Annual post-market environmental monitoring report on the cultivation of genetically modified maize MON 810 in 2016

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Abstract

Following a request from the European Commission, EFSA assessed the annual post-market environmental monitoring (PMEM) report for the 2016 growing season of the Cry1Ab-expressing maize event MON 810 provided by Monsanto Europe S.A. Partial compliance with refuge requirements was reported in Spain, as observed in previous years. EFSA reiterates the need to achieve full compliance in areas of high maize MON 810 adoption to delay resistance evolution, and therefore advocates increasing the level of compliance in such areas. Resistance monitoring data do not indicate a decrease in susceptibility to the Cry1Ab protein in the field corn borer populations tested in the 2016 season. However, EFSA identified some methodological and reporting limitations pertaining to resistance monitoring that need improvement in future PMEM reports. No complaints related to corn borer infestation of maize MON 810 were received via the farmer alert system during the 2016 cultivation season. EFSA encourages the consent holder to provide more information on this complementary resistance monitoring tool. The data on general surveillance do not indicate any unanticipated adverse effects on human and animal health or the environment arising from the cultivation of maize MON 810. EFSA reiterates its recommendations on the methodology and analysis of farmer questionnaires, and considers that future literature searches on maize MON 810 performed in the context of annual PMEM reports should follow the guidelines given in the 2017 EFSA explanatory note on literature searching. Moreover, EFSA encourages relevant stakeholders to implement a methodological framework that enables the use of existing networks in the broader context of environmental monitoring. EFSA concludes that no new evidence has been reported in the 2016 PMEM report that would invalidate previous EFSA evaluations on the safety of maize MON 810.

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Summary

Following a request from the European Commission, the European Food Safety Authority (EFSA) assessed the annual post-market environmental monitoring (PMEM) report on the cultivation of the Cry1Ab-expressing maize event MON 810 during the 2016 growing season provided by Monsanto Europe S.A. This report presents the results of the 2016 insect resistance management and monitoring activities on maize MON 810 (hereafter referred to as case-specific monitoring), along with the results of general surveillance.

The 2016 case-specific monitoring data set on maize MON 810 consists of: a survey on compliance with refuge requirements in Spain and Portugal; diagnostic bioassays to monitor changes in susceptibility to the Cry1Ab protein in target pests (European and Mediterranean corn borer) collected from north-eastern Spain; and complaints about product performance collected through the farmer alert system.

The 2016 PMEM report shows partial compliance with refuge requirements in Spain, as observed in previous years. EFSA reiterates the need to achieve full compliance in areas of high maize MON 810 adoption to delay resistance evolution, and therefore advocates increasing the level of compliance in such areas.

The outcomes of the bioassays do not indicate a decrease in susceptibility to Cry1Ab in the target pests from the populations monitored in 2016. However, EFSA identified some methodological and reporting limitations pertaining to resistance monitoring that need improvement in future PMEM reports. In this respect, EFSA recommends the consent holder to provide additional evidence for the new diagnostic concentration selected for the Mediterranean corn borer. EFSA considers that the methodology of the diagnostic bioassays with both target pests should be harmonised and that separate diagnostic bioassays should be conducted with F_1-larvae from each sampling zone. In cases where moulting inhibition is lower than expected, standardised follow-up studies should be conducted with the suspected-resistant larvae to confirm and characterise Cry1Ab resistance alleles. EFSA recommends that the consent holder develops alternative testing methods to improve the sensitivity and precision of the current monitoring strategy. EFSA also provides a list of recommendations for reporting future resistance monitoring studies.

The consent holder has implemented a farmer alert system allowing farmers to report complaints about product performance (including unexpected field plant damage caused by target pests). No complaints related to corn borer infestation of maize MON 810 were received via the farmer alert system during the 2016 cultivation season. EFSA encourages the consent holder to provide more information on this complementary resistance monitoring tool to determine whether appropriate communication mechanisms and fit-for-purpose educational programmes are implemented that ensure the timely and effective reporting of farmer complaints.

The 2016 general surveillance data set on maize MON 810 consists of a survey based on 250 farmer questionnaires, and peer-reviewed publications relevant to the risk assessment and/or management of maize MON 810 (published between June 2016 and May 2017). The available data do not indicate any unanticipated adverse effects on human and animal health or the environment arising from the cultivation of maize MON 810.

No information collected from existing monitoring networks in the European Union was provided by the consent holder. EFSA encourages relevant stakeholders to implement a methodological framework that enables the use of existing networks in the broader context of environmental monitoring.

The farmer questionnaire and the approach followed to identify unanticipated adverse effects caused by the cultivation of maize MON 810 are similar to those in previous annual PMEM reports. EFSA therefore reiterates previous observations on the methodology and analysis of the farmer questionnaire survey. The 2016 PMEM report represents the 11th reporting year, resulting in the completion of a total of 2,877 questionnaires since 2006. The aimed for sample size of 2,500 questionnaires to achieve the desired statistical power has therefore been obtained. EFSA recommends the consent holder to pool the data obtained over this 11-year period and perform an appropriate analysis of the combined data sets.

EFSA advises that future literature searches on maize MON 810 performed in the context of annual PMEM reports follow the guidelines given in the 2017 EFSA explanatory note on literature searching.

An additional relevant publication (Camargo et al.,2018) published after the period covered by the literature search performed by the consent holder was identified by EFSA. The findings reported by Camargo et al. (2018) reinforce the need to implement a sensitive monitoring plan, as previously recommended by EFSA.

Based on the evidence provided in the 2016 PMEM report and in the additional relevant publication identified by EFSA, EFSA concludes that no new evidence has been reported that would invalidate previous GMO Panel evaluations on the safety of maize MON 810.

This scientific output has been endorsed by the Working Group on annual PMEM Reports.

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1. Introduction

Genetically modified (GM) maize MON 810 produces the insecticidal protein Cry1Ab from *Bacillus thuringiensis* (*Bt*), which confers resistance to certain lepidopteran pests, such as the European corn borer (ECB), *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), and the Mediterranean corn borer (MCB), *Sesamia nonagrioides* (Lefebvre) (Lepidoptera: Noctuidae).

The cultivation of maize MON 810 has been authorised under Directive 90/220/EEC in the European Union (EU) by the Commission Decision 98/294/EC of 22 April 1998.[1]

Since 2003, the transformation event MON 810 has been introduced into a wide range of maize varieties that have been grown in the EU. In 2016, maize MON 810 was cultivated in Spain (129,081 ha), Portugal (7,069 ha), Slovakia (138 ha) and the Czech Republic (75 ha) over a total area of 136,363 ha (ISAAA, 2016).[2]

According to the Commission Decision 98/294/EC of 22 April 1998 authorising the placing on the market of maize MON 810, Monsanto Europe S.A. (hereafter referred to as the consent holder) has defined an insect resistance management (IRM) plan to delay the evolution of resistance in target insect pests, and committed to inform the European Commission and/or the EU Member States of the results of monitoring of this aspect.

Since 2003, the harmonised IRM plan developed by EuropaBio[3] for single lepidopteran-active *Bt*-maize events (Alcalde et al., 2007) has been followed for the cultivation of maize MON 810. The implemented resistance management measures are based on the 'high-dose/refuge' strategy, which prescribes planting *Bt*-crops that produce a very high concentration of the insecticidal *Bt*-protein, so that nearly all target insect pests that are heterozygous for resistance do not survive on it (Gould, 1998; Tabashnik et al., 2013). In addition, a nearby structured refuge of the non-*Bt*-crop is required where the target insect pest does not encounter the *Bt*-protein.[4]

As part of the IRM plan, resistance and compliance monitoring is typically conducted to allow the periodic evaluation of the adequacy and efficacy of the IRM strategy. Resistance monitoring is designed to detect early warning signs indicating increases in tolerance of target pests in the field; a timely detection of such signs enables actions to limit the survival of resistant insects, and slow or prevent their spread should resistance have evolved among field populations. In the case of maize MON 810, the consent holder follows a two-pronged approach for resistance monitoring, consisting of: (1) monitoring for changes in susceptibility to the Cry1Ab protein in ECB/MCB in laboratory bioassays; and (2) monitoring of unexpected field damage caused by ECB/MCB through a farmer alert system. Ensuring compliance with refuge requirements is a critical factor contributing to the success of IRM plans in delaying the rate at which resistance evolves. Failure to fully comply with the refuge requirements and carry out the operational details of IRM plans is likely to have contributed to the field-selected resistance to certain *Bt*-crops (reviewed by Tabashnik et al., 2013; Tabashnik and Carrière, 2017). Education (training) and information programmes form an integral part of IRM plans, as they aid farmers to understand the importance of adhering to IRM requirements and are key to the success of the 'high-dose/refuge' strategy (Glaser and Matten, 2003; Bates et al., 2005; Andow, 2008; Head and Greenplate, 2012).

In 2005, the consent holder initiated, on a voluntary basis, a general surveillance monitoring program in anticipation of the mandatory requirement for post-market environmental monitoring (PMEM) in all applications or renewals for deliberate release submitted under Directive 2001/18/EC and Regulation (EC) No 1829/2003 (including the pending renewal of the maize MON 810 consent). This general surveillance aims at detecting unanticipated adverse effects associated with the commercial use of GM plants.

Since 2005, the results of the IRM and monitoring activities on the cultivation of maize MON 810 in the EU (hereafter referred to as case-specific monitoring which focuses on resistance and refuge compliance monitoring to allow the periodic evaluation of the adequacy and efficacy of the IRM

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[1] 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). OJ L 131, 5.5.1998, 32–33.

[2] At present, maize MON 810 is the only GM maize event cultivated in the EU. Maize *Bt*176, also producing the protein Cry1Ab, was cultivated in the EU between 1998 and 2005 (Ortego et al., 2009).

[3] http://www.europabio.org/ (Accessed 8 May 2018).

[4] The harmonised IRM plan establishes that farmers planting more than 5 ha of *Bt*-maize should plant a non-*Bt*-maize refuge within a distance of 750 m from the *Bt*-maize field and which corresponds to at least 20% of the surface planted with *Bt*-maize. The 5 ha threshold relates to the total area of *Bt*-maize, within or among fields, planted by one grower and is independent of the size of the individual fields or the total land area managed by this grower. Refuges can be located near, adjacent to or within *Bt*-maize fields; refuges within a *Bt*-maize field can be planted as a block, perimeter border or as strips, and they should be managed similarly as the *Bt*-maize field.

strategy), along with the results of general surveillance, have been reported to the European Commission and the EU Member States on an annual basis by the consent holder. These PMEM reports on maize MON 810 have been assessed by EFSA since 2010 (EFSA GMO Panel, 2011a, 2012a, 2013, 2014a, 2015a,b, 2016, 2017). From the data provided in the previous annual PMEM reports, the GMO Panel did not identify adverse effects on human and animal health and the environment resulting from the cultivation of maize MON 810. However, the GMO Panel noted shortcomings in the methodology for case-specific monitoring and general surveillance, and made several recommendations to improve future annual PMEM reports on maize MON 810.

