Appendix 6

these regionally occurring pests, the spatial and temporal exposure of their larvae to Cry1Ab protein is likely to be less than that of target pests and lead to reduced selection pressure on these species.

Therefore, the EFSA GMO Panel suggests that the applicant uses farmer questionnaires as a tool to give early-warning of the potential for resistance evolution in populations of these non-target pests by reporting observations on the occurrence and unexpected survival (and possible damage) of their larvae on Bt-maize plants. In the event that farmers report indications of possible resistance evolution in these regionally occurring non-target lepidopteran pests, they should be considered for inclusion in the routine IRM plan (for further details, see EFSA GMO Panel, 2012b).

In general, before the onset of resistance, regionally occurring non-target lepidopteran pests should be controlled by appropriate integrated pest management (IPM) measures. Against this background, under the communication and education programme set up by the applicant, farmers should be informed of the need to apply IPM measures (see section 2.2.3).

#### 2.2.1.2. Implementation of non-Bt refugia

The EFSA GMO Panel analysed the results of the survey by ANTAMA addressing the implementation of non-Bt refugia by 100 Spanish farmers (from the Ebro Valley) who cultivated maize MON 810 in 2011. It concluded that 7 % of the farmers growing maize MON 810 in 2011 did not plant a refuge area. The reasons given by the farmers for not planting a refuge area were (1) ECB causes significant economic losses; (2) sowing is easier (with Bt-maize); (3) they considered their farms to be small farms (i.e. less than 5 ha and therefore no refuge is required). The EFSA GMO Panel points out some inconsistencies in reporting data from the ANTAMA survey. For future reports, the EFSA GMO Panel recommends that the applicant provides a rationale justifying possible inconsistencies or the raw data from the survey as recommended by the EFSA guidance document on PMEM of GM plants (EFSA GMO Panel, 2011a).

The EFSA GMO Panel notes an improvement of the percentage of farmers complying with the refugia implementation over the years (i.e. 81 % in 2009, 88 % in 2010, 93 % in 2011). However, the 2011 MON 810 report still shows partial non-compliance with the implementation of non-Bt refugia in Spain, which was further confirmed by the farmer questionnaires.<sup>10</sup> The EFSA GMO Panel recommends that the applicant should not reduce its efforts to increase the level of compliance, especially in regions of high maize MON 810 uptake.

#### 2.2.2. Baseline susceptibility studies and proposed strategy for resistance monitoring of target pests

The harmonised IRM plan<sup>11</sup> for cultivation of Bt-maize in the EU consists in two steps: (i) measurement of baseline susceptibility and (ii) detection of the frequency of resistance alleles.

##### 2.2.2.1. Establishment of baseline susceptibility

In the first instance, the applicant has been establishing baseline susceptibility data since 2005 for ECB populations in the Czech Republic (5 populations), France (16), Germany (9), Hungary (1), Italy (8), Poland (3), Portugal (2), Slovakia (6), Romania (17) and Spain (18) as well as for three pooled MCB populations from Portugal and Spain. In 2011, ECB larvae were sampled in Spain, Romania, the Czech Republic, Germany and Poland whereas MCB larvae were collected in North-East Iberia.

The susceptibility of ECB<sup>12</sup> and MCB<sup>13</sup> to Cry1Ab protein was assessed by the applicant in a laboratory colony and in larval samples collected from refugia areas and fields adjacent to Bt-maize in

<sup>10</sup> MON 810 2011 PMEM report, Appendix 1

<sup>11</sup> MON 810 2011 PMEM report, Appendix 6

<sup>12</sup> MON 810 2011 PMEM report, Appendix 8

<sup>13</sup> MON 810 2011 PMEM report, Appendix 7

the aforementioned EU countries. To detect changes in susceptibility to maize MON 810 in ECB and MCB populations, the applicant used the same methods employed in its previous reports: mortality assessed to determine the lethal concentrations (LC) and/or growth inhibition assessed for the molting inhibition concentrations (MIC).

The EFSA GMO Panel is of the opinion that, in addition to the data available in the scientific literature (e.g. González-Núñez et al., 2000; Farinós et al., 2011; Saeglitz et al., 2006), the applicant has established an appropriate dataset on the baseline susceptibility of the target pests in Europe.

#### 2.2.2.2. Proposed strategy for resistance monitoring of target pests

Once regular measurement of the susceptibility baseline is implemented, changes in the frequency of resistance alleles should also be reported. The applicant suggests a methodological approach based on a discriminating dose assay according to Hawthorne et al. (2001) and Marçon et al. (2000). The applicant states that the resistance monitoring aims at detecting a frequency of the resistance allele ranging from 1 to 5 %.

During its assessment of the 2009 report on maize MON 810 (see Appendix 2 to EFSA GMO Panel, 2011b), the EFSA GMO Panel made use of a theoretical model by Alstad and Andow (1995) in order to estimate the resistance allele frequency indicating the onset of resistance. In EFSA (2011b, 2012a), the EFSA GMO Panel concluded that the detection of resistance alleles should be clearly less than 5 % (i.e. between 1 and 3 %). In order to further support and to precise its previous recommendations to the applicant, the EFSA GMO Panel used the same model to run additional simulations with increasing values of the different parameters.

The EFSA GMO Panel conducted further simulations with the aim of giving more detailed indications on CSM plans and also provided support on the effectiveness of possible mitigation measures. Additional simulations considered a range of different scenarios for various parameters, such as adoption rate of maize MON 810, initial frequency of the resistance allele, sampling frequency and target pests (i.e. bivoltine ECB populations). For each scenario, the aim was to estimate the number of generations required to reach an allele frequency of 0.5 in the target insect population, once the resistance allele frequency detected during CSM had reached 1, 3 or 5 %. The main outcomes are summarised in Appendix A.

According to the results of the model, in the case of a very sensitive annual sampling plan aimed at detecting an occurrence of 0.01 allele frequency in the population of the target insect, even at a very high adoption rate (i.e. 80 %), there is enough time to implement contingency plans before resistance occurs in the field. However, in the case of very high adoption rates, a drastic reduction in maize MON 810 cultivation is needed to significantly delay insect resistance (see Table 1).

When a detection limit of 0.05 is set, it appears that a successful contingency plan can be efficiently applied only for annual samplings and in the case of low to medium adoption rates up to 50 %. A detection limit of an allele frequency of 0.03 increases the time to react for low and medium rates of adoption.

When considering the possibility of implementing biennial sampling plans, the results indicate that the detection of a 0.05 allele frequency can be applied only for low adoption rate up to 40 %. Even the 0.03 detection limit can be applied only if the adoption rate does not exceeds 40 %. Over 40 %, a biennial sampling plan is acceptable only when a detection limit of an allele frequency of 0.01 is the aim and for an adoption rate of maize MON 810 up to 50 %.

The EFSA GMO Panel concludes that, in regions of high uptake of maize MON 810 (e.g. 60 % to 80 % uptake in Catalunya), the detection level of 5 % resistance allele, as suggested by the applicant, is not acceptable. Therefore, the EFSA GMO Panel suggests that, an efficient monitoring programme should be aimed at detecting an allele frequency below 0.03 with sufficient confidence for maize



MON 810 adoption rates lower than 50 %. In the case of higher adoption rates, a detection limit of 0.01 is deemed necessary.

In the case of monovoltine ECB populations, the time available to react is longer and therefore monitoring programs can be revised accordingly. For monitoring plans with *Sesamia* spp., the possible occurrence of multivoltine populations in this species makes the need for annual sampling plans even more important.

### 2.2.2.3. Sampling procedure

#### *Sampling locations*

Although the 2011 report does not provide background information on maize MON 810 adoption rate in various EU countries, the applicant claims that the sampling focuses on areas with high uptake of maize MON 810. The applicant also indicates that an area to be considered for sampling is a 'geographical zone where maize is typically grown under similar agronomic practices isolated from other maize by barriers that may impair an easy exchange of target pests between the areas, e.g., Ebro valley'. Considering areas as large as north-east Iberia, the current sampling protocol proposed by the applicant pools together samples from three fields separated by distances of over 50 km and recommends not to sample within the same location sites over time. The EFSA GMO Panel is of the opinion that, while this protocol might be appropriate to set up a baseline for regional susceptibility, it is not suitable for resistance monitoring over time and an early detection of resistance evolution. In the framework of CSM, the purpose is to check the assumption of the environmental risk assessment (ERA), i.e. that the high-dose/refuge strategy maintains the susceptibility of target pests.

Overall, the sampling efforts should be mainly concentrated on areas<sup>14</sup> where there is high uptake of maize MON 810 associated with high target pest infestation (i.e. more than one generation of the target pests per year). Because of the high selection pressure in these areas (e.g. the Ebro Valley), this is where resistance evolution in target pests is more likely to occur. However, the EFSA GMO Panel acknowledges that a large variability of maize MON 810 uptake might exist within such an area. Indeed, although the overall uptake may remain low to moderate in that area, there could be several agricultural 'zones' within the area where the maize MON 810 uptake already reaches 80 %. These 'zones' should be sampled to determine whether or not the high-dose/refuge strategy as implemented prevents resistance evolution.

The EFSA GMO Panel therefore recommends that the applicant screens the 'areas' of high selection pressure and selects geographical 'zones' of a smaller scale (i.e. county) within the broader area. These smaller geographical zones should be monitored over time. Early detection of resistance evolution in such zones would give more time to growers for implementing appropriate mitigation measures and would delay resistance evolution on a larger scale.