1.1. Terms of Reference as provided by the requestor

On 3 October 2017, the European Commission received from the consent holder the annual PMEM report for the 2016 cultivation season of maize MON 810 (hereafter referred to as 2016 PMEM report). The reporting period of the 2016 PMEM report covers from July 2016 to July 2017.

On 16 November 2017, the European Commission mandated the GMO Panel 'to assess the 2016 PMEM report and, in particular, to evaluate the findings of the monitoring activities, taking into consideration the comments received from Member States and to assess the appropriateness of the methodology if this is found to differ compared to the previous season'.

2. Data and methodologies

2.1. Data

In delivering this statement, EFSA took into account the 2016 PMEM report,[5] additional information provided by the consent holder, scientific comments submitted by the EU Member States and relevant scientific publications.

2.2. Methodologies

EFSA carried out a scientific assessment on the information/data provided in the 2016 PMEM report, in accordance with Annex VII of Directive 2001/18/EC. Following the terms of reference of the mandate, EFSA also considered whether the methodology followed for the monitoring activities during the 2016 growing season differed from that followed in the previous PMEM reports on maize MON 810.

EFSA took into account the appropriate principles described in its guidelines for the PMEM of GM plants (EFSA GMO Panel, 2011b). EFSA also assessed the consent holder's systematic literature search in accordance with the principles for literature searching laid down in EFSA (2010, 2017).

The comments raised by the EU Member States are addressed in Annex A of this statement, and were taking into consideration during the scientific assessment.

3. Assessment

3.1. Case-specific monitoring

3.1.1. Implementation of non-*Bt*-maize refuges[6]

3.1.1.1. Consent holder's assessment

Compliance with refuge requirements was assessed through the farmer questionnaires supplied as part of the general surveillance (Section 3.2.1). In 2016, 237 farmers from Spain and 13 farmers from Portugal completed a questionnaire which included a question on compliance with the refuge strategy, i.e. *did you plant a refuge in accordance to the technical guidelines?*

Spain

In Spain, 217 out of the 237 farmers growing maize MON 810 stated that they complied with refuge requirements. Fifty-three of those farmers (22% of the farmers surveyed) planted less than 5 ha of maize MON 810 and were therefore not required to plant a refuge (Appendix A).

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[5] The 2016 PMEM report is publicly available at https://ec.europa.eu/food/sites/food/files/plant/docs/gmo_rep_stud_mon-810_re port-2016.pdf (Accessed 8 May 2018)
[6] 2016 PMEM report: Section 3.2.1.1.

The 20 farmers who did not plant a refuge but cultivated an area of maize MON 810 of more than 5 ha provided the following reasons for their non-compliance (as indicated in the survey): (1) she/he had no or not enough information about the technical guidelines and was concerned about yield losses in conventional maize (ten farmers); (2) refuges from neighbours were considered sufficient or the refuge was smaller than 20% of maize MON 810 area (seven farmers); and (3) planting refuges complicates sowing (three farmers).

The exact location of the *Bt*-maize fields where no refuges were planted was not provided.

Portugal

In Portugal, the 13 maize MON 810-growing farmers surveyed complied with the refuge requirements (none of them were exempted since the maize MON 810 area was more than 5 ha). In addition to the farmer questionnaires, the Portuguese authorities performed inspections on 79 farms (out of the 242 notifications received in 2016) where maize MON 810 was grown to check compliance with refuge and coexistence requirements outlined in Portuguese law. Based on these inspections, the Portuguese authorities concluded that there was full compliance with refuge requirements.

Based on the above-mentioned information, the consent holder concluded that 'the results from the presented surveys (…) during the 2016 season are consistent and do show a high level of compliance'.

3.1.1.2. EFSA's assessment

The 2016 PMEM report shows partial compliance (89%) with refuge requirements in Spain and full compliance in Portugal, as observed in previous years (Appendix A). EFSA considers that full compliance should be achieved in high adoption areas, and reiterates that the consent holder should strive to increase the level of compliance in those areas, because, as indicated in several studies (e.g. Tabashnik et al., 2013; Castañera et al.,2016), refuge compliance is crucial to sustain the efficiency of the technology and delay resistance evolution, especially in regions of high adoption rate.

EFSA reiterates that refuge requirements should also apply to clusters of small maize MON 810 fields (i.e. a group of adjacent fields that can be from different farms) in which the aggregate area planted with maize MON 810 is greater than 5 ha, irrespective of individual field and farm size (EFSA, 2009). EFSA acknowledges that the implementation of this recommendation may entail practical challenges (e.g. identification of clustered *Bt*-maize fields prior to planting and of those farmers that will be responsible for planting the refuge area). However, based on the level of non–compliance (11%), the proportion of farmers planting less than 5 ha of maize MON 810 (22%), and the recent findings reported by Camargo et al. (2018) on the frequency of Cry1Ab resistance alleles in MCB populations in the Ebro Valley (see Section 3.2.3.3 for further details), it is essential to plant sufficient refuges in areas where the adoption rate of maize MON 810 is high, and thus to ensure full compliance with refuge requirements in such areas, regardless of the size of individual fields. In this context, EFSA recommends that the consent holder and the EU Member States develop appropriate information systems on GM crop cultivation to ensure that structured refuges (i.e. blocks or strips of non-*Bt*-maize that are located within or adjacent to the *Bt*-maize field) are planted in these clustered areas.

3.1.2. Insect resistance monitoring[7]

3.1.2.1. Consent holder's assessment

The IRM plan has been revised with regard to the sampling strategy and monitoring protocol of ECB and MCB populations, accounting for some of the previously made EFSA recommendations, the experience gained with the implementation of the initial IRM plan, and relevant scientific publications (EuropaBio, 2017). The major revisions of the plan are as follows:

- ECB and MCB populations will be monitored in those geographic areas[8] where adoption rate of *Bt*-maize hybrids is over 60% of the total maize acreage. In those areas, multivoltine[9] populations will be monitored annually;
- Approximately 1,000 larvae will be targeted for collection in each area to reach a detection level of 3% (recessive) resistance allele frequency in the target pest populations;

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[7] 2016 PMEM report: Section 3.2.1.2 and Appendix 6.
[8] A geographical area is defined as a geographical zone where maize is typically grown following similar agronomic practices isolated from other maize areas by barriers that might impair an easy exchange of target pests between those areas.
[9] A multivoltine population is a population completing several generations in a year.

- Susceptibility of target pests to the *Bt*-protein will be assessed by performing diagnostic bioassays with F_1-progeny larvae from field-collected individuals and, alternatively, by F_2-screening.

In the 2016 growing season, resistance monitoring focused on the Ebro Valley (north-eastern Spain), due to the high concentration of maize MON 810 cultivation, and the susceptibility of ECB and MCB populations to maize Cry1Ab was tested in diagnostic bioassays.

European corn borer monitoring[10]

a) Field sampling and laboratory rearing

In 2016, 1,111 ECB late-instars from the last generation were collected at the end of the maize growing season from nine sampling sites (refuges and non-*Bt*-maize fields) located in three zones across the Ebro Valley (for more details, see Appendix C). Twenty additional sites were sampled, but the minimum number of larvae established in the IRM plan could not be reached. Field-collected larvae were shipped to the laboratory (BTL GmbH, Sagerheide, Germany), where ECB resistance was evaluated. Larvae were reared following a standardised protocol (Thieme et al., 2017). A total of 554 larvae reached the adult stage (50% of the field-collected larvae). Emerging adults from the different sampling sites and zones were pooled and placed in 30 oviposition cages for mating. All cages were used to obtain F_1-progeny for the diagnostic bioassay.

In addition, two reference susceptible strains, established from egg masses collected from Niedernberg (Germany) in 2005 and from 145 larvae collected from Galicia (Spain) in 2015 were used. Both strains have been reared in the laboratory ever since on non *Bt*-diet, i.e. without any exposure to maize MON 810 or Cry1Ab.

b) Monitoring assays

The consent holder performed: (1) a diagnostic bioassay with the field populations to detect potential increases in resistance allele frequency; (2) a follow-up study to the diagnostic bioassay with exposure to maize MON 810 leaves; and (3) concentration-response assays with the susceptible strains.

Diagnostic bioassay: The bioassay was conducted using neonates obtained from the progeny of the field-collected insects, i.e. F_1-larvae. Purified Cry1Ab protein at a diagnostic concentration of 28.22 ng Cry1Ab/cm^2 of diet surface area was used in an artificial diet-overlay assay.[11] The selected concentration corresponds to the 99% moulting inhibition concentration (MIC_{99}) estimated with data pooled from ECB populations collected in the Czech Republic, France, Germany, Hungary, Italy, Poland, Portugal, Romania and Spain between 2005 and 2012.[12] This diagnostic concentration was validated by testing several ECB populations collected in Spain in 2013, 2014 and 2015 (EFSA GMO Panel, 2015a, 2016, 2017).

In the 2016 bioassay, 1,562 neonates were tested against the diagnostic concentration. Larvae (N = 223) treated with the same buffer solution used to dissolve the Cry1Ab protein (i.e. 50 mM bicarbonate buffer, pH 10.25) were used as a negative control. Moulting inhibition, corresponding to dead larvae and larvae not reaching the second instar, was determined after seven days. None of the reference strains were included in the diagnostic bioassay.

Detailed results of the diagnostic bioassay are given in Table 1. Moulting inhibition of ECB larvae tested against Cry1Ab was 99.23%, whereas moulting inhibition in the control group was 0.45%. The study authors indicated that 'evidence for a decrease of Cry1Ab susceptibility of ECB during the monitoring duration could not be detected'.

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[10] 2016 PMEM report: Appendix 8 and additional information: 16/2/2018.
[11] Batch 2b was used: 1.64 mg Cry1Ab/ml in 50 mM bicarbonate buffer; pH 10.25; 91% purity.
[12] The 99% moulting inhibition concentration (MIC_{99}) of these populations corresponded to 48.2 (42.8–55.1) ng Cry1Ab/cm^2 of diet surface area. Due to a change in the protein batch in 2012, the diagnostic concentration was re-calibrated, resulting in a MIC_{99} value of 28.22 ng Cry1Ab/cm^2 of diet surface area.

Table 1: Moulting inhibition (%) of *Ostrinia nubilalis* (ECB) tested with a diagnostic concentration of Cry1Ab protein: 2016 field population [Table created from data provided in the 2016 PMEM report]

| Sampling area | Treatment moulting inhibition % (No. of larvae tested) | |
	Control	Cry1Ab[b]
North-eastern Spain[a]	0.45[c] (223)	99.23[d] (1,562)

(a): Emerging adults from the different sampling zones were pooled for mating and a single bioassay was performed with the their progeny.

(b): A diagnostic concentration of 28.22 ng Cry1Ab/cm^2 of diet surface area was used.

(c): Of the 223 larvae tested, one larva died, whereas the rest moulted to other instars.

(d): Of the 1,562 larvae tested, three larvae died, 1,547 larvae survived but did not moult to the second instar, and 12 larvae moulted.

Follow-up study with maize MON 810 leaves: A follow-up study using maize MON 810 leaves (variety not reported) was conducted to confirm that the 12 larvae that reached the second instar in the diagnostic bioassays were not potentially resistant to Cry1Ab. Larvae were placed individually on maize MON 810 leaf discs and mortality was assessed after five days of exposure. The negative control group consisted of neonates from the Spanish reference strain fed non-*Bt*-maize leaves (cv. Golden Bantam) for three days.

All ECB larvae fed maize MON 810 leaves died within five days. From the 192 larvae fed non-GM maize leaves (i.e. negative control group), two larvae died (1.0%), 157 larvae reached the second instar (81.8%) and 33 larvae did not reach the second instar, though were classified as healthy (17.2%).

Concentration–response assays: The susceptibility of the two reference strains to Cry1Ab was assessed in concentration-response assays. For each assay, eight concentrations, ranging from 0.2 to 28.22 ng Cry1Ab/cm^2 of diet surface area,[12] and a negative control (the same buffer solution in which the purified Cry1Ab protein was dissolved) were tested. For each concentration, 32 neonates were used (64 for the controls). Moulting inhibition was assessed after seven days of exposure. MIC_{50} and MIC_{90} values, with a 95% confidence interval (CI), were estimated by probit analysis (Robertson et al., 2007).

The historical results of the concentration assays with both reference strains are given in Appendix D. MIC_{50} and MIC_{90} values estimated in 2016 were higher than those obtained in previous years.

No raw data from the bioassays conducted with ECB were provided as part of the 2016 PMEM report.

Mediterranean corn borer monitoring[13]

a) Field sampling and laboratory rearing

In 2016, 1,364 MCB late-instars from the last generation were collected at the end of the maize growing season from nine sampling sites (refuges and non-*Bt*-maize fields) in three zones across the Ebro Valley (for more details, see Appendix B). Attempts were made to collect larvae from thirteen additional sites, but the minimum number of larvae established in the IRM plan could not be reached. Collected larvae were brought to the laboratory (Centro de Investigaciones Biológicas, Madrid, Spain), where MCB resistance was evaluated. Larvae were reared following a standardised protocol (González-Núñez et al., 2000; Farinós et al., 2004). A total of 960 larvae reached the adult stage (70% of the field-collected larvae) and were placed in 90 oviposition cages for mating. Emerging adults from the different sampling zones were kept separately. Eighty-five cages, containing 911 adults were used to obtain F1-progeny for the diagnostic bioassay.

In addition, a reference susceptible strain established from approximately 3,000 larvae collected from Spain in 1998 was used. The strain is reared in the laboratory on non-*Bt* diet and has been refreshed periodically with the addition of new field-collected individuals.[14]

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[13] 2016 PMEM report: Appendix 7 and additional information: 16/2/2018.

[14] The reference strain was established from larvae collected in Andalucía (661 larvae), Madrid (793 larvae), the Ebro Valley (857 larvae) and Galicia (665 larvae) (Spain) in 1998 (González-Núñez et al., 2000). Every time the strain was refreshed, between 10% and 15% of new field-collected individuals, with respect to the reference strain, were introduced. The similarity in susceptibility to Cry1Ab was verified before the introduction of the new individuals.

b) Monitoring assays

The consent holder performed: (1) a diagnostic bioassay with the field populations to detect potential increases in resistance allele frequency; (2) a follow-up study to the diagnostic bioassay with maize MON 810 leaves; and (3) concentration-response assays with the reference strain.

Diagnostic bioassays: Independent diagnostic bioassays were performed with F_1-MCB larvae from the three sampling zones collected in 2016. Progeny of the field-collected larvae were tested to purified Cry1Ab protein at a diagnostic concentration of 1,091 ng Cry1Ab/cm^2 of diet surface area in an artificial-diet overlay assay.[15] The selected diagnostic concentration corresponds to the upper limit of 95% confidence interval of the MIC_{99} estimated with data pooled from MCB populations collected in fields from north-eastern Spain over 2009, 2011, 2013 and 2015. The laboratory reference strain was also tested against the diagnostic concentration.

In the 2016 assays, between 1,004 and 1,202 neonates were tested against the diagnostic concentration. Larvae treated with the same buffer solution used to dissolve the purified Cry1Ab protein served as a negative control (i.e. 50 mM bicarbonate buffer, pH 10.25). Moult inhibition was recorded after seven days.

The results of the diagnostic bioassays are provided in Table 2. For all three zones, moulting inhibition was lower than the expected 99%, whereas in the control treatments it ranged between 2.09% and 5.63%. Moult inhibition observed in the laboratory reference strain was 99.23%.

The average percentage of moulting inhibition obtained in the diagnostic bioassays for the three regions was statistically compared to the expected value of 99% and the percentage of moulting inhibition observed in the reference strain, using a one-sample t-test and a one-tailed probability distribution. No statistically significant differences were observed for any of the two comparisons. The study authors indicated that 'the moulting inhibition caused to F_1 neonates of *S. nonagrioides* from larvae collected in the Ebro Valley in 2016 after treatment at a diagnostic concentration was not significantly lower than the expected value of 99%. Thus, no decrease in the susceptibility of *S. nonagrioides* to the Cry1Ab protein has been observed'.

Table 2: Moulting inhibition (%) of *Sesamia nonagrioides* (MCB) populations tested with a diagnostic concentration of the Cry1Ab protein: 2016 field populations [Table created from data provided in the 2016 PMEM report]

Sampling area		Treatment moulting inhibition % (No. of larvae tested)	
		Control	**Cry1Ab[(a)]**
North-eastern Spain	Zone 1	5.63 (160)	98.86 (1,024)
	Zone 2	2.09 (191)	98.47 (1,004)
	Zone 3	3.17 (221)	96.56 (1,202)
	Total	3.50 ± 1.71[(b)] (572)	97.96 ± 0.71[(b)] (3,230)
Reference susceptible strain		4.69 (192)	99.23 (783)

No significantly differences were observed between the north–eastern population and the expected value of 99% (t = −1.459; df = 2; p = 0.141).
No significant differences were observed between the north–eastern population and the reference susceptible strain (t = −1.740; df = 2; p = 0.112).
(a): A diagnostic concentration of 1,091 ng Cry1Ab/cm^2 of diet surface area was used. Values correspond to corrected moulting inhibition, calculated using Abbot's formula (Abbot, 1925).
(b): Mean ± standard error.

Follow-up study with maize MON 810 leaves: A follow-up study using maize MON 810 leaves (variety not reported) was conducted to confirm that the larvae surviving the diagnostic bioassays were not potentially resistant to Cry1Ab. In addition, more than 10,000 F_1-first instars not used in the diagnostic bioassays were fed maize MON 810 leaves. Groups of 200–300 larvae were placed in plastic boxes containing leaves of maize MON 810 (variety not reported). Larvae were allowed to feed *ad libitum* for 10 days and survival was then assessed. No negative control group (i.e. larvae fed non-*Bt* maize leaves) was included in the study.

None of the MCB larvae fed maize MON 810 leaves survived.

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[15] Batch B2-3 was used: 1.8 mg Cry1Ab/ml in 50 mM sodium bicarbonate buffer; pH 10.25; purity 91%.

Concentration–response assays: Concentration–response assays were performed with the MCB reference strain. Seven concentrations, ranging from 1 to 128 ng Cry1Ab/cm^2 of diet surface area,[16] and a negative control (i.e. the same buffer solution in which the purified Cry1Ab protein was dissolved) were tested.

In all bioassays, three replicates were used for each concentration and the control, each one consisting of 32 larvae (64 for the controls), giving a total of 96 larvae tested for each concentration (192 for the controls). Mortality and development was assessed after seven days of exposure. MIC_{50} and MIC_{90} values, with a 95% CI, were estimated by probit analysis. Both MIC_{50} and MIC_{90} values estimated in 2016 fell within the range of those estimated in previous years. The historical results of the concentration assays with the reference strain are given in Appendix D.

No raw data from the bioassays conducted with MCB were provided as part of the 2016 PMEM report.

Farmer alert system

The consent holder and other companies marketing maize MON 810 seeds have implemented a farmer alert system allowing farmers to report complaints about product performance (including unexpected crop damage caused by target pests). The consent holder stated that, during the 2016 cultivation season, no complaints related to corn borer infestation of maize MON 810 were received. The consent holder also referred to a survey conducted by member companies of the National Breeder Association in Spain[16] marketing maize MON 810.

None of the 1,556 complaints received in 2016 were attributed to loss of efficacy of maize MON 810.

3.1.2.2. EFSA's assessment

European and Mediterranean corn borer monitoring

   a) Field sampling and laboratory rearing

According to the revised IRM plan that has been implemented since the 2016 growing season, the consent holder monitored ECB and MCB populations annually in those geographic areas where adoption rate of *Bt*-maize hybrids is over 60% of the total maize acreage, and where target pest populations are multivoltine. A total of 1,111 ECB and 1,364 MCB larvae were collected to reach a detection level of 3% (recessive) resistance allele frequency in the target pest populations, which is in line with previous recommendations made by EFSA (2015) and its GMO Panel (2016, 2017).

EFSA considers that insect sampling should focus on those areas where deployment of *Bt*-maize is the highest and where resistance is likely to evolve more quickly, i.e. those areas with the highest selection pressure. Currently, the only hotspot in the EU is located in the Ebro Valley, north-eastern Spain, where more than 60% of the total maize acreage corresponds to maize MON 810 hybrids (Appendix B; Castañera et al., 2016; Farinós et al., 2017) and ECB and MCB populations complete two generations annually (Alfaro, 1972) and are therefore considered multivoltine.