The EFSA GMO Panel does not recommend sampling all relevant 'zones', as defined above, but advises the applicant to sample a minimum of three zones of high uptake of maize MON 810 within a given larger area (i.e. the Ebro Valley). This would be considered sufficiently representative of a target pest population occupying a large area. At the time of adoption of this opinion, maize MON 810 is the only Cry1-expressing maize cultivated in the EU. However, the EFSA GMO Panel recommends that in future the applicant takes into consideration the overall uptake of Cry1-expressing maize when identifying zones of high adoption for sampling target pests.

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<sup>14</sup> That is 'province' in Spain, 'region' in France or even 'lander' in Germany.

### *Sampling frequency*

As part of the IRM plan, the applicant<sup>15</sup> proposed to sample multi-voltine ECB and MCB populations every two years in areas where maize MON 810 adoption rate varies between 20 % and 80 % of the total arable land. Annual sampling is foreseen only in exceptional circumstances in areas of high uptake (i.e. > 80 %; where non-Bt refugia are not implemented).

Based on the outcomes of the additional simulations with the Alstad and Andow (1995) model (see Appendix A), and considering that resistance evolution should focus on areas of high adoption rates, the EFSA GMO Panel recommends annual sampling. The Panel's recommendations are summarised in Table 1.

**Table 1:** Recommended sampling frequency of target pests

Maize MON 810 uptake <sup>16</sup> in a zone (% total arable land)	Sampling frequency	
	For a monovoltine target pest population	For a bi-/multi-voltine target pest population
< 20 %	None	None
20 % to < 50 % (R allele frequency of 3 %)	Biennial	Biennial
50-80 % (R allele frequency of 1 %)	Biennial	Annual
> 80 % <sup>17</sup>	Annual	

### *Further considerations for sampling frame*

In its opinions on previous reports (EFSA GMO Panel, 2011b, 2012a), the EFSA GMO Panel advised the applicant 'to include in the sampling surviving target lepidopteran pests within maize MON 810 fields in order to detect potentially resistant individuals. The sampling should be mainly done as late as possible in the growing season in order to increase the likelihood of detecting surviving individuals'.

During its assessment of the present report, the EFSA GMO Panel considered the concerns raised by the applicant as well as the limitations of the suggested approach.

The rationale for monitoring Bt-fields is that the increase in allele frequencies in Bt-stands, should it appear, is expected to be faster and surviving individuals might be an indication of resistance evolution. However, there is the probability to sample individuals on 'outlier' plants not expressing the insecticidal toxin and hence not linked with resistance evolution.

Therefore, aiming to achieve early detection of resistance evolution, the EFSA GMO Panel recommends:

- whenever possible, to sample 'mixed' populations, namely from plants in refugia and from Bt-maize fields; and
- to make the best use of the farmer questionnaire to report any unusual presence of damaged maize plants and of surviving target pests in maize MON 810 fields (see sections 3.2.1 and 3.3).

<sup>15</sup> See Section 'Documentation provided to EFSA'

<sup>16</sup> At the time of adoption of this opinion, maize MON 810 is the only Cry1-expressing maize cultivated in the EU. However, the EFSA GMO Panel recommends that in future the applicant takes into consideration the overall uptake of Cry1-expressing maize when identifying zones of high adoption for sampling target pests.

<sup>17</sup> In some regions where farmers do not comply with non-Bt refugia implementation (see section 2.2.1.2).

### 2.2.3. Communication with and education of farmers

From the ANTAMA survey<sup>18</sup> in the Ebro valley in Spain, where most of the maize MON 810 was grown in 2011, all the farmers planting maize MON 810 were aware of the recommendation to plant a non-Bt refuge. Of the surveyed farmers, 89 % considered that they were well informed about refugia implementation and 11 % *'somehow informed'*.

As described in section 2.2.1.2 above, the EFSA GMO Panel still noted that a certain percentage of farmers growing maize MON 810 did not comply with the implementation of non-Bt refugia in 2011. However, the EFSA GMO Panel acknowledges the initiatives taken by the applicant over the last few years to improve the education of farmers (i.e. by means of farmer interviews on refugia compliance in local newspapers and by revising the user's manual of the farmer questionnaire). This is supported by the improvement in the percentage of farmers complying with the refugia implementation over the years (i.e. 81 % in 2009, 88 % in 2010, 93 % in 2011).

The EFSA GMO Panel remains of the opinion that special attention should be paid (1) to refugia implementation in those areas in which the likelihood of resistance evolution is higher as well as to (2) the implementation of appropriate IPM measures against relevant regionally occurring pests before the onset of resistance.

### 2.3. Conclusions and recommendations on CSM

The EFSA GMO Panel assessed the CSM/IRM plan in 2011 and its implementation. First of all, the EFSA GMO Panel identifies similar shortcomings in the methodology as it did in the 2009 and 2010 reports and hence reiterates its previous recommendations to the applicant for improvement of the methodology (for further details, see section 2.3 of EFSA GMO Panel, 2011b, 2012a).

Secondly, the EFSA GMO Panel considered the concerns expressed by the applicant and hence advises the applicant to take the following points into consideration when reviewing its IRM plan and in particular the resistance monitoring of target pests:

- to revise the monitoring protocol, aiming to detect a resistance allele frequency between 1 % (for a high maize MON 810 adoption rate) and 3 % (for low to moderate adoption rate);
- to focus the sampling of target lepidopteran pests in geographical zones (i.e. counties) within a larger area where the likelihood of resistance evolution is the greatest. These zones are characterised by a high maize MON 810 uptake and multivoltine target pest populations;
- whenever possible, to sample pest larvae from mixed batches within non-Bt refugia and Bt-plants on GM maize;
- to sample on an annual basis for high maize MON 810 uptake (see Table 1).

The EFSA GMO Panel reiterates its previous recommendation to *'consider regionally important lepidopteran pests (other than ECB and MCB) of maize MON 810 in the context of CSM for IRM strategy'*. However, the EFSA GMO Panel suggests that the applicant uses farmer questionnaires as a tool to give early-warning of the potential for resistance evolution in populations of these non-target pests by reporting observations on the occurrence and unexpected survival (and possible damage) of their larvae on Bt-maize plants. In the event that farmers report indications of possible resistance evolution in these regionally occurring non-target lepidopteran pests, they should be considered for inclusion in the routine IRM plan.

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<sup>18</sup> MON 810 2011 PMEM report, Section 3.2.1.1

Finally, the EFSA GMO Panel concludes that the available dataset does not show evidence of change in susceptibility in the target pests. Moreover, the EFSA GMO Panel is not aware of any scientific report on field resistance in target pests detected in the EU so far. Nevertheless, the local adoption rate can be high in some regions and the EFSA GMO Panel advises the applicant to adapt the monitoring of the resistance allele frequency in order to ensure the early detection of resistance evolution. To conclude, the applicant did not provide new data from the 2011 growing season of maize MON 810 that would invalidate previous evaluations by the EFSA GMO Panel of maize MON 810 (EFSA GMO Panel, 2009, 2011b, 2012a,b,c).

### **3. General Surveillance (GS)**

#### **3.1. Summary of the information provided by the applicant**

As for the previous growing seasons, the 2011 plan for GS<sup>19</sup> of maize MON 810 consisted of four elements: (1) a farmers' survey; (2) a search for relevant publications related to maize MON 810 and/or the Cry1Ab protein; (3) company stewardship activities; and (4) alerts on environmental issues by the authorities and existing networks.

Novel datasets specific to the 2011 growing season are:

- (1) The survey based on 249 questionnaires received from farmers in six European countries (i.e. 29 in the Czech Republic, 10 in Poland, 150 in Spain, 42 in Portugal, 15 in Romania and 3 in Slovakia). According to the applicant, the farmers/fields were selected among the countries depending on the level of market penetration of maize MON 810, the method of selection depended upon the availability of a sampling frame for that country. The farmer surveys were carried out by third parties with experience of conducting agricultural surveys, with the exception of Romania, where Monsanto representatives assisted the farmers to fill in the questionnaire.<sup>20</sup> In its report, the applicant concluded that the 2011 statistical analysis of the 249 questionnaires did not reveal any unanticipated adverse effects that could be associated with maize MON 810.
- (2) A list of peer-reviewed publications on the safety of maize MON 810 and/or the Cry1Ab protein published between June 2011 and beginning of June 2012 was submitted. The applicant used specific key words and searched in journals included in the Web of Science database.<sup>21</sup> The first set of papers resulting from the search was screened for relevance to the ERA of maize MON 810. The applicant reported 12 publications on molecular and food/feed aspects, 10 publications related to the ERA of maize MON 810 and 10 review papers on Bt-maize. The applicant concluded that the peer-reviewed literature did not raise any safety concerns for maize MON 810.

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

#### **3.2. Assessment by the EFSA GMO Panel**

##### **3.2.1. Farmer questionnaires**

The EFSA GMO Panel, in close collaboration with the EFSA Unit for Scientific Assessment Support (EFSA SAS Unit), assessed the methodology followed by the applicant to analyse the farmer

<sup>19</sup> The 2010 MON 810 report submitted by Monsanto is publicly available on the webpage of the EC Directorate General for Health and Consumers, at [http://ec.europa.eu/food/plant/gmo/reports\\_studies/report\\_2011\\_mon\\_810\\_en.htm](http://ec.europa.eu/food/plant/gmo/reports_studies/report_2011_mon_810_en.htm)

<sup>20</sup> MON 810 2011 PMEM report, Appendix 1.

<sup>21</sup> MON 810 2011 PMEM report, section 3.1.6.

<sup>22</sup> MON 810 2011 PMEM report, section 3.1.2.3.

questionnaires. Appendix B provides methodological guidance for a systematic evaluation of the farmer questionnaires, including a list of evaluation criteria (e.g. sample size, survey response rate, statistical analysis) for the farmer questionnaires and the statistical analysis.

The evaluation of the overall farmers survey (including, for example, sampling of farmers, types of questions, method of conduct interviews, data validation, method used for the design of the statistical analysis) is given in Appendix B. Similar weaknesses in the methodology as in previous reports were identified. In addition, the 2011 report provides limited information on the sampling methodology, and the possibility of selection bias in the survey cannot be ruled out.

Recommendations to the applicant for the improvement of the methodology are listed in section 3.3 and in Appendix B. However, from the analysis of the 2011 farmer questionnaires on maize MON 810, the EFSA GMO Panel concludes that no unanticipated adverse effects can be identified.

### 3.2.2. Existing monitoring networks

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

### 3.2.3. Literature review

The EFSA GMO Panel acknowledges that the papers related to maize MON 810 and/or the Cry1Ab protein selected by the applicant (published between June 2011 and beginning June 2012) were adequately discussed and put into the context of the overall safety assessment of maize MON 810.

With the exception of three papers (i.e. Peterson et al., 2011; Rossi et al., 2011; Bell et al., 2012), most had already been addressed by the EFSA GMO Panel in its scientific opinions on maize MON 810 or on Bt-maize (for further details, see EFSA GMO Panel, 2011b, 2012a,b, 2013). The three papers mentioned previously were reviewed by the EFSA GMO Panel and no safety concerns owing to maize MON 810 were identified. Therefore, none of the selected papers relating to maize MON 810 and/or the Cry1Ab protein (published between June 2011 and beginning June 2012) report adverse effects on human and animal health or the environment.

However, the EFSA GMO Panel notes that one relevant paper related to Bt-maize/maize MON 810 and/or the Cry1Ab protein was not reported by the applicant (i.e. Székács et al., 2012). The paper by Székács et al. (2012) was discussed by the EFSA GMO Panel in its scientific opinion updating the risk assessment conclusions and risk management recommendations on maize MON 810 (EFSA GMO Panel, 2012c). In that scientific opinion, the EFSA GMO Panel concluded that *'results reported by Székács et al. (2012) did not contain new information that would invalidate the previous conclusions on the molecular characterisation of maize MON 810 made by the EFSA GMO Panel'*.

Therefore, the outcome of the literature review confirms the previous conclusions on the safety of maize MON 810 made by the EFSA GMO Panel. Therefore, the EFSA GMO Panel considers that its previous conclusions on maize MON 810 remain valid and applicable (EFSA GMO Panel, 2009, 2011b, 2012a,b,c).

### 3.3. Conclusions and recommendations on GS

From the data provided by the 2011 farmer survey on maize MON 810 and the literature review, no adverse effect of the cultivation of maize MON 810 in 2011 on human and animal health or the environment can be identified.

However, the EFSA GMO Panel identified shortcomings similar to those found in the methodology used for previous reports and therefore reiterates similar recommendations to the applicant (for further details on the recommendations, see Appendix B).

In addition, considering novel datasets specific to the 2011 growing season of maize MON 810, the EFSA GMO Panel highlights the following recommendations:

- The sampling frame should be representative of the target population and described in the report. A description of the method to ensure that units are randomly selected from the sampling frame should be included in the report, including where relevant the statistical software and/or the program code used for this procedure. This method should be used consistently in all regions sampled and in all years of the survey. Moreover, the losses to sampling should be fully documented, i.e. the number of farmers not participating in the survey and their reasons should be documented.
- It would be preferable if the standardised interviews are carried out by independent parties to reduce interviewer bias.
- The choice of statistical test should be based on the number of possible categories. Analysing multinomial data as a series of binomial proportions increases the experiment-wise type I error rate and therefore it is recommended to use a multinomial analysis to test for the distributional difference with a subsequent binomial approach used to test for differences in a specific category or to correct for the multiplicity of testing.
- Data should be pooled and statistically analysed with all available data (i.e. to increase power) over years. At the end of the 10 years of GS, the applicant should conduct a statistical analysis with all pooled data.
- A codification for farmers surveyed repeatedly over the years should be set up.

In its scientific opinions on previous reports (EFSA GMO Panel, 2011b, 2012a), the EFSA GMO Panel already suggested that the applicant uses the farmer questionnaire in order to collect data on additional parameters (e.g. the occurrence of regionally occurring non-target lepidopteran pests). In the Panel's view, the farmer questionnaire could also be used as a complementary tool for an early detection of resistance evolution in target pests (e.g. from observations of the unusual presence of damaged Bt-maize plants or of surviving target pests in the Bt-maize field).

Furthermore, in order to improve the sampling frame of the farmers survey, the EFSA GMO Panel reiterates the importance of national GMO cultivation registers, as referred to in Article 31.3 (b) of Directive 2001/18/EC (EC, 2001); and its recommendations to applicants to consider how they may make best use of the information recorded in national registers and opens dialogue with those responsible for the administration of these registers where maize MON 810 is cultivated.

The outcome of the literature review confirms the previous conclusions on the safety of maize MON 810 made by the EFSA GMO Panel.

To conclude, the applicant did not provide new data from the GS of maize MON 810 grown in 2011 that would invalidate previous EFSA GMO Panel evaluations of maize MON 810 (EFSA GMO Panel, 2009, 2011b, 2012a,b,c).

## OVERALL CONCLUSIONS AND RECOMMENDATIONS

The data submitted by the applicant in its 2011 MON 810 report does not indicate any adverse effects on human and animal health or the environment arising from maize MON 810 cultivation in 2011.

Considering the weaknesses in the methodology, similar to those identified in previous reports, the EFSA GMO Panel reiterates the general recommendations given in its scientific opinion providing guidance on PMEM of GM plants (EFSA GMO Panel, 2011a) and the additional specific recommendations for the improvement of the PMEM methodology of maize MON 810 (EFSA GMO Panel, 2011b, 2012a).

Considering the comments raised by the applicant, the EFSA GMO Panel makes further recommendations for the improvement of the methodological approach to the resistance monitoring of target pests (e.g. sampling strategy of target pests and detection method aiming at the detection of resistance evolution at an early stage). However, the EFSA GMO Panel remains of the opinion that the possible resistance evolution is not an environmental concern per se but may lead to altered pest control practices that may cause adverse environmental effects. Furthermore, the EFSA GMO Panel is not aware of any scientific report on field resistance in target pests detected in the EU so far.

Therefore, the EFSA GMO Panel is of the opinion that the outcomes of the 2011 MON 810 report do not invalidate the Panel's previous scientific opinions on maize MON 810 and its subsequent recommendations on risk management and monitoring.

## DOCUMENTATION PROVIDED TO EFSA

1. Letter from the European Commission, dated 7 May 2013, to the EFSA Executive Director requesting the assessment of the MON 810 monitoring report for the 2011 cultivation season provided by Monsanto, and its annex.
2. Letter<sup>23</sup> from Monsanto, dated 14 December 2012, to the European Commission.
3. Acknowledgement letter, dated 17 June 2013, from the EFSA Executive Director to the European Commission.
4. Letter, dated 6 November 2013, from the EFSA Executive Director to the European Commission.

## REFERENCES

- Alcalde E, Amijee F, Blache G, Bremer C, Fernandez S, Garcia-Alonso M, Holt K, Legris G, Novillo C, Schlotter P, Storer N and 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.

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<sup>23</sup> Available at <http://registerofquestions.efsa.europa.eu/roqFrontend/questionsListLoader?unit=GMO> (Question No EFSA-Q-2013-00440)

- Andow DA and Ives AR, 2002. Monitoring and adaptive resistance management. *Ecological Applications*, 12, 1378-1390.
- Batista R and Oliveira M, 2010. Plant natural variability may affect safety assessment data. *Regulatory Toxicology and Pharmacology*, 58, S8-S12.
- Beckie, H.J., Hall, L.M., Simard, M.-J., Leeson, J.Y., and Willenborg, C.J. (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
- Bell JR, Burkness EC, Milne AE, Onstad DW, Abrahamson M, Hamilton KL, Hutchison WD, 2012. Putting the brakes on a cycle: bottom-up effects damp cycle amplitude. *Ecology Letters*, 15, 310–318. doi: 10.1111/j.1461-0248.2011.01739.x
- 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, Paris.
- 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
- Comins HN, 1977. The development of insecticide resistance in the presence of migration. *Journal of Theoretical Biology*, 64, 177–197.
- de Vendomois J, Cellier D, Velot C, Clair E, Mesnage R, Seralini GE, 2010. Debate on GMOs health risks after statistical findings in regulatory tests. *International Journal of Biological Sciences*, 6, 590-598.
- EC (European Commission), 1990. Council Directive 90/220/EEC of 23 April 1990 on the deliberate release into the environment of genetically modified organisms. *Official Journal L117*, 08.05.1990, 15-27.
- EC (European Commission), 1998. Commission Decision of 22 April 1998 concerning the placing on the market of genetically modified maize (*Zea mays* L. line MON 810), pursuant to Council Directive 90/220/EEC (98/294/EC). *Official Journal L131*, 05.05.1998, 32-33.
- EC (European Commission), 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*, 27.04.2001, 1-39.
- EFSA (European Food Safety Authority), 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 MON 863 x MON 810 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 (European Food Safety Authority), 2009. Scientific Opinion of the Panel on Genetically Modified Organisms on applications (EFSA-GMO-RX-MON 810) for the renewal of authorisation for the continued marketing of (1) existing food and food ingredients produced from genetically modified insect resistant maize MON 810; (2) feed consisting of and/or containing maize MON 810, and maize MON 810 for feed use (including cultivation); and of (3) food additives and feed materials produced from maize MON 810, all under Regulation (EC) No 1829/2003 from Monsanto. *The EFSA Journal* 2009, 1149, 1-84.
- EFSA Panel on Genetically Modified Organisms (GMO), 2011a. Scientific Opinion providing guidance on the Post-Market Environmental Monitoring (PMEM) of genetically modified plants. *EFSA Journal* 2011;9(8):2316, 40 pp. doi:10.2903/j.efsa.2011.2316