Based on the outcome of simulations using the resistance evolution model developed by Alstad and Andow (1995),[17] and given the past and current maize MON 810 adoption rates, EFSA recommends: (1) annual sampling of target pests in the Ebro Valley; and (2) setting a maximum detection threshold for resistance allele frequency at 3% to enable the early detection of resistance so that alternative management measures can be implemented in time to delay the development of resistance.

EFSA notes that the numbers of ECB and MCB larvae collected from refuges and non-*Bt*-maize fields in the 2016 growing season (i.e. 1,111 ECB and 1,364 MCB larvae) are higher compared to previous seasons. EFSA welcomes the efforts made by the consent holder to achieve the 3% threshold. Yet, EFSA recognises that it might not always be possible to collect the target number of individuals due to several factors such as natural fluctuation in pest density, environmental conditions, and regional pest suppression (Dively et al., 2018). However, EFSA recommends that sampling efforts are maintained in future seasons, and that as many field-collected larvae as possible are used in the laboratory assays as F_1-larvae to provide sufficient detection sensitivity.

   b) Monitoring assays

Diagnostic bioassays: According to the revised IRM plan, susceptibility of target pests to the *Bt*-protein is assessed by conducting diagnostic bioassays with F_1-progeny larvae from field-collected

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[16] Asociación Nacional de Obtentores Vegetales (ANOVE): http://web.anove.es/ (Accessed 8 May 2018).

[17] Using the shareware software Populus, Version 5.4. Copyright © 2007 DN Alstad, University of Minnesota, available at http://wwumnw.cbs.edu/populus (Accessed 8 May 2018).

individuals. Consequently, resistance is now mainly tested in diagnostic bioassays, without relying on concentration-response bioassays.[18] Although both testing methods present advantages and limitations (summarised by Siegfried and Spencer, 2012), diagnostic bioassays are considered more efficient for detecting low frequencies of resistance (Roush and Miller, 1986; ffrench-Constant and Roush, 1990), require fewer larvae to test, and are less resource intensive (Halliday and Burnham, 1990) than concentration-response bioassays, which are insensitive to small changes in resistance allele frequency, particularly when resistance is first appearing (Halliday and Burnham, 1990). The majority of the current monitoring programmes for *Bt* crops have depended on two traditional methods, the concentration–response and diagnostic bioassays (Huang, 2006). For instance, diagnostic bioassays are an integral part of the resistance monitoring approach followed in the US and in the Philippines for lepidopteran pests as a mean to distinguish susceptible and potentially resistant individuals, but are typically combined with concentration–response bioassays to ensure that the microbial-derived *Bt*-proteins used in the bioassays are biologically active once baseline susceptibility to the *Bt*-protein is established among pest populations (Siegfried et al., 2007). However, in the case of the harmonised IRM plan in the EU, this information is obtained through the susceptible laboratory strains, which also serve as additional comparators in the diagnostic bioassays.

Taking all these aspects together, EFSA agrees with the principles driving the revision proposed by the consent holder. However, EFSA has some reservations on the actual implementation and would like to make some considerations regarding the selection of the diagnostic concentrations, the design of the diagnostic bioassays, and follow-up studies performed with suspected-resistant individuals. Also, EFSA encourages the consent holder to continuously improve the IRM plan and consider alternative testing methods.

EFSA notes that the consent holder followed a different methodology for the diagnostic bioassays conducted with ECB and MCB. Whereas ECB individuals from the different sampling zones were pooled and a single diagnostic bioassay was conducted with F_1-larvae, MCB larvae from each zone were kept separate and independent bioassays were conducted. Moreover, for MCB the bioassays included a reference strain that served as a negative control and additional point of comparison. This additional point of comparison was not reported for ECB.

EFSA advocates the harmonisation of the methodology of the diagnostic bioassays used for both target pests. EFSA favours the approach followed for MCB and thus recommends that separate bioassays are conducted with F_1-larvae from each sampling zone. In the case of suspected resistance of larvae from a particular zone, this would allow additional collections obtained from the same zone in the following seasons. EFSA also recommends the consent holder to include a reference susceptible strain in future diagnostic bioassays with ECB.

Diagnostic bioassays resulting in moulting inhibition lower than the expected > 99% should be regarded as statistically (although not necessarily biologically) significant, and trigger follow-up studies with the suspected-resistant larvae to confirm and characterise Cry1Ab-resistant alleles, i.e. to determine whether there was heritable resistance among survivors of the diagnostic concentration; in order to quantify the magnitude of resistance; and to determine the level of survival on maize MON 810 plants (Siegfried et al., 2007).

Selection of diagnostic concentrations: The concentration chosen for diagnostic bioassays should be able to discriminate between susceptible and resistant individuals (homozygous individuals in case of recessive resistance) and needs to be selected with care to minimise the likelihood of false positives while maximising the probability of detecting resistant individuals (Rust et al., 2005). To reliably estimate a diagnostic concentration, baseline susceptibility data from field populations should be collected before the large-scale cultivation of *Bt*-crops (Marçon et al., 2000), and data sets from several populations should be combined to increase sample size and minimise CIs (i.e. reduce variability) (Robertson et al., 2007). Initially, one or several candidate concentrations are estimated, which are then tested on several field populations in so-called validation assays. A candidate concentration causing between 99% and 100% of the biological response in the validation assays is finally selected and used in subsequent bioassays for routine resistance monitoring. Examples on the rationale and the process for selecting a diagnostic concentration can be found in Marçon et al. (2000), Alcantara et al. (2011), Siegfried and Hellmich (2012) and Bernardi et al. (2014).

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[18] Concentration-response bioassays were conducted with ECB and MCB populations collected in north-eastern, central and south-western Spain between 2004 and 2015. The analyses of the data sets did not indicate a decrease in susceptibility to the Cry1Ab protein in the ECB and MCB populations tested. The results of the bioassays with the populations collected in north-eastern Spain are shown in Appendix E.

In the 2016 PMEM report, the concentrations selected for the diagnostic bioassays with ECB and MCB were established using data from populations collected over several growing seasons. However, EFSA notes that a different approach was followed for both target pests (see Section 3.1.2.1). The consent holder explained that 'there are a variety of formulae (...) to calculate diagnostic concentrations' and that 'the minor differences in how the diagnostic concentrations were calculated will have not impacted the validity of these measures and reflect the personal preferences of the principal investigators'.

Validation assays were conducted with the ECB and MCB populations collected between 2013 and 2015 growing seasons against the candidate concentrations, and are summarised in Table 3. Based on the results of these bioassays, the candidate concentration selected for ECB was considered appropriate to be used in further diagnostic bioassays. In contrast, the consent holder considered that the candidate concentration selected for MCB needed to be refined because 'the mortality obtained was significantly lower than the expected 99% in one of the four area-year combinations tested'. Because sampling has now focused in the Ebro Valley, the consent holder considered that the new concentration should be estimated with data from populations collected in that area over 2009, 2011, 2013 and 2015, using the 95% upper limit of the estimated MIC_{99}, which corresponds to 1,091 ng $Cry1Ab/cm^2$ of diet surface area. The consent holder, however, did not explain why data from populations collected in north–eastern Spain in 2004 and 2005 were not included when recalculating the diagnostic concentration.

Table 3: Validation assays using candidate diagnostic concentrations against *Sesamia nonagrioides* (MCB) and *Ostrinia nubilalis* (ECB) [Table created from data provided in the annual PMEM reports]

Species	Growing season	Region (Spain)	% Moult inhibition (Mean $\pm$ SE)[c]
ECB[a]	2013	North-east	100
		Central	100
	2014	South-west	100
	2015	North-east	100
MCB[b]	2013	North-east	97 ± 2
	2014	Central	96 ± 1
		South-west	96 ± 2
	2015	North-east	100

SE: standard error.

(a): The concentration tested was 28.22 ng $Cry1Ab/cm^2$ of diet surface area. It corresponds to the 99% moulting inhibition concentration (MIC_{99}) estimated with data pooled from ECB populations collected in the Czech Republic, France, Germany, Hungary, Italy, Poland, Portugal, Romania and Spain between 2005 and 2012.

(b): The concentration tested was 726 ng $Cry1Ab/cm^2$ of diet surface area. It corresponds to the MIC_{99} estimated with data pooled from populations collected in north-eastern, central and south-western Iberia between 2008 and 2012.

(c): For both target pests, progeny of the field-collected larvae were used in the bioassays. For ECB, 32 neonates were tested. For MCB, three replicates consisting of 32 larvae each were used.

EFSA notes that the diagnostic concentrations for ECB and MCB derive from data that included populations which were already exposed to *Bt*-maize hybrids and thus subjected to selection pressure. Moreover, the recalculated concentration for MCB only derives from larvae collected from the Ebro Valley over recent years that were subject to very high selection pressure. Since this new concentration has not been previously tested and validated, the consent holder should confirm the validity of this concentration by comparing it with the upper limit of the 95% confidence interval of the MIC_{99} estimated from previously performed concentration-response bioassays with MCB larvae collected from areas of low selection pressure.

Follow-up studies: Follow-up studies using maize MON 810 leaves were performed with those ECB and MCB larvae that survived the diagnostic concentration and with spare MCB larvae that were not used in the bioassays 'to confirm that resistant individuals were not present in the field-collected populations'.

EFSA notes that the study with MCB did not include a negative control, as recommended by the GMO Panel (EFSA GMO Panel, 2017). The inclusion of a suitable negative control is a key element in the design of laboratory studies and adds certainty to the suitability of the test system and increases the reliability of the obtained results (Romeis et al., 2011). Therefore, these confirmatory tests cannot be used to reinforce the results of the diagnostic bioassays. EFSA also identified some methodological differences between the follow-up studies conducted with the two species (e.g. experimental arenas, test duration).

EFSA recommends that the consent holder standardises the testing method for confirming resistance. When doing so, the consent holder should follow a stepwise approach and consider the following procedures: (1) rearing survivors on non-*Bt*-diet and re-testing subsequent generations against diagnostic concentration; (2) performing studies with additional generations with maize MON 810 leaf discs; (3) continued selection of survivors at the diagnostic concentration; and (4) testing on whorl stage *Bt*-maize plants. Examples of follow-up studies with suspected-resistant individuals can be found in Siegfried et al. (2007), Alcantara et al. (2011), Siegfried and Hellmich (2012) and BPPD (2016).

To increase the reliability of follow-up studies with plant material, EFSA encourages that the consent holder implements the following recommendations: (1) using a suitable negative control (e.g. near-isogenic maize variety); (2) using larvae in the control group from the same population as the one tested on *Bt*-maize; (3) confirming the expression of Cry1Ab protein in the *Bt*-maize leaves used (e.g. by using commercial test strips, see Camargo et al., 2018); (4) duration of the exposure should be the same for the treatment (maize MON 810) and control groups to allow for a proper comparison.

Alternative testing methods – F_2-screen: EFSA recommends the consent holder to develop alternative testing methods to improve the sensitivity and precision of the current monitoring strategy. An alternative approach to diagnostic bioassays is the F_2-screen (Andow and Alstad, 1998). Unlike diagnostic bioassays, the F_2-screen can potentially detect recessive-resistant alleles in a heterozygous state by: (1) establishing single-female family lines from field-collected individuals; (2) inbreeding the offspring of the family lines; (3) screening the susceptibility of the F_2-offspring to the *Bt*-protein using a discriminating concentration or *Bt*-maize plants/leaves; and (4) estimating the frequency of the resistance allele in the sampled population by back-calculating the frequency of family lines containing a resistant allele. The F_2-screen mainly has been used to estimate the initial frequency of resistance alleles when establishing baseline susceptibility data. In Europe, F_2-screen has been used to estimate the upper 95% confidence interval for Cry1Ab-resistant allele frequencies in several ECB (Bourguet et al., 2003; Engels et al., 2010) and MCB populations (Andreadis et al., 2007) as part of baseline susceptibility data. EFSA is aware that this method is resource intensive (Andow and Alstad, 1998; Huang et al., 2012), presents some technical limitations (Siegfried et al., 2007; Siegfried and Spencer, 2012) and has only been implemented routinely in the resistance management plan for *Bt*-cotton in Australia (Downes and Mahon, 2012; Downes et al., 2016). Still, EFSA is of the opinion that the F_2-screen could be performed periodically with ECB and MCB populations to confirm the results of the diagnostic bioassays; to validate one of the key assumptions of the 'high dose/refuge' strategy (i.e. frequency of resistant alleles is $< 10^{-3}$); and to revise the predictions of resistance evolution models.

Reference susceptible strains: EFSA recommends that, when needed (to avoid inbreeding), reference strains are refreshed with individuals that have not been exposed to Cry1Ab or that are collected from areas where the adoption rate of maize MON 810 is low.

Reporting of monitoring data: EFSA considers that the reporting of the resistance monitoring assays should be improved to facilitate their quality assessment (i.e. methodological quality). EFSA developed a list of recommendations that aim at improving the reporting of future resistance monitoring assays. The recommendations are presented as a checklist in Appendix G of this statement, and study authors should consider them when preparing the reports of resistance monitoring assays. The checklist focuses on several elements relevant for the evaluation of study design and interpretation of results. Study authors are also encouraged to provide a rationale whenever a reporting recommendation cannot be met.

Although EFSA requested to provide raw data of the different bioassays conducted with both target pests, the consent holder did not follow up on this request, arguing that maize MON 810 is 'a GM product approved and cultivated worldwide for over a decade with no indications of evolving resistance for ECB and MCB' and that 'providing raw data for specific bioassays is not considered necessary for the overall assessment of the report'. EFSA considers that raw data are necessary to further evaluate and verify data quality. Such recommendation is in line with the obligation to provide the raw data of the studies provided in the environmental risk assessment of deliberate releases into the environment of GMOs under Directive 2001/18/EC, as amended by Directive 2018/350,[19] and with recent initiatives prompting authors of scientific articles to disclose raw data of their studies to further increase transparency (Nature Editorial, 2013; Harris et al., 2014).

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[19] Commission Directive (EU) 2018/350 amending Directive 2001/18/EC of the European Parliament and of the Council as regards the environmental risk assessment of genetically modified organisms. OJ L 67, 9.3.2018, pp. 30–45.

Farmer alert system

EFSA is of the opinion that the farmer alert system is a useful complement to the other strategies used for managing insect resistance as it provides a method for those observing and managing crops to comment on pest infestation levels, and provides an additional source of first-hand information.

EFSA encourages the consent holder to provide more information on this complementary resistance monitoring tool to determine whether appropriate communication mechanisms and fit-for-purpose educational programmes (e.g. characterisation of the damage caused by corn borers) are implemented to ensure the timely and effective reporting of farmer complaints.

3.1.2.3. Conclusions on insect resistance monitoring

The analysis of the data provided by the consent holder do not indicate a decrease in susceptibility to the Cry1Ab protein of the corn borer samples tested in the 2016 growing season. This is supported by the lack of farmer reports of unexpected plant damage to maize MON 810. However, EFSA identified some methodological and reporting limitations pertaining to the resistance monitoring that need improvement in future PMEM reports.

3.2. General surveillance

3.2.1. Farmer questionnaires[20]

3.2.1.1. Consent holder's assessment

In the annual 2016 PMEM report, the consent holder submitted a survey based on 250 questionnaires received from farmers in Spain and Portugal (Table 4). No farmers from the Czech Republic and Slovakia, representing less than 1% of the maize MON 810 grown in the EU in 2016, were interviewed.

The surveys were performed in each country by an external company and were completed between January and March 2017. The response rate was ≈ 51% in Spain, and 100% in Portugal. One-hundred seventy-nine out of 250 farmers were interviewed for the first time.

Table 4: Farmers surveyed and maize MON 810 areas monitored in 2016 through questionnaires [Table created from data provided in the 2016 PMEM report]

Country	No. of farmers surveyed	Mean maize MON 810 area monitored per farmer (ha)	Monitored maize MON 810 area (ha)	Total planted MON 810 area (ha)	Monitored maize MON 810 (% of total area)
Spain	237[(a)]	28.6	6,778	129,081	5.2
Portugal	13[(b)]	79.0	1,027	7,056	14.6

Farmers from the Czech Republic and Slovakia, representing less than 1% of the cultivated area of maize MON 810 in the EU, were not surveyed.

(a): One-hundred sixty-two farmers were from Aragón/Cataluña, 28 from Extremadura, 20 from Andalucía, 15 from Comunidad Foral de Navarra, 12 from Castilla la Mancha/Comunidad de Madrid. One-hundred seventy-seven out of 237 farmers were interviewed for the first time.

(b): Six farmers were from Alentejo, four from Lisbon and Vale do Tejo, and three from Centre. Two out of 13 farmers were interviewed for the first time.

The questionnaire was designed to collect data on four specific areas (1) area cropped to maize; (2) typical agronomic practices; (3) observations of maize MON 810; and (4) implementation of maize MON 810 specific measures, and aimed at identifying unintended effects caused by the cultivation of maize MON 810.

The consent holder concluded that the analysis of the 2016 farmer questionnaires on maize MON 810 'did not identify any potential adverse effects that might be related to MON 810 plants and their cultivation'.

3.2.1.2. EFSA's assessment

The farmer questionnaire and the approach followed to identify unanticipated adverse effects caused by the cultivation of maize MON 810 are similar to those in previous annual PMEM reports. EFSA therefore reiterates previous observations on the methodology (e.g. sampling, comparator (non-

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[20] 2016 PMEM report: Section 3.1.2.1 and Appendix 1.

GM) fields, type of questions and possible responses) and the analysis of data from the farmer questionnaire survey (EFSA GMO Panel, 2016, 2017).

To achieve the statistical power described in the sample size calculations provided in Annex I of the 2016 PMEM report, EFSA considers that the data from the farmer questionnaires should be pooled for statistical analysis when the aimed sample size of 2,500 questionnaires is obtained. The 2016 PMEM report represents the eleventh reporting year, resulting in the completion of a total of 2,877 questionnaires since 2006. However, a pooled analysis of all the data has not yet been provided or reported in the scientific literature. Moreover, the statistical analysis should be designed to enable an analysis of the monitoring characteristics according to the length of GM crop cultivation, in order to assess residual effects and possible trends. Certain effects may reach sufficient magnitude for detection only with repeated cultivation of a GM crop, and so amendments to the study design and the analysis plan should be considered to assess the effect of multiple years of GM crop cultivation.

EFSA recommends that the data obtained over this 11-year period should be pooled and an appropriate analysis of the combined data sets should be carried out. In such analysis, consideration should be given to the consistency of the questions to assess monitoring characteristics and the comparability of the obtained data from year to year, the possible inclusion of the same farmers in more than one year in the survey (and the enumeration of these farmers in the report) and the interim analyses performed for the annual reports.

3.2.1.3. Conclusions on farmer questionnaires

EFSA is of the opinion that the assessment of the results of the analysis of the pooled data is needed in order to confirm that no unintended effects caused by the cultivation of maize MON 810 have been observed, and to evaluate the farmer questionnaire methodology.

3.2.2. Existing monitoring networks[21]

Directive 2001/18/EC and Council Decision 2002/811/EC propose to make use of existing monitoring networks because they can complement farmer questionnaires and provide an additional tool for the general surveillance of GM plants. The EU Member States have various networks in place – some of which have a long history of data collection – that may be helpful in the context of general surveillance of GM plants.

3.2.2.1. Consent holder's assessment

As in previous annual PMEM reports, the consent holder reported no information gathered through existing monitoring networks in the EU.

3.2.2.2. EFSA's assessment

In an external report commissioned by EFSA (Centre for Ecology and Hydrology, Perseus, 2014) and in associated publications (e.g. Smets et al., 2014), several existing networks have been identified as potentially suitable for the general surveillance of GM plants. Although the usefulness of such networks requires resolving issues pertaining to data accessibility, data reporting format, and data connectivity with GMO registers (EFSA GMO Panel, 2014b), EFSA encourages relevant stakeholders to implement a methodological framework that enables the use of existing networks in the broader context of environmental monitoring.

3.2.3. Literature searching[22]

3.2.3.1. Consent holder's assessment

The consent holder performed a systematic literature search to retrieve publications relevant to the food/feed and environmental safety assessment of maize MON 810 and the Cry1Ab published between June 2016 and the beginning of June 2017. The literature search was conducted according to the guidelines given in the EFSA (2010, 2017).

The electronic bibliographic databases Web of Science (WoS) Core Collection and CABI CAB Abstracts and Global Health, both hosted under the WoS platform (Clarivate Analytics), were searched to identify relevant publications. Altogether, 403 publications were identified (including duplicates).

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[21] 2016 PMEM report: Section 3.1.2.3 and 3.1.4.3.
[22] 2016 PMEM report: Section 3.1.6 and Appendixes 5.1 to 5.4.

After applying the eligibility/inclusion criteria defined *a priori* by the consent holder, 27 primary research studies (hereafter referred to as publications) were identified as relevant for the assessment of food/feed safety (six publications) or environmental safety (21 publications). In addition, 10 review publications were identified. The list of relevant publications identified by the applicant through the systematic literature search described above is listed in Appendix F.