- EFSA Panel on Genetically Modified Organisms (GMO), 2011b. Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2009. EFSA Journal 2011;9(10):2376, 66 pp. doi:10.2903/j.efsa.2011.2376
- EFSA Panel on Genetically Modified Organisms (GMO), 2011c. Statement supplementing the evaluation of the environmental risk assessment and risk management recommendations on insect resistant genetically modified maize Bt11 for cultivation. EFSA Journal 2011;9(12):2478, 44 pp. doi:10.2903/j.efsa.2011.2478
- EFSA Panel on Genetically Modified Organisms (GMO), 2012a. Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2010. EFSA Journal 2012;10(3):2610, 35 pp doi:10.2903/j.efsa.2012.2610
- EFSA Panel on Genetically Modified Organisms (GMO), 2012b. Scientific Opinion supplementing the conclusions of the environmental risk assessment and risk management recommendations for the cultivation of the genetically modified insect resistant maize Bt11 and MON 810. EFSA Journal 2012;10(12):3016, 32 pp. doi:10.2903/j.efsa.2012.3016
- EFSA Panel on Genetically Modified Organisms (GMO), 2012c. Scientific Opinion updating the risk assessment conclusions and risk management recommendations on the genetically modified insect resistant maize MON 810. EFSA Journal 2012;10(12):3017, 98 pp. doi:10.2903/j.efsa.2012.3017
- EFSA Panel on Genetically Modified Organisms (GMO), 2012d. Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from BASF Plant Science Company GmbH on the cultivation of genetically modified potato EH92-527-1 in 2010. EFSA Journal 2012;10(2):2558, 38 pp. doi:10.2903/j.efsa.2012.2558
- EFSA Panel on Genetically Modified Organisms (GMO), 2012e. Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from BASF Plant Science Company GmbH on the cultivation of genetically modified potato EH92-527-1 in 2011. EFSA Journal 2012;10(12):3015, 35 pp. doi:10.2903/j.efsa.2012.3015
- EFSA Panel on Genetically Modified Organisms (GMO), 2013. Scientific Opinion on application (EFSA-GMO-NL-2005-23) for placing on the market of genetically modified maize 59122 for food and feed uses, import, processing and cultivation under Regulation (EC) No 1829/2003 from Pioneer. EFSA Journal 2013;11(3):3135, 104 pp. doi:10.2903/j.efsa.2013.3135
- Engels H, Bourguet D, Cagán L, Manachini B, Schuphan I, Stodola TJ, Micoud A, Brazier C, Mottet C and Andow DA, 2010. Evaluating Resistance to Bt Toxin Cry1Ab by F2 Screen in European Populations of *Ostrinia nubilalis* (Lepidoptera: Crambidae). *Journal of Economic Entomology*, 103 (5), 1803-1809.
- EMA (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](http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003658.pdf)
- EPPO (European and Mediterranean Plant Protection Organization). <http://www.eppo.org/>
- Farinós GP, Andreadis SS, de la Poza M, Mironidis GK, Ortego F, Savopoulou-Soultani M and Castañera P, 2011. Comparative assessment of the field-susceptibility of *Sesamia nonagrioides* to the Cry1Ab toxin in areas with different adoption rates of Bt-maize and in Bt-free areas. *Crop Protection*, 30, 902-906.
- Galyean ML and Wester DB, 2010. Comparison of multinomial and binomial proportion methods for analysis of multinomial count data. *Journal of Animal Science*, 88, 3452-3463. doi: 10.2527/jas.2010-2868

- Gonzalez-Nuñez M, Ortego F and 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.
- Gould F, Anderson A, Jones A, Sumerford D, Heckel D, Lopez J, Micinski S, Leonard R and Laster M, 1997. Initial frequency of alleles for resistance to *Bacillus thuringiensis* toxins in field populations of *Heliothis virescens*. *Proceedings of the National Academy of Sciences of the USA*, 94, 3519-23.
- Hawthorne D, Siegfried B, Shelton T and Hellmich R, 2001. Monitoring for resistance alleles: a report from an advisory panel on insect resistance monitoring methods for Bt corn. Unpublished study submitted to EPA (part of Monsanto's 2000 IRM monitoring report). MRID # 453205-02.
- Hollis JM, Jones RJA, Marshall CJ, Holden A, Van de Veen JR and 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, 38 pp. Office for Official Publications of the European Communities, Luxembourg.
- Huang F, Andow DA and Buschman LL, 2011. Success of the high-dose/refuge resistance management strategy after 15 years of Bt crop use in North America. *Entomologia Experimentalis et Applicata* 140, 1–16. doi: 10.1111/j.1570-7458.2011.01138.x
- Kruger M, Van Rensburg JBJ and Van den Berg J, 2012. Transgenic Bt maize: farmers' perceptions, refuge compliance and reports of stem borer resistance in South Africa. *Journal of Applied Entomology*, 136, 38-50.
- Marçon PCRG, Taylor DB, Mason CE, Hellmich RL and 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 and 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
- Peterson JA, Lundgren JG and Harwood JD, 2011. Review - Interactions of transgenic *Bacillus thuringiensis* insecticidal crops with spiders (Araneae). *The Journal of Arachnology*, 39, 1-21.
- Rasch D, Herrendörfer G, Bock J, Victor N and Guiard V, 2007. *Verfahrensbibliothek Versuchsplanung und -auswertung*. Oldenbourg Verlag. Munich, Germany.
- Romeis J and Meissle M, 2011. Non-target risk assessment of Bt crops - Cry protein uptake by aphids, *Journal of Applied Entomology*, 135, 1-6.
- Rossi F, Morlacchini M, Fusconi G, Pietri A and Piva G, 2011. Effect of insertion of Bt gene in corn and different fumonisin content on growth performance of weaned piglets. *Italian Journal of Animal Science*, 10, e19.
- Roush RT and Miller GL, 1986. Considerations for design of insecticide resistance monitoring programs. *Journal of Economic Entomology*, 79, 293-298.
- Saeglitz C, Bartsch D, Eber S, Gathmann A, Priesnitz KU and Schuphan I, 2006. Monitoring the Cry1Ab susceptibility of European corn borer in Germany. *Journal of Economic Entomology*, 99, 1768-1773.
- Székács A, Weiss G, Quist D, Takacs E, Darvas B, Meier M, Swain T and Hilbeck A, 2012. Inter-laboratory comparison of Cry1Ab toxin quantification in MON 810 maize by enzyme-immunoassay. *Food and Agricultural Immunology*, 23, 99-121.
- Schmidt K, Wilhelm R, Schmidtke J, Beißner L, Mönkemeyer W, Böttinger P, Sweet J and Schiemann J, 2008. Farm questionnaires for monitoring genetically modified crops: a case study using GM maize. *Environmental Biosafety Research*, 7, 163-179.

- Tabashnik BE, Gassmann AJ, Crowder DW and Carrière Y, 2008. Insect resistance to Bt crops: evidence versus theory? *Nature Biotechnology*, 26, 199-202.
- Tabashnik BE, Van Rensburg JBJ and Carriere Y, 2009. Field-evolved insect resistance to Bt crops: definition, theory, and data. *Journal of Economic Entomology*, 102, 2011-2025.
- Tabashnik BE, Brévault T and Carrière Y, 2013. Insect resistance to Bt crops: lessons from the first billion acres. *Nature Biotechnology*, 31, 510-521 doi:10.1038/nbt.2597
- Tinland B, Delzenne P and 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.
- Venette RC, Hutchison WD and Andow DA, 2000. An In-Field Screen for Early Detection and Monitoring of Insect Resistance to *Bacillus thuringiensis* in Transgenic Crops. *Journal of Economic Entomology*, 93 (4), 1055-1064.
- Wilhelm R, Beißner L, Schmidt K, Schmidtke J, and Schiemann J, 2004. Monitoring des Anbaus gentechnisch veränderter Pflanzen - Fragebögen zur Datenerhebung bei Landwirten. *Nachrichtenbl. Deut. Pflanzenschutzd*, 56 (8), 184-188.
- Yanni S, Whalen J and Ma B, 2010. Crop residue chemistry, decomposition rates, and CO<sub>2</sub> evolution in Bt and non-Bt corn agroecosystems in North America: a review, *Nutrient Cycling in Agroecosystems*, 87, 277-293.
- Zhao JZ, Li YX, Collins HL and Shelton AM, 2002. Examination of the F2 screen for rare resistant alleles to *Bacillus thuringiensis* toxins in the diamondback moth (Lepidoptera: Plutellidae). *Journal of Economic Entomology*, 95 (1), 14-21.
- Zhu X, Chen L, Shen P, Jia J, Zhang D and Yang L, 2011. High sensitive detection of Cry1Ab protein using a quantum dot-based fluorescence-linked immunosorbent assay. *Journal of Agricultural and Food Chemistry*, 59, 2184-2189.

## APPENDIX A – SIMULATION EXERCISE TO OPTIMIZE CSM FOR INSECT RESISTANCE MANAGEMENT

In its previous scientific opinions on annual reports on maize MON 810, the EFSA GMO Panel has discussed the likelihood of insurgence of resistance by the target organisms to maize MON 810 and advised on possible CSM plans (EFSA GMO Panel, 2011b, 2012a).

The IRM plan provided by the applicant aims at early detections of the possible onset of resistance in target organisms where maize MON 810 is cultivated. Several features linked to the biology of the target organism and the receiving environment, in particular the location and size of refuges where the Bt-crop is to be released, are the major drivers for such trends in allele frequency under a given set of conditions.