The consent holder evaluated the relevant publications identified by this literature search and concluded that they confirm former risk assessment conclusions on maize MON 810.

3.2.3.2. EFSA's assessment

EFSA assessed the systematic literature search provided by the consent holder according to the guidelines given in EFSA (2010, 2017). The overall quality of the performed literature search is acceptable. However, EFSA considers that the methodology and reporting of literature searches on maize MON 810 could be improved further and therefore provides recommendations for future searches. These recommendations are classified according to the different steps/processes of the literature search:

- Searching for relevant publications – Constructing the search strategy:

    — Ensure that enough search term variation is used (e.g. covering possible synonyms, related terms, acronyms, spelling variants, old and new terminology, brand and generic names, lay and scientific terminology, common typos, translation issues);
    — Include controlled vocabulary (subject indexing) in the searches when available (in addition to text words);
    — Use truncation consistently (e.g. truncate "resistan*" and not "resistant*"; truncate "cornborer");
    — Increase the proximity operator distance (NEAR/5 (or greater) instead of NEAR/3);
    — Ensure that search strings are combined to have a more sensitive search (e.g. do not combine the trade name with the plant species);
    — Adapt the search strategy to each electronic database used.

- Searching for relevant publications – Identification of sources of scientific literature:

    — Consider additional information sources (e.g. hand searching, citation searching, checking websites of relevant organisations).

- Selection of studies – Relevance criteria:

    — Better define the eligibility/inclusion criteria for assessing the relevance of the retrieved publications.

- Reporting:

    — Report the number of publications retrieved for each single search set performed (or search lines);
    — report better how the selection based on title/abstract and full-text was performed;
    — Report how the date search limits were set;
    — Report how inter-reviewer agreement was ensured;
    — Report the number of retrieved publications for each database, the number of publications remaining after excluding duplicates, and the number of publications excluded after screening of title and abstract and after full-text screening;
    — Report the reason for exclusion of those publications for which the full-text was assessed.

In addition, EFSA advises that future literature searches on maize MON 810 performed in the context of annual PMEM reports should follow the guidelines given in EFSA (2017).

The results reported in the relevant publications identified by the consent holder as part of its 2016 PMEM report do not provide new information that would invalidate the previous food/feed and

environmental safety assessment conclusions and risk management recommendations on maize MON 810 made by EFSA or its GMO Panel.[23]

3.2.3.3. Additional scientific publications assessed by EFSA

EFSA identified an additional relevant publication (Camargo et al., 2018) that was published online on 5 March 2018, after the period covered by the literature search performed by the consent holder.

Camargo et al. (2018) estimated the frequency of Cry1Ab resistance alleles in MCB populations collected from the Ebro Valley during the 2016 growing season, using a F_2-screen assay (Andow and Alstad, 1998). Three-hundred eighty-five iso-female lines were established from the 1,327 late-instars collected, of which 137 lines produced enough viable offspring which were screened for resistance on maize MON 810 leaves (Table 4). One of the screened lines was considered to carry a major resistance allele since larvae moulted to the second instar and caused substantial damage when feeding on maize MON 810 leaves for five days on two consecutive generations (i.e. F_2 and F_3). The expected frequency of resistance alleles was estimated to be 3.6×10^{-3}, with a 95% CI between 4×10^{-4} and 10^{-2}. The value reported by Camargo et al. (2018) was higher than the initial estimation of 2.9×10^{-3} (95% CI $0 - 8.6 \times 10^{-3}$), calculated from MCB populations collected in 2004/2005 (Andreadis et al., 2007) (Table 5). However, both estimates are not significantly different because of the low number of lines screened in 2004/2005.

Table 5: Results of the F_2-screen to estimate frequency of Cry1Ab resistance alleles in *Sesamia nonagrioides* populations from the Ebro Valley (north-eastern Spain) in 2004/2005 and 2016 [Table created from data provided in Álvarez-Alfageme (2007), Andreadis et al. (2007) and Camargo et al. (2018)]

Growing season	Larvae collected	P₀ lines established	Lines screened (F₂)[a]	Positive lines	Estimated frequency (95% CI)	Detection probability (%)[b]
2004/2005	1,206	395	85	0	0.0029 (0–0.0086)	97.5
2016	1,327	385	137	1	0.0036 (0.0004–0.01)	97.5

CI: confidence interval.
(a): F_2-lines from 2004/2005 season were screened using maize Bt176 leaves, whereas F_2-lines from 2016 season were screened using maize MON 810 leaves.
(b): Probability of detecting a resistance allele if present in the lines tested.

The authors ran new simulations with the resistance evolution model developed by Castañera et al. (2016) using the latest resistance frequency estimation. Results of these simulations indicate that resistance is not evolving much faster than initially predicted, and that field resistance is expected to occur in 31 years from 2016 onward, assuming continued cultivation of maize MON 810 in the region.

The findings reported by Camargo et al. (2018) reinforce previous recommendations made by EFSA and its GMO Panel, i.e. that achieving full compliance with refuge requirements is key, especially in areas of high selection pressure such as the Ebro Valley; that the monitoring strategy should be designed to detect resistance alleles at a frequency that allows the implementation of mitigation measures before field resistance evolves (i.e. a maximum detection threshold of resistance frequency alleles of 3% has been proposed); and that F_2-screening should be performed periodically to confirm the results obtained via the diagnostic bioassays and directly estimate the frequency of resistance alleles in target pest populations.

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[23] In one of the scientific publications, Ibrahim and Okasha (2016) described a morphological study of the jejunum from male rats (10/group) receiving for 90 days a diet containing either maize MON 810 or a non-GM maize at 30% incorporation rate. Several approaches and tools were used (histology, immunohistochemistry, morphometry, electron microscopy). The authors claimed that the consumption of the GM maize altered the jejunal histological structure, with changes consistent with mucosal damage (epithelial haemorrhagic erosions, goblet cells and mucus alterations, ultrastructural degenerative features) and reorganisation (villi distortion, shortening, flattening and fusion and crypts proliferation), associated with inflammation. EFSA notes some methodological issues, including scarce information on the test and control materials and diets, and on the test system, as well as insufficient information on the intestinal sampling procedure. Moreover, misinterpretation of artefactual or anatomical features was noted. Therefore, EFSA concludes that the findings reported by Ibrahim and Okasha (2016) do not add scientific evidence sufficient to change the conclusions on the safety on maize MON 810.

3.2.3.4. Conclusions on literature searching

The overall quality of the literature search performed by the consent holder is acceptable. However, EFSA considers that the methodology and reporting could be improved and therefore provides recommendations for future searches.

EFSA assessed the relevant publications identified by the consent holder through the performed literature search and acknowledges that no publication has been identified raising a safety concern for human and animal health and the environment which would change the original risk assessment conclusions on and risk management recommendations for maize MON 810. However, the findings reported by Camargo et al. (2018) reinforce the need to implement a sensitive monitoring plan, as previously recommended by EFSA and its GMO Panel (EFSA, 2015; EFSA GMO Panel, 2017).

4.   Conclusions

The information reported in the 2016 PMEM report and in the additional relevant publication identified by EFSA does not indicate any adverse effects on human and animal health or the environment arising from the cultivation of maize MON 810 during the 2016 growing season. EFSA therefore concludes that no new evidence has been reported in the context of the 2016 PMEM report that would invalidate previous GMO Panel evaluations on the safety of maize MON 810 (EFSA, 2009; EFSA GMO Panel, 2012b,c). However, EFSA identified some methodological and reporting limitations pertaining to insect resistance monitoring, farmer questionnaires and literature searching, and therefore updated its previous recommendations that should be implemented by the consent holder.

5.   Recommendations

5.1.   Case-specific monitoring

The consent holder should provide additional evidence to underpin the appropriateness of the diagnostic concentration selected for MCB.

The methodology of the diagnostic bioassays should be harmonised for both target pests. Separate diagnostic bioassays should be conducted with F_1-larvae from each sampling zone, and in cases where moulting inhibition is lower than expected (i.e. lower than 99%), follow-up studies should be conducted with the suspected-resistant larvae to confirm and characterise Cry1Ab resistance alleles.

The consent holder should standardise the follow-up studies for confirming resistance with suspected-resistant larvae. Such studies should follow a stepwise approach and consider the following procedures: (1) rearing survivors on non-*Bt*-diet and re-testing subsequent generations against diagnostic concentration; (2) performing studies with additional generations with maize MON 810 leaf discs; (3) continued selection of survivors at the diagnostic concentration; and (4) testing on whorl stage *Bt*-maize plants.

EFSA recommends that the consent holder develops alternative testing methods to improve the sensitivity and precision of the current monitoring strategy. F_2-screen could be performed periodically with ECB and MCB populations to confirm the results of the diagnostic bioassays; to confirm that the Cry1Ab resistance alleles are rare; and to revise the predictions of resistance evolution models.

The authors of resistance monitoring assays should consider EFSA's recommendations outlined in Appendix G of this statement when preparing the reports of such assays. Moreover, the consent holder should supply the raw data of the different resistance monitoring bioassays conducted with both target pests as part of future PMEM reports.

Further details on the recommendations for insect resistance monitoring are provided in Section 3.1.2.2.

EFSA considers that the consent holder should provide more information on the farmer alert system to enable the appraisal of its usefulness as complementary resistance monitoring tool, and determining whether appropriate communication mechanisms and fit-for-purpose educational programmes are implemented that ensure the timely and effective reporting of farmer complaints.

EFSA reiterates that the consent holder should pursue its efforts to further enforce compliance with refuge requirements, especially in regions of high maize MON 810 adoption. EFSA recommends that the consent holder and EU Member States develop appropriate information systems on GM crop cultivation to ensure that structured refuges are planted in these clustered areas.

5.2. General surveillance

For the farmer questionnaires, EFSA reiterates its former recommendations on their survey design and reporting; more detailed information on the sampling methodology should be provided, and the possibility of selection bias should be reduced. Moreover, the consent holder should explore how to make the best use of the information recorded in national GMO cultivation registers, and foster the dialogue with those responsible for the administration of registers for maize MON 810 cultivation. The consent holder should provide the analysis of the pooled data from the surveys obtained over the last eleven years to: (1) confirm that no unintended effects caused by the cultivation of maize MON 810 have been observed; and (2) evaluate the farmer questionnaire methodology.

Although the usefulness of existing monitoring networks in the EU can present some limitations, EFSA encourages relevant stakeholders to implement a methodological framework that enables the use of such networks in the broader context of environmental monitoring.

The literature searching performed by the consent holder should be improved according to the recommendations given in Section 3.2.3.2 and follow the guidelines given in EFSA (2017).

Documentation provided to EFSA

1) Letter from the European Commission, dated 16 November 2017, to EFSA requesting the assessment of the annual PMEM report on the cultivation of maize MON 810 during the 2016 season provided by the consent holder; the PMEM report was annexed to the letter.
2) Comments from the EU Member States on the 2016 PMEM report.
3) Acknowledgment letter dated 12 December 2017 from EFSA to the European Commission.
4) Letter dated 14 December 2017 from the European Commission to the consent holder requesting supplementary information.
5) Letter dated 16 February 2018 from the consent holder to EFSA providing supplementary information.

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Abbreviations

Bt	*Bacillus thuringiensis*
CI	confidence interval
ECB	European corn borer
ERA	environmental risk assessment
GM	Genetically modified
IRM	insect resistance management
MCB	Mediterranean corn borer
MIC_x	x% moulting inhibition concentration
PMEM	post-market environmental monitoring

Appendix A – Compliance with refuge requirements by Spanish farmers between 2004 and 2016

[Table created from data provided in the annual PMEM reports]

Growing season	No. of farmers surveyed	No. of farmers planting structured refuges	No. of farmers not planting refuges		Compliance (%)[a]	Source[b]
			Field < 5 ha[a]	Field > 5 ha		
2004	100	58	0	42	58	Antama
2005	100	49	0	51	49	Antama
2006	100	56	27	17	77	FQ
	100	64	0	36	64	Antama
2007	100	70	9	21	77	FQ
	100	60	0	40	60	Antama
2008	99	76	10	13	85	FQ
	100	82	0	18	82	Antama
2009	100	85	7	8	91	FQ
	100	81	0	19	81	Antama
2010	150	129	8	13	91	FQ
	100	88	NR	NR	> 88	Antama
2011	150	134	10	6	96	FQ
	100	93	NR	NR	> 93	Antama
2012	175	130	21	24	84	FQ
	110	NR	NR	NR	≥ 93	Antama
2013	190	153	15	22	87	FQ
2014	213	178	24	11	94	FQ
2015	212	162	38	12	93	FQ
2016	237	164	53	20	89	FQ

NR: not reported.
Shaded row corresponds to the annual PMEM report under assessment.
(a): Farmers planting < 5 ha of maize MON 810 in the farm are not required to plant a refuge. For the FQ, only farmers who are required to plant a refuge were considered for the calculation of non-compliance with refuge requirements.
(b): FQ: farmer questionnaires; Antama: Study sponsored by Spanish foundation supporting the use of new technologies in agriculture. In the surveys conducted by Antama, all farmers were from the Ebro Valley (north-eastern Spain).

Appendix B – Area and adoption rate of maize MON 810 in north-eastern, central and south-western Spain between 2012 and 2016

Season	Area maize of MON 810 (ha)[a]	Source			
		Avances[b]		ESYRCE[c]	
		Total maize (ha)	Adoption rate (%)	Total maize (ha)	Adoption rate (%)
North-eastern Spain (Aragón, Navarra and Cataluña)					
2012	81,001	130,441	62.1	126,996[d]	63.8
2013	95,460	150,281	63.5	145,735[d]	65.5
2014	97,686	154,134	63.4	197,637	49.4
2015	80,022	149,953	53.5	163,886	48.8
2016	96,180	142,123[e]	67.7	145,661	66.0
Mean 2012–2016	–	–	**62.0**	–	**58.7**
Central Spain (Albacete)					
2012	6,453	17,701	36.5	19,297[d]	33.4
2013	6,564	16,950	38.7	20,698[d]	31.7
2014	5,696	14,700	38.8	16,585[d]	34.3
2015	4,027	11,800	34.1	14,895[d]	27.0
2016	4,388	9,600[e]	45.7	10,221[d]	42.9
Mean 2012–2016	–	–	**33.9**	–	**33.5**
South-western Spain (Extremadura and Andalucía)					
2012	26,313	101,649	25.9	118,039[d]	22.3
2013	31,058	113,437	27.4	123,097[d]	25.2
2014	24,507	96,999	25.3	108,574	22.6
2015	21,298	87,094	24.5	103,242	20.6
2016	25,958	71,911[e]	36.1	81,611	31.8
Mean 2012–2016	–	–	**27.8**	–	**24.5**

(a): Source: http://www.magrama.gob.es/es/calidad-y-evaluacion-ambiental/temas/biotecnologia/organismos-modificados-gene ticamente-omg-/consejo-interministerial-de-ogms/superficie.aspx (Accessed 8 May 2018).

(b): Avances de superficies y producciones de cultivos: http://www.magrama.gob.es/es/estadistica/temas/estadisticas-agrarias/ agricultura/avances-superficies-producciones-agricolas/ (Accessed 8 May 2018).

(c): Encuesta sobre superficies y rendimiento de cultivos (ESYRCE): http://www.magrama.gob.es/es/estadistica/temas/estad isticas-agrarias/agricultura/esyrce/ (Accessed 8 May 2018).

(d): Data for maize as a second crop are not included.

(e): Provisional data.

Appendix C – Field sampling of *Ostrinia nubilalis* (ECB) and *Sesamia nonagrioides* (MCB) larvae in the 2016 growing season in north-eastern Spain

[Table created from data provided in the 2016 PMEM report]

Species	Sampling zone	Sampling site location – code (Province)[a]	No. of larvae collected	No. of adults emerged (% over larvae collected)
ECB	1	Lanaja – 1 (Huesca)	166	75 (45)
		Lanaja – 3 (Huesca)	78	25 (32)
		Lanaja – 5 (Huesca)	112	56 (50)
		Sariñena – 1 (Huesca)	122	49 (40)
		Total	**478**	**205 (43)**
	3	La Almunia de Doña Godina – 1 (Zaragoza)	354	–
		La Almunia de Doña Godina – 3 (Zaragoza)	39	–
		Total	**393**	**230 (59)**
	4	Mendigorria – 1 (Navarra)	172	83 (43)
		Mendigorria – 2 (Navarra)	20	
		Artajona – 1 (Navarra)	48	36 (75)
		Total	**240**	**119 (50)**
	Total		**1,111**	**554 (50)**
MCB	1	Lanaja – 3 (Huesca)	176	–
		Lanaja – 5 (Huesca)	142	–
		Sariñena – 1 (Huesca)	110	–
		Total	**428**	**288 (67)**
	2	Candasnos – 1 (Huesca)	149	–
		Candasnos – 4 (Huesca)	175	–
		Peñalba – 1 (Huesca)	186	–
		Peñalba – 2 (Huesca)	14	–
		Total	**524**	**376 (72)**
	3	La Almunia de Doña Godina – 1 (Zaragoza)	200	–
		La Almunia de Doña Godina – 3 (Zaragoza)	212	–
		Total	**412**	**296 (72)**
	Total		**1,364**	**960 (70)**

Late-instars were collected from refuges and non-*Bt*-maize fields between 12 September and 20 October 2017. No geographical coordinates were provided for the sampling sites. All ECB larvae collected were in diapause, as well as most of the MCB larvae collected.

(a): Thirteen and 20 additional sites were inspected for MCB and ECB, respectively, but the minimum number of larvae established in the harmonised insect resistance management (EuropaBio, 2017) plan could not be reached.

Appendix D – Susceptibility to purified Cry1Ab protein of reference susceptible strains of *Ostrinia nubilalis* (ECB) and *Sesamia nonagrioides* (MCB)

[Table created from data provided in the annual PMEM reports]

Target pest (strain)	Season	Protein batch	MIC$_{50}$ (95% CI)[a]	MIC$_{90}$ (95% CI)[a]
ECB (G.04)[b]	2006	1	1.20 (0.50–2.21)	4.78 (2.57–14.38)
	2007	1	1.44 (0.86–2.06)	3.94 (2.68–8.28)
	2008	1	2.21 (1.89–2.55)	4.47 (3.70–6.00)
	2008	1	2.26 (1.49–3.01)	8.16 (5.95–13.50)
	2009	1	3.65 (2.77–4.90)	9.56 (6.72–17.75)
	2010	1	2.77 (2.22–3.27)	6.03 (4.93–8.41)
	2011	1	4.01 (2.58–6.12)	10.07 (6.50–28.96)
	2011	2	2.94 (2.33–3.60)	6.27 (4.97–8.91)
	2012	2	0.37 (0.14–0.62)	1.13 (0.67–6.39)
	2013	2	1.97 (0.78–5.59)	5.66 (2.67–95.34)
	2013	2a	1.96 (0.84–4.60)	6.57 (3.13–50.53)
	2014	2a	0.28 (0.24–0.33)	0.46 (0.38–0.62)
	2015	2a	4.03 (2.85–4.86)	7.03 (5.83–9.91)
	2016	2b	6.07 (5.09–7.02)	11.10 (9.45–13.94)
ECB (ES.ref)[c]	2015	2a	1.82 (1.53–2.16)	2.95 (2.43–4.54)
	2016	2b	5.02 (3.61–6.33)	14.25 (11.29–19.87)
MCB[d]	2004	B1	18 (11–25)	99 (66–208)
	2007	B1	16 (11–22)	94 (69–147)
	2008	B1	19 (10–30)	120 (76–255)
	2010	B1	8 (5–11)	74 (51–117)
	2011	B2-1	9 (6-13)	68 (45–127)
	2012	B2-1	7 (5–10)	62 (41–107)
	2013	B2-1	7 (5–10)	48 (31–88)
	2013	B2-2	5 (3–9)	42 (26–87)
	2014	B2-2	17 (11–25)	91 (57–209)
	2015	B2-2	28 (21–36)	67 (50–110)
	2016	B2-3	30 (24–38)	83 (62–132)

Shaded rows correspond to values from the annual PMEM report under assessment.

(a): 50% and 90% moulting inhibition concentration (MIC$_{50}$ and MIC$_{90}$) and their 95% confidence intervals (CI 95%) are expressed in ng Cry1Ab/cm^2 of diet surface area.

(b): The 'G.04' strain was established from egg masses collected from Niedernberg (Germany) in 2005. This strain has not been refreshed with field-collected individuals.

(c): The 'ES.ref' strain was established from 145 diapausing larvae collected from three sampling sites in Galicia (Spain) in 2015, of which 75 survived the diapause, reached the adult stage and were placed in oviposition cages for mating.