Based on the first set of simulations (EFSA GMO Panel, 2012a), the EFSA GMO Panel argued that the threshold of 5 % allele presence detection proposed by the applicant and the biennial sampling plan would not be sufficient in many cases to detect sufficiently early the onset of resistance and consequently the early implementation of contingency plans.

As a consequence of the comments provided by the applicant to the European Commission,<sup>24</sup> the EFSA GMO Panel conducted further simulations with the aim of giving more detailed indications on CSM plans and also provided support on the effectiveness of possible mitigation measures.

The additional simulations aimed at estimating, under different maize MON 810 uptake<sup>25</sup> scenarios, the number of generations required to reach an allele frequency of 0.5 in the target insect population, once the resistance allele frequency detected during CSM had reached 1, 3 or 5 %. New simulations were run using the model by Alstad and Andow (1995) with the shareware software Populus.<sup>26</sup> In particular, the model was run using the parameters' values listed in Table 2.

**Table 2:** Parameters' values used for the model

Parameters	ECB
Adoption rate of Bt- maize	Ranging from 20 to 90 %
Initial allele frequencies	0.006 (estimated baseline for ECB)
Fecundity	100
Dominance	0.01 (Almost fully recessive)
Preference for Bt-maize in second generation	120 %
Overwinter survival	0.01
Survival of susceptible homozygotes on Bt	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 to default values in the software.

### Results

In the following Table 3, an overview of the results for bivoltine populations is presented. The allele frequency of the population over time (for each generation) is indicated in the case of different adoption rates of maize MON 810. The occurrence of field-detected resistance is conventionally set at

<sup>24</sup> See Section 'Documentation provided to EFSA'

<sup>25</sup> At the time of adoption of this opinion, maize MON 810 is the sole Cry1-expressing maize cultivated in the EU. However, the EFSA GMO Panel recommends that in future the applicant takes into consideration the overall uptake of Cry1-expressing maize when identifying zones of high adoption for sampling target pests.

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

an allele frequency of 0.5 in the population. The model was initiated simulating an allele frequency of 0.006 in the original population as recorded in population of ECB in Italy and Slovakia (Engels et al., 2010).

**Table 3:** Overview of model result for bivoltine pest populations

Generations	20 %	40 %	50%	60 %	70 %	80 %	90 %
1	0,006	0,006	0,006	0,006	0,006	0,006	0,006
2	0,0061	0,0062	0,0064	0,0066	0,0071	0,0077	0,0093
3	0,0069	0,0084	0,0095	<b>0,0110</b>	<b>0,0131</b>	<b>0,0167</b>	<b>0,0271</b>
4	0,0070	0,0088	<b>0,0101</b>	0,0119	0,0146	0,0197	<b>0,0380</b>
5	0,0083	<b>0,0123</b>	0,0154	0,0200	0,0271	<b>0,0417</b>	<u>0,1072</u>
6	0,0084	0,0129	0,0166	0,0223	<b>0,0317</b>	<u>0,0541</u>	<u>0,2074</u>
7	<b>0,0101</b>	0,0190	0,0276	<b>0,0430</b>	<u>0,0724</u>	0,1565	<u>0,6771</u>
8	0,0103	0,0203	<b>0,0308</b>	<u>0,0516</u>	0,0990	0,2783	0,9317
9	0,0125	<b>0,0324</b>	<u>0,0592</u>	0,1251	0,2927	<u>0,6838</u>	0,9904
10	0,0128	0,0357	0,0714	0,1817	<u>0,5014</u>	0,8982	0,9985
11	0,0158	<u>0,0637</u>	0,1610	<u>0,4225</u>	0,7856	0,9747	0,9998
12	0,0163	0,0748	0,2284	<u>0,6288</u>	0,9167	0,9935	1
13	0,0205	0,1464	<u>0,4307</u>	0,8186	0,9716	0,9985	1
14	0,0213	0,1919	<u>0,5957</u>	0,9143	0,9900	0,9996	1
15	0,0276	0,3346	0,7603	0,9629	0,9967	0,9999	1
16	0,0288	<u>0,4502</u>	0,8607	0,9836	0,9989	1	1
17	<b>0,0389</b>	<u>0,6050</u>	0,9250	0,9931	0,9996	1	1
18	0,0409	0,7190	0,9591	0,9970	0,9999	1	1
19	<u>0,0569</u>	0,8155	0,9786	0,9987	1	1	1
20	0,0613	0,8786	0,9886	0,9994	1	1	1
21	0,0878	0,9236	0,9941	0,9998	1	1	1
22	0,0969	0,9513	0,9968	0,9999	1	1	1
23	0,1377	0,9699	0,9984	1	1	1	1
24	0,1575	0,9810	0,9991	1	1	1	1
25	0,2146	0,9883	0,9996	1	1	1	1
26	0,2505	0,9927	0,9998	1	1	1	1
27	0,3203	0,9955	0,9999	1	1	1	1
28	0,3720	0,9972	0,9999	1	1	1	1
29	0,4461	0,9983	1	1	1	1	1
30	<u>0,5051</u>	1	1	1	1	1	1

The figures in boxes indicate after how many generations the population allele frequency reaches a level  $\geq 0.5$  and it is therefore considered resistant. The figures in **bold**, **in bold and in italic**, and underlined indicate the generation in which the sensitivity chosen for the sampling plan (respectively at 0.01, 0.03 and 0.05 %) would allow the detection of an increase in allele frequency. The closer these dates are to the generation in which the allele frequency has reached the 0.5 value, the shorter is the time available to react with a remediation plan.

In case of a biennial sampling programme, the effectiveness of the monitoring plan in preventing the onset of resistance is affected by the fact that information on allele frequency may be available only the year after the limit has in fact been reached (i.e. the data in the second next row, in the case of a bivoltine population).

It is reasonable to consider that a minimum of 1 year-delay from detection of resistance and taking an adaptive response is required (Andow and Ives, 2002). However, it has to be considered that it may be necessary to wait for a few months in order to obtain the results from the laboratory tests with the ECB individuals collected from the field. Therefore it seems a safe estimate to consider that to implement any mitigation measures in fields, it is necessary that in the two following growing seasons (i.e. three to four generations in the case of a bivoltine population of ECB) the allele frequency of 0.5 is not reached.

From the results presented in Table 3, it is also possible to evaluate the efficiency of the reduction of the percentage of areas cropped with maize MON 810 once signs of increasing allele frequency are detected. When a given allele frequency indicated in the table is detected, the efficiency of such a reduction can be assessed by looking in the respective column to see how many generations will then be needed before resistance is achieved with a reduced level of adoption of maize MON 810.

For example with an adoption of maize MON 810 of 60 % of the total cropped area, when a 0.05 allele frequency is detected, the population is expected to become resistant two years later (four generations, see column 5). However, if a reduction to 20% of the maize MON 810 cropped area is immediately implemented, maize MON 810 is expected to maintain its effectiveness for five-six more years (see column 2 in Table 3). This is only valid if annual sampling is carried out and if action is taken before the following growing season. In the case of biennial samplings, as currently implemented, the allele frequency might already be higher than 0.05 when detected. Using the same example, if in year  $n$ , the allele frequency is 0.0430 (generation 7 of column 5), it won't be picked up by a sampling procedure aiming to detect 0.05 and the next sampling will occur two years after when the allele frequency will have reached 0.1817, far too late to undertake any efficient measures.

According to the results of the model, in the case of a very sensitive annual sampling plan aimed at detecting an occurrence of 0.01 allele frequency in the population of the target insect, even at high adoption rates (80 %), there is time enough to implement contingency plans before resistance occurs in field. However, in the case of very high adoption rates, a drastic reduction in maize MON 810 is needed to significantly delay insect resistance (see Table 1).

When a detection limit of 0.05 is set, it appears that a successful contingency plan can be efficiently applied only for annual samplings and in the case of low to medium adoption rates up to 50 %. A detection limit of an allele frequency of 0.03 increases the time available to react for low and medium rates of adoption.

These predictions are based on hypothetical samplings 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, according to the model in the case of ECB surviving in Bt-stands, the detection of 5 % will only leave one additional generation before resistance is achieved in Bt-fields.

When considering the possibility of implementing biennial sampling plans, the results indicate that the detection of a 0.05 allele frequency can be applied only for low adoption rate up to 40 %. Even the 0.03 detection limit can be applied only if the adoption rate does not exceeds 40 %. Over 40 %, a biennial sampling plan is acceptable only when a detection limit of an allele frequency of 0.01 is the aim and for an adoption rate of maize MON 810 up to 50 %.

## Discussion

The early detection of an increased allele frequency in the population of the target pest is the main goal of a IRM plan. IRM is a proactive measure necessary to ensure the effectiveness of this measure in preventing a possible adaptation (see EFSA GMO Panel, 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 exercise, the EFSA GMO Panel estimates that a level of detection of an allele frequency of 0.05 does not allow the necessary time for taking any adaptive response for bivoltine strains of ECB. These results are in agreement with Andow and Ives (2002) who considered the case of ECB in the USA.

The sampling strategy (frequency and threshold) should be adapted to the potential selection pressure, i.e., maize MON 810 uptake. In addition to the selection of the appropriate detection threshold, the sampling procedure should ensure enough statistical precision. Indeed, a given allele frequency level which has been reached in a region might not be detected in due time because of the sampling errors.

Therefore, the EFSA GMO Panel suggests that, an efficient monitoring program should be based on annual samplings and aimed at detecting allele frequency below 0.03 with enough confidence for adoptions rates of maize MON 810 lower than 50 %. In case of higher adoption rates a detection limit of 0.01 is deemed necessary.

In case of monovoltine ECB populations, the available time to react is longer and therefore monitoring programs can be revised accordingly.

For monitoring plans with *Sesamia* spp., the possible occurrence of multivoltine populations of this species makes the necessity of annual sampling plans even more stringent.

## APPENDIX B – SAS TECHNICAL REPORT ON THE EVALUATION OF FARMER QUESTIONNAIRES

### BACKGROUND

This Appendix B was prepared by the EFSA SAS Unit to support the EFSA GMO Panel in its evaluation of the monitoring report on maize MON 810 for the 2011 growing season, specifically to provide methodological guidance on evaluation of the farmer questionnaires submitted as part of the GS programme, which aimed to identify adverse affects of the GM maize or its use on human and animal health or the environment that had not been anticipated in the ERA.

### METHOD

Evaluation criteria were developed based on the principles of design for cross-sectional studies, and in particular surveys. The evaluation grid can be applied to surveys used for GS of GM plants. In July 2011, the EFSA GMO Panel updated its guidance on the PMEM of GM plants (EFSA GMO Panel, 2011a). The criteria reflect the recommendations in this guidance document. These criteria were previously applied in the assessment of the 2009 - 2010 MON 810 monitoring reports and the 2010 – 2011 Amflora monitoring reports (EFSA GMO Panel, 2011b, 2012 a,d,e).

Study design principle	Criteria
Sampling frame	<ol style="list-style-type: none"> <li>1) The sampling frame used is specified</li> <li>2) The total population included the sampling frame is specified</li> <li>3) The characteristics of the population included in the sampling frame are described, including region, agricultural practices, GM cultivation</li> <li>4) The sampling frame coverage is appropriate for GM cultivation in the EU</li> </ol>
Sampling method (sample bias)	<ol style="list-style-type: none"> <li>1) The sampling method to select sample units from the sampling frame is described</li> <li>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</li> <li>3) A list of sample units selected from the sample frame is provided</li> <li>4) The sampling method minimises selection bias</li> </ol>
Sample size (sample precision)	<ol style="list-style-type: none"> <li>1) The size of the adverse effect to be measured is specified and scientifically justified and is within an acceptable limit of change.</li> <li>2) The significance level is specified and the chosen level is scientifically justified (Type I error rate)</li> <li>3) The power is specified and the chosen level is scientifically justified (Type II error rate)</li> <li>4) A literature reference for the sample size method is provided</li> <li>5) The sample size calculation method is appropriate for a proportion in a cross-sectional study</li> <li>6) The sample size is sufficient to detect an adverse effect related to GM cultivation</li> </ol>
Survey response rate (non response bias)	<ol style="list-style-type: none"> <li>1) Follow-up method for non-responders is described and appropriate</li> <li>2) Response rate is specified</li> <li>3) Details of losses in sampling are described</li> <li>4) he number of partial responses and reasons for non-completion are specified</li> </ol>



	<ol style="list-style-type: none"> <li>5) comparison is made between characteristics of responder group and non-responder group</li> <li>6) Comparison is made between characteristics of responder group and independent sources of information about the target population</li> <li>7) The effects of non response bias have been minimised</li> </ol>
Instrument design	<ol style="list-style-type: none"> <li>1) The study design includes considerations to avoid interviewer bias</li> <li>2) Where interviewers are used the interviewer training is described</li> <li>3) The selection of open and closed questions is appropriate for the question type</li> <li>4) The questions are clearly phrased and not open to misinterpretation</li> <li>5) The questions encourage independent and objective responses</li> <li>6) The comparator used in the study is described and appropriate for general surveillance</li> <li>7) The instrument has been previously tested and validated</li> </ol>
Instrument validity	<ol style="list-style-type: none"> <li>1) Content validity – the survey includes questions relevant to assess <ul style="list-style-type: none"> <li>• Background data</li> </ul> <p>Identifier of location of monitoring site and comparator site, surrounding landscape, type of field margins, proximity to conservation areas, cultivation and management of the GM field including recent history and previous cropping, soil (type, structure, quality), nutrient status, fertilization, irrigation.</p> <ul style="list-style-type: none"> <li>• Data informing on possible change in behaviour and performance of GMP</li> </ul> <p>Other GMPs cultivated, number of years of cultivation of GMP, cultivation and tillage from the removal of the previous crop to seed sowing, crop husbandry including sowing/planting date, post planting management, crop emergence, growth (vigour, height), pest, disease and weed management, flowering, standing ability, harvesting date and methods, yield, post-harvest management and subsequent cropping of the site, post-harvest storage, handling, processing, feeding</p> <ul style="list-style-type: none"> <li>• Data informing on possible ecological/environmental impacts of GMP on the protection goals and measurement</li> </ul> <p>Weed and pest populations, observations of other flora and fauna such as insects, birds and mammals, pollination and presence of pollinators, health of humans and performance of livestock.</p> <ul style="list-style-type: none"> <li>• Implementation of specific management requirements</li> </ul> <p>Implementation of risk management measures, coexistence segregation measures, stewardship recommendations, specific management due to regional environmental requirements</p> </li> <li>2) Criterion validity – agronomy parameters reported in the survey are compared with field trial data to test for concurrency</li> <li>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)</li> </ol>

	<ol style="list-style-type: none"> <li>4) Plausibility of responses – results for cultivation methods, agronomy parameters and weed/pest management practices reported in the survey conform to European agricultural practices</li> <li>5) Construct validity – consistency and agreement between outcome variables is examined</li> </ol>
Data validation	<ol style="list-style-type: none"> <li>1) Data validation procedure are documented</li> <li>2) Results excluded from the statistical analysis during validation are reported</li> <li>3) Missing values are reported</li> </ol>
Longitudinal aspects	<p>Comparison with survey results from previous years</p> <ol style="list-style-type: none"> <li>1) The survey is applied to the sample unit for multiple years in order to assess residual effects</li> </ol>
Statistical analysis	<ol style="list-style-type: none"> <li>1) Objective and hypotheses for analysis are clearly stated</li> <li>2) A statistical analysis plan is provided</li> <li>3) Statistical analysis includes analysis of pre-defined sub-groups according to PMEM guidance e.g country</li> <li>4) Statistical analysis is appropriate for the data types</li> <li>5) Results are clearly and consistently presented</li> <li>6) The report should include descriptive statistics for the outcome variables</li> <li>7) The issue of multiplicity is addressed</li> <li>8) Method for handling missing values are described</li> <li>9) Where appropriate confidence intervals should be provided</li> <li>10) The results of post-hoc analysis should be identifiable</li> </ol>
Report conclusions	<ol style="list-style-type: none"> <li>1) The report conclusions are clearly stated</li> <li>2) The study design is appropriate to assess the conclusions</li> <li>3) The data presented supports the conclusions presented in the report</li> </ol>

## Results

### Sampling frame

#### 1) Sampling frame specification

Appendix 1 of the 2011 MON 810 report specifies that in Portugal and Romania the sampling frame for the survey was a public register. In Czech Republic, Slovakia and Poland, customer lists obtained from companies selling seeds were used. In Spain, the country with highest cultivation of maize MON 810 and therefore the largest number of surveyed farmers, no suitable sampling frame was available as a consequence surveyors used previous contacts.

#### 2) Population included the sampling frame

Appendix 1 of the 2011 MON 810 report did not include information on the number of farmers in the sampling frame.

#### 3) Characteristics of the population included in the sampling frame

Appendix 1 of the 2011 MON 810 report did not include information on the characteristics of the farmers included in the sampling frame. Information on the number of farmers in the sampling frame according to country, region, size of farm/number of fields and previous cultivation of GM crops is important.

#### 4) Sampling frame coverage

Information on the sampling frame was not provided in Appendix 1 of the 2011 MON 810 report, and therefore this is difficult to assess. The report states that *'The customer lists of the seed selling companies do not completely reflect the MON 810 cultivating farmers, so that some are missing'* but does not try to characterise the missing farmers. Table 3.2 indicates that farmers from all the countries growing maize MON 810 were included in the survey. The percentage of maize MON 810-planted surfaces surveyed ranged between 3.8 % in Spain to 94 % in Romania. For Europe as a whole, 9.9 % of maize MON 810-planted surfaces were surveyed – this is a decrease from 12.5 % in 2009 and 13.3 % in 2010. Since the survey size per year is fixed at 250 farmers and the area of maize MON 810 cultivation has increased this proportion may continue to decrease. Full details on the source of the sampling frame, the number of farmers and the major characteristics of the farmers should be included in the survey report. The national registers set by Member States on the cultivation of GM crops would be the optimum sampling frame, however the report notes that when using public registers they *'do not necessarily contain the contact data of the farms so that it is often very difficult to identify them.'*

### Sampling method

#### 1) Selection of sample units

Appendix 1 of the 2011 MON 810 report states public registers and customer lists of the seed selling companies have been used as sampling frames in 2011, but in one country no sampling frame was available. Survey design methodology requires the sampling frame to be representative for the target population, in this case European farmers growing MON 810, and that the random selection process is applied to the sample units in the sampling frame prior to proceeding with the interviews. A description of the method to ensure that units are randomly selected from the sampling frame should be included in the report, including where relevant the statistical software and/or the program code used for this procedure.

#### 2) Sampling of units from representative environments

Appendix 1 of the 2011 MON 810 report states *'two strategies for selecting farmers are applied: in MS with a high rate of market penetration a certain number of farms will be selected whereas in MS with low cultivation rates preferably all MON 810 cultivating farmers are interviewed.'* This differs from the 2010 MON 810 report which stated: *'For selecting farmers in countries with higher market penetration a procedure is applied to select: at least 10 % of farmers and 10 % GM area per region and at least 20 % of new farmers each year.'* To ensure units are selected from representative environments (regions with high uptake of maize MON 810), a proportion of farmers to be selected from each strata (e.g. country) should be clearly defined and consistently applied in each year of the survey.