(d): The strain was established from larvae collected from Andalucía (661 larvae), Madrid (793 larvae), Ebro Valley (857 larvae) and Galicia (665 larvae) (Spain) in 1998 (González-Núñez et al., 2000). To preserve its vigour, the strain was refreshed periodically with new individuals. To this end, the progeny of the populations collected for the monitoring bioassays is used, and between 10% and 15% of new individuals with respect to the laboratory strain are introduced.

Appendix E − Historical data on the susceptibility to the Cry1Ab protein of *Ostrinia nubilalis* and *Sesamia nonagrioides* populations from north-eastern Spain

[Table created from data provided in the annual PMEM reports]

Target pest	Season	No. of larvae collected (no. sites)	Protein batch[a]	MIC$_{50}$ (95% CI)[b]	MIC$_{90}$ (95% CI)[b]	RR MIC$_{50}$ (95% CI)[c]	RR MIC$_{90}$ (95% CI)[c]
ECB	2008	401 (4)	1	7.03 (4.89–10.03)	23.91 (15.76–46.84)	3.11/3.18*[d] (NR)	2.93/5.35*[d] (NR)
	2009	509 (3)	1	6.40 (5.32–7.75)	13.68 (10.77–20.02)	1.75* (NR)	1.43 (NR)
	2011	382 (6)	2	1.79 (1.54–2.07)	4.19 (3.45–5.48)	0.61* (NR)	0.67 (NR)
	2013	452 (3)	2a	2.48 (2.03–3.02)	5.41 (4.27–7.61)	1.26 (NR)	0.82 (NR)
	2015	376 (3)	2a	2.12 (1.75–2.55)	5.43 (4.36–7.29)	0.53* (NR)	0.77 (NR)
MCB	2004	424 (4)	B1	63 (34–99)	570 (333–1318)	3.5 (NR)	5.8 (NR)
	2005	400 (2)	B1	9 (3–15)	76 (54–117)	0.5 (NR)[d]	0.8 (NR)[e]
	2007	457 (3)	B1	14 (8–20)	99 (71–158)	0.9 (NR)	1.0 (NR)
	2009	489 (3)	B1	22 (16–28)	188 (138–277)	1.1 (0.8–1.7)	1.6 (NR)
	2011	564 (4)	B2-1	20 (14–27)	135 (91–232)	2.2 (1.6–3.0)*	2.0 (1.3–2.9)*
	2013	742 (5)	B2-2	19 (14–25)	163 (108–287)	2.6 (2.0–3.4)*	3.4 (2.2–5.2)*
	2015	529 (3)	B2-2	17 (13–21)	84 (63–124)	0.6 (0.5–0.8)*	1.3 (0.9–1.8)

NR: not reported.

*: Significant difference ($p < 0.05$) between the field population and the reference strain was identified for that season. From 2016 onwards, susceptibility to Cry1Ab is assessed in diagnostic bioassays.

(a): Data provided by the consent holder in previous monitoring reports showed that the Cry1Ab protein batches 1 and 2, 2 and 2a, B1 and B2-1, and B2-1 and B2-2 have similar insecticidal activity (see Appendix C).

(b): 50% and 90% moulting inhibition concentration (MIC$_{50}$ and MIC$_{90}$) and their 95% confidence intervals (CI 95%) are expressed in ng Cry1Ab/cm^2 of diet surface area.

(c): Resistance ratio (RR) between MIC values of the field-collected populations and of the susceptible laboratory strain for each cultivation season.

(d): The reference strain was tested two times in 2008 (see Appendix D).

(e): MIC$_{50}$ and MIC$_{90}$ values of the reference strain used to calculate RR MIC$_{50}$ and RR MIC$_{90}$ correspond to those estimated in 2004.

Appendix F – Scientific publications relevant to the food/feed or environmental safety of maize MON 810 assessed by EFSA as part of the 2016 PMEM report

Reference	Study type	Relevant area
Andow DA and Zwalhen C, 2016. Ground beetle acquisition of Cry1Ab from plant- and residue-based food webs. Biological Control, 103, 204–209.	Primary	ENV safety
Andreassen M, Bøhn T, Wikmark O-G, Bodin J, Traavik T, Lovik M and Nygaard UC, 2016. Investigations of immunogenic, allergenic and adjuvant properties of Cry1Ab protein after intragastric exposure in a food allergy model in mice. BMC Immunology, 17, 11–12.	Primary	FF safety
Blanco CA, W Chiaravalle W, Dalla-Rizza M, Farias JR, Garcia-Degano MF, Gastaminza G, Mota-Sanchez D, Murua MG, Omoto C, Pieralisi BK, Rodriguez JC, Teran-Santofimio H, Teran-Vargas AP, Valencia SJ and Willink E, 2016. Current situation of pests targeted by *Bt* crops in Latin America. Current Opinion in Insect Science, 15, 131–138.	Review	ENV safety
Buuk C, Gloyna K and Thieme T, 2016. Is there any change in susceptibility of European corn borer (*Ostrinia nubilalis*) to Cry1Ab protein? IOBC-WPRS Bulletin, 114, 1–6.	Primary	ENV safety
Camargo AM, Andow DA, Castañera P, GP Farinós, 2018. First detection of a *Sesamia nonagrioides* resistance allele to Bt maize in Europe. Scientific Reports, 8, 3977.	Primary	ENV safety
Castañera P, Farinós G, Ortego F and Andow D, 2016. Sixteen years of *Bt* maize in the EU hotspot: Why has resistance not evolved? Plos One, 1–13.	Primary	ENV safety
Chrenkova M, Pomikalova S, Chrastinova L, Polacikova M, Formelova Z, Rajsky M and Mlynekova Z, 2016. Effect of crimped maize grain ensiled with high moisture grains of transgenic *Bt* maize in fattening bulls. 17th International Conference, Forage Conservation, 27–29 September 2016, Horný Smokovec, Slovak Republic, 159-162 ref 11.	Primary	FF safety
Coates BS, 2016. *Bacillus thuringiensis* toxin resistance mechanisms among Lepidoptera: progress on genomic approaches to uncover causal mutations in the European corn borer, *Ostrinia nubilalis*. Current Opinions in Insect Science, 15, 70–77.	Review	ENV safety
Di Grumo D and Lovei GL, 2016. Body size inequality in ground beetle (Coleoptera: Carabidae) assemblages as a potential method to monitor environmental impacts of transgenic crops. *Periodicum Biologorum*, 118, 223–230.	Primary	ENV safety
Diaz-Gomez J, Marin S, Capell T, Sanchis V and Ramos AJ, 2016. The impact of *Bacillus thuringiensis* technology on the occurrence of fumonisins and other mycotoxins in maize. World Mycotoxin Journal, 9, 475–486.	Review	ENV safety
Domingo JL, 2016. Safety assessment of GM plants: An updated review of the scientific literature. Food and Chemical Toxicology, 95, 12–18.	Review	FF safety
Dos Santos CA, Marucci RC, Barbosa TAN, Araujo OG, Waquil JM, Dias AS, Hebach FC and Mendes SM, 2016. Desenvolvimento de *Helicoverpa* spp. em milho *Bt* corn expressao de diferentes proteinas. Pesquisa Agropecuaria Brasileira, 51, 537–544.	Primary	ENV safety
Erasmus A, Marais J and Van den Berg J, 2016. Movement and survival of *Busseola fusca* (Lepidoptera: Noctuidae) larvae within maize plantings with different ratios of non-*Bt* and *Bt* seed. Society of Chemical Industry, 72, 2287–2294.	Primary	ENV safety
Griffiths NA, Tank JL, Royer TV, Rosi EJ, Shogren AJ, Frauendorf TC and Whiles MR, 2017. Occurrence, leaching, and degradation of Cry1Ab protein from transgenic maize detritus in agricultural streams. Science of the Total Environment, 592, 97–105.	Primary	ENV safety
Han L, Jiang XX and Peng Y, 2016. Potential resistance management for the sustainable use of insect-resistant genetically modified corn and rice in China. Current Opinion in Insect Science, 15, 139–143.	Review	ENV safety

Reference	Study type	Relevant area
Han P, Velasco-Hernandez MC, Ramirez-Romero R and Desneux N, 2016. Behavioral effects of insect-resistant genetically modified crops on phytophagous and beneficial arthropods: a review. Journal of Pest Science, 89, 859–883.	Review	ENV safety
Ibrahim MAA and Okasha EF, 2016. Effect of genetically modified corn on the jejunal mucosa of adult male albino rat. Experimental and Toxicologic Pathology, 68, 579–588.	Primary	FF safety
Joshi S, Barnett B, Doerrer NG, Glenn K, Herman RA, Herouet-Guicheney C, Hunst P, Kough J, Ladics GS, McClain S, Papineni S, Poulsen LK, Rascle J-B, Tao AL, Van Ree R, Ward J and Bowman CC, 2016. Assessment of potential adjuvanticity of Cry proteins. Regulatory Toxicology and Pharmacology, 79, 149–155.	Review	FF safety
Korwin-Kossakowska A, Sartowska K, Tomczyk G, Prusak B and Sender G, 2016. Health status and potential uptake of transgenic DNA by Japanese quail fed diets containing genetically modified plant ingredients over 10 generations. British Poultry Science, 57, 415–423.	Primary	FF safety
Kotey DA, Obi A, Assefa Y, Erasmus A and Van den Berg J, 2017. Monitoring resistance to *Bt* maize in field populations of *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) from smallholder farms in the Eastern Cape Province of South Africa. African Entomology, 25, 200–209.	Primary	ENV safety
Lee MS and Albajes R, 2016. Monitoring carabid indicators could reveal environmental impacts of genetically modified maize. Agricultural and Forest Entomology, 18, 238–249.	Primary	ENV safety
Mashiane RA, Ezeokoli OT, Adeleke RA and Bezuidenhout CC, 2017. Metagenomic analyses of bacterial endophytes associated with the phyllosphere of a *Bt* maize cultivar and its isogenic parental line from South Africa. World Journal of Microbiology and Biotechnology, 33, 1–12.	Primary	ENV safety
Niu Y, Head GP, Price PA and Huang F, 2016. Performance of Cry1A.105-selected fall armyworm (Lepidoptera: Noctuidae) on transgenic maize plants containing single or pyramided *Bt* genes. Crop Protection, 88, 79–87.	Primary	ENV safety
Omoto C, Bernardi O, Salmeron E, Sorgatto R, Dourado PM, Crivellari A, Carvalho RA, Willse A, Martinelli S and Head GP, 2016. Field-evolved resistance to Cry1Ab maize by *Spodoptera frugiperda* in Brazil. Pest Management Science 2016, 72, 1727–1736.	Primary	ENV