#### 3) Proportion of sample units selected

The number of farmers surveyed in each country is provided, but no indication of the total number of farmers in each country and region included in the sampling frame is given. Table 3.2 describes the proportion of maize MON 810-planted area covered in the survey, it can be seen that in 2011 there are no countries where all farmers were surveyed. Information on the farmers included in the sampling frame and selected from the sampling frame should be provided as evidence that the sampling method has been successfully implemented.

#### 4) Selection bias

If the number of farmers cultivating maize MON 810 increases, it will be difficult to ensure all farmers within a region are interviewed as a consequence an appropriate sampling methodology becomes more important. The report provides limited information on the sampling methodology and the possibility of selection bias and achievement of adequate power in the survey cannot be excluded. The grouping of sample units according to the strata and random selection of sample units from within the strata should

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

### Sample size

#### 1) Size of the adverse effect

Appendix 1 of the 2011 MON 810 report states that the null hypothesis is that the proportion of responses that are not 'as usual' is above 10 %. Therefore, the threshold or margin for adverse effects is 10 % (i.e. 5 % above the baseline). No specific reference from the scientific literature was provided to support the selection of 10 %; however, for this type of study 10 % represents an acceptable limit of change. A 10 % effect size has also been selected in a framework proposal for post-release monitoring of second-generation crops with novel traits in Canada (Beckie et al., 2010).

#### 2) Type I error rate

The type I error rate is  $\alpha = 0.01$  in Appendix 1 of the 2011 MON 810 report. This denotes that there is a 1 % probability of rejecting the null hypothesis that there is a 'proportion of adverse effects greater than 10%' when it is true, i.e. failure to detect a true adverse effect. A type I error rate of 1 % is conservative and acceptable.

#### 3) Type II error rate

The type II error rate is  $\beta = 0.01$  in Appendix 1. This denotes that there is a 1 % probability of accepting the null hypothesis that there is a 'proportion of adverse effects greater than 10%' when it is false, i.e. falsely detecting an adverse effect. The selection of 0.01 will result in a large sample size.

#### 4) Reference for the sample size method

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

#### 5) Sample size calculation

Sample size calculation is as reported in the 2009 and 2010 monitoring reports. The sample size is calculated assuming difference testing.

#### 6) Sample size

As concluded for the 2009 and 2010 monitoring reports, the selection of parameters for the sample size calculation is conservative, and consequently the resulting sample size is large. In 2011, 249 farmers were sampled – this is one farmer less than the planned 250 farmers per year. Nonetheless, it is likely that the same farmer may be surveyed in different years and therefore sample units may not be independent from each other. Consideration of this factor should be included in the sample size calculation. Most importantly, the power of the study will be achieved only when the sample size of 2500 farmers/fields surveyed is achieved after 10 years.

### Survey response rate

#### 1) Follow-up for non-responders

Appendix 1 of the 2011 MON 810 report states *'The surveys are performed after the planting season, the farmers are provided with a copy of the questionnaire at least two weeks before a telephone interview or interviewed face-to-face.'* This should reduce the number of non-responders in comparison with other survey methods. No information is provided in the report on the follow-up for non-responders.

## 2) Response rate

The response rate is not provided, however in Appendix 1 of the 2011 MON 810 report the fact that ten farmers from the Czech Republic refused to participate is recorded.

## 3) Losses in sampling

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

## 4) Partial responses and reasons for non-completion

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

## 5) Characteristics of responder group and non-responder group

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

## 6) Characteristics of responder group compared with the target population

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

## 7) Non response bias

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

## **Instrument design**

### 1) Interviewer bias

The 2011 MON 810 survey used third parties to perform the interviews, with the exception of Romania, where Monsanto field representatives assisted the farmers to fill in the questionnaire. The use of third-party interviewers can prevent interviewer bias.

### 2) Interviewer training

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

### 3) Question type

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

### 4) Phrasing of questions

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

### 5) Independent and objective responses

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

### 6) Comparator

The questionnaire relies on a comparison between a representative GM maize field and a representative conventional field to in order to detect unanticipated adverse effects. Consequently, the choice of representative fields and the recollection of similarities and differences is crucial to the success of the survey. The report provides no indication about the comparator fields selected by the farmer for comparison in the survey, however Figure 3.3 of mean percent of maize MON 810 cultivation area of total maize area per farmer appears to indicate that on all farms some non-GM maize is grown that may be suitable as a comparator. It is recommended that the questionnaire contains questions to record whether the comparator field is growing on the same farm at the same growing season and the variety of the comparator. If no comparators are being grown spatially or temporally close to the GM crop, then the rationale for selecting another comparator (e.g. maize grown in previous years) should be fully described. The comparators selected by the farmers for the survey should be summarised in the report.

### 7) Validation of the instrument

The questionnaire was developed by the German Federal Biological Research Centre for Agriculture and Forestry and maize breeders and statisticians in Germany, and the results of the pilot of this questionnaire were published in 2004 (Wilhelm et al., 2004). The questionnaire was used in annual monitoring reports in the period 2006–2011. Any future amendments to the questions should be made giving consideration to pooled analysis of the results over 10 years.

## **Instrument validity**

### 1) Content validity

- Background data

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

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

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

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

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

- Compliance with good agricultural practice

Section 4 requests information on compliance with good agricultural practice, and in this case the planting of non-Bt refuge(s).

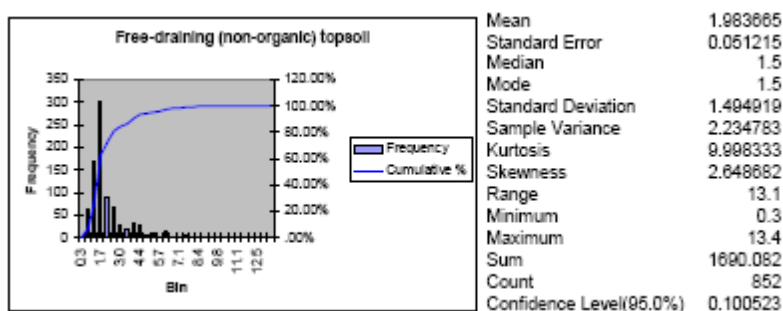
## 2) Criterion validity

The scientific opinion of the EFSA GMO Panel on the renewal of the authorisation for maize MON 810 commercialisation in the EU (EFSA GMO Panel, 2009) states that *'The information available in the renewal applications gives no reason to change the opinion that maize MON 810 is agronomically and phenotypically equivalent to currently grown non-GM maize varieties, with exception of the insect resistance conferred by the Cry1Ab protein.'* The 2005 scientific opinion for maize MON 863 × MON 810 × NK603 (EFSA GMO Panel, 2005) states *'Plants of the same field trials as for compositional analysis, except for a difference in glyphosate treatment (see section 3.2.2) were compared for their agronomic and phenotypic characteristics. These characteristics included seedling vigour, crop growth stages (for example, the stage at which silking and pollination occurred), height of the plant and ear (attachment containing the cob and kernels), root lodging (plants leaning to the surface), stalk lodging (plants with stalks broken below the ear), dropped ears, final stand count, stay-green and kernel yield. The plants tested showed no particular deviations in any of these parameters. In addition, plant damage due to insect feeding in two locations and due to weather in one location appeared to occur*

preferentially in plots planted with reference lines. Report MSL-18567 (Carringer et al., 2004) includes data on the agronomic parameters assessed in the above opinion. In the case of seedling vigour, both maize MON 810 and the reference varieties had ‘excellent’ vigour, with the exception of one site where one reference variety was classed as poor and one as average. Stalk lodging in plants near harvest was observed more frequently in the reference varieties, and at one site root lodging in plants close to harvest was observed more frequently in the reference varieties. In the case of the other agronomic parameters, there was no particular deviation between maize MON 810 and the reference varieties. Appendix 1 of the 2011 MON 810 report assessing the characteristics of maize MON 810 reported ‘unchanged germination, unchanged time to emergence, unchanged time to male flowering, unchanged plant growth and development, lower incidence of stalk/root lodging, delayed time to maturity, higher yield and lower occurrence of MON 810 volunteers.’ Comparing the field trial data with the farmer survey data provides an opportunity to check the validity of the farmers’ responses. It appears that there may be differences between field trial data and the questionnaire: there are a number of possible explanations for this, e.g. the conventional crops grown on the farms differ from the comparator variety used in the field trials, the information provided by the farmers is biased or erroneous or the GM crop is performing differently on farm-scale cultivation (possibly performing better when the cultivation conditions are less than optimal). It is of value to select parameters measured using a ‘gold standard’ methodology and to contrast these with the responses in the survey to ensure the validity of the reported responses.

### 3) External consistency

Comparison of the data reported in the survey with information from independent data sources provides a further opportunity to test the validity of the responses. The information on soil quality offers the opportunity to compare it with the information held in the Soil Profile Analytical Database for Europe (SPADE-2) (Hollis et al., 2006). Figure 1 shows the information on top soil organic carbon contained in this database. The MON 810 survey reports organic carbon content values between 0.58 and 7.27 with a mean of 2.2. 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 1:** Distribution and descriptive statistics of top soil organic carbon contents in SPADE-2 for free-draining non-organic soils.

### 4) Plausibility of responses

The sowing and harvest times were used to check the plausibility of the responses provided by the farmers: the sowing time ranged from 1 March 2011 to 30 June 2011 and the harvest time from 1 August 2011 to 31 December 2011.

### 5) Construct validity



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

### Data validation

#### 1) Validation procedures

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

#### 2) Exclusion of results

All completed questionnaires (249) were included in the analysis.

#### 3) Missing values

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

### Longitudinal aspects

#### 1) Sampling over multiple years

The repeated sampling of a sample unit needs to be considered in the sample size calculations and in the statistical analysis of the results. It is important that a mechanism for recording repeated sampling is introduced and the numbers of sample units repeatedly sampled are included in the report. If this information were available, it would allow an analysis considering the intensity of maize MON 810 cultivation and the possible changes in monitoring characteristic assessment as maize MON 810 cultivation is repeated in consecutive years.

### Statistical analysis

#### 1) Objective and hypotheses

Appendix 1 states 'The aim of the survey is to identify potential adverse effects that might be related to MON 810 plants and their cultivation. For that reason, most questions are formulated to get ordinary data, i.e. with three possible answers (Plus/ As usual/ Minus). The Plus- and Minus-answers indicate a deviation from the situation with conventional maize and are provided with a specification to describe the specific effect and its potential cause. High frequency (> 10 %) of Plus or Minus-answers would indicate possible effects.'

#### 2) Statistical analysis plan

Section 2.4 of Appendix 1 describes the statistical test procedure. The effect is specified as a 5 % increase from the baseline of 5 %, setting the threshold for responses that are not 'as usual' at 10 %. It would be expedient to provide scientific references to support the selection of the 10 % threshold. Additionally, for certain responses, 10 % may be greater than the acceptable limit of change. Alternative statistical analyses allowing the exploration of different effect sizes for certain monitoring characteristics would assist in the interpretation of the results. The null hypothesis is that the proportion of responses not 'as usual' is above 10 %. A significance level of 0.01 % was used in the statistical test. If  $p < 0.01$ , then the null hypothesis that the minus/plus response is greater than 10 % is rejected and therefore no effect can be identified.

### 3) Pre-defined subgroups

The analysis was performed for all fields surveyed in 2011. There was no analysis of country level data. Given the number of farmers surveyed in some countries, analyses of country-level subgroup may not have been statistically valid; however, consideration should be given to the fact that Member States may require country-level results. In addition, analysis of the assessment of monitoring characteristics by new farmers compared with farmers with previous experience of cultivation of maize MON 810 would be of interest. This could assist in detecting residual effects.

### 4) Statistical analysis

The reports states that plus responses and minus responses were 'statistically tested by using the exact binomial test'. This test is appropriate for the 'same/different' type of question. However, for questions of the 'as usual or worse or better' type, where there are three outcomes, an analysis using a multinomial test should be performed (in this case a trinomial test). Galyean and Wester (2010) used simulation methods to generate experimental count data from multinomial distributions in order to compare multinomial and binomial proportion methods for analysis. It was concluded that analysing multinomial data as a series of binomial proportions increased the experiment-wise type I error rate and recommended to use a multinomial analysis to test for the distributional difference with a subsequent binomial approach used to test for differences in a specific category or to correct for the multiplicity of testing.

### 5) Results presentation

For each monitoring characteristic measured by the survey, a table of the responses was provided with percentage and 'valid percentages' (the proportion of answers excluding missing values) plus a bar chart of the frequency of responses. The valid percentages were used in the binomial test. The reasoning between the valid percentages in the table of responses and the table of the results of the binomial test for different 'treatments/practices' should be further explained by the applicant in order to facilitate interpretation of the results.

### 6) Descriptive statistics

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

### 7) Multiplicity

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

#### 8) Handling missing values

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

#### 9) Confidence intervals

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

#### 10) Post-hoc analysis

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

### Report conclusions

#### 1) Report conclusions

Appendix 1 contains the following conclusions:

2011 data indicates that in comparison to conventional maize plants, maize MON 810 plants

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

#### 2) Study design

The study design was appropriate to evaluate whether a set of monitoring characteristics relating to plant performance and management practices for maize MON 810 cultivation in the current year of the survey differed from a comparator variety by a threshold of 10 %. However there are indications of weaknesses in the sampling methodology applied for the survey and as a consequence the possibility

of selection bias in the survey cannot be excluded. In addition the result of this assessment was very much dependent on the selection of an appropriate comparator.

Certain effects may reach a sufficient magnitude for detection only with repeated cultivation of a GM crop, and so amendments to study design and the analysis plan should be considered in order to assess the effect of multiple years of GM crop cultivation. Table 4.1 in Appendix 1 presents the results from the previous five years and the 2011 results. The inclusion of the pooled results would be of interest.

### 3) Substantiation of results

Sixty-two farmers (25 %) indicated that they had changed the application procedure for insecticides in the maize MON 810 field; these were farmers who usually used insecticides specifically to control corn borers. Sixty-one farmers (25 %) reported a reduction in stalk and root lodging in the maize MON 810 field compared with the conventional field. A reduction in stalk and root lodging was also observed in the field trial studies. Thirty-two farmers (13 %) reported delayed maturity. Forty-nine farmers (20 %) reported that the maize MON 810 field was less susceptible to diseases, with associated reports of reduced susceptibility to *Fusarium* spp. (36 farmers) and *Ustilago maydis* (24 farmers). The reports of reduced susceptibility to fungal infections were substantiated with similar findings from the scientific literature. Two hundred and fourteen farmers (86 %) and 166 farmers (valid percentage 86 %) reported that maize MON 810 provided 'very good' control of ECB and pink borer, respectively, and 44 farmers (18 %) reported maize MON 810 to be less susceptible to pests other than the borers. These results are to be expected, as the genetic modification provides protection from corn borers and therefore should result in a healthier crop. An increased yield was reported by 108 farmers (43 %); as maize MON 810 has less insect damage, an increased yield is not unexpected. Seventeen farmers (7 %) reported a lower occurrence of volunteers.

For the monitoring characteristics above, the report states that the effect was greater than 5 % plus the 5 % baseline, and the null hypothesis that an effect was evident could not be rejected. Presenting the results with confidence intervals would have facilitated the interpretation of the results and allowed the effect of the selection of alternative threshold values other than the arbitrarily selected 10 % to be explored.

The monitoring characteristics that were not 'as usual' described above were also observed in the 2009 and 2010 monitoring reports. In 2011 not 'as usual' responses above the 10 % threshold related to time of harvest, germination vigour and plant growth and development were not observed, however these may be dependent on the growing conditions in a particular year. The consistency of the results in each year of survey indicates the stability of the observed effects. Interpretation of the results should be viewed with caution as there are indications of weaknesses in the sampling methodology applied for the survey and as a consequence the possibility of selection bias in the survey cannot be excluded. The number of farmers cultivating maize MON 810 is increasing and in 2011 there were no regions where a census survey was performed as consequence it is becoming increasingly essential that an appropriate and consistent sampling methodology is used. The grouping of sample units according to the strata and random selection of sample units from within the strata should be performed using the specified sampling frame prior to conducting the interviews. A description of the method to ensure that units are randomly selected from the sampling frame should be included in the report, including where relevant the statistical software and/or the program code used for this procedure. The proportion of new farmers and farmers with previous experience of maize MON 810 selected from the sampling frame for each region should be presented in the report to provide evidence that the sampling method ensures that areas of intensive maize MON 810 cultivation are appropriately covered in the survey.

## Recommendations and Conclusions

From the data provided in the 2011 survey from the farmer questionnaire to monitor adverse effects associated with the cultivation of maize MON 810, no adverse effect can be identified. However the

2011 report provides limited information on the sampling methodology and the possibility of selection bias in the survey cannot be excluded. Therefore, the following improvements to the survey design and reporting are recommended:

- Full details on the source of the sampling frame, the number of farmers and the major characteristics (e.g. previous cultivation of maize MON 810) of the farmers should be included in the survey report. The national registers set by Member States for the cultivation of GM crops would be the optimum sampling frame, if available.
- A description of the method to ensure that units are randomly selected from the sampling frame should be included in the report, including where relevant the statistical software and/or the program code used for this procedure. The proportion of new farmers and farmers with previous experience of maize MON 810 selected from the sampling frame for each region should be presented in the monitoring report to provide evidence that the sampling method ensures that areas of intensive maize MON 810 cultivation are appropriately covered by the survey.
- The losses to sampling should be fully documented in the report to provide evidence that there is no non-response bias. It is important to know if a specific subgroup of farmers is not participating in the survey and therefore is not represented in the survey findings;
- It is recommended that independent trained interviewers are used to reduce interviewer bias.
- It is recommended that the farmer questionnaire contain questions to record whether the comparator field is growing on the same farm in the same growing season and the variety of the comparator. If no comparators are being grown spatially or temporally close to the GM crop, then the rationale for selecting another comparator (e.g. maize grown in previous years) should be fully described. The comparators selected by the farmers for the survey should be summarised in the monitoring report.
- Farmer questionnaires should focus only on changes that would be recognised by the farmer during the daily management of the farm. However, additional questions could be included to gain a better understanding of the intensity of GM maize cultivation on the farm (number of years of maize MON 810 cultivation and frequency of maize MON 810 in crop rotations), and further information on plant protection product usage (in particular, in the comparator field) should be obtained to facilitate a full understanding of any observed changes.
- Confidence intervals for the analysis of the monitoring characteristics should be included in the statistical report. Presenting the results with confidence intervals would have facilitated their interpretation and allowed the effect of the selection of alternative threshold values other than the arbitrarily selected 10 % to be explored. The choice of statistical test should be based on the number of possible outcomes, the use of a series of binomial tests for multinomial distributions would increase the experiment-wise type I error rate (i.e. failure to detect a true adverse effect).
- The statistical analysis should be planned to allow an analysis of the monitoring characteristics according to the length of GM crop cultivation in order to assess residual effects. As the statistical power of the study will be achieved only after 10 years, this will require a pooled analysis. Consequently, when conducting the survey, consideration should be given to the consistency of questions to assess monitoring characteristics, the inclusion of the same farmers in consecutive years in the survey (and the enumeration of these farmers in the report) and the interim analyses performed for the annual reports.

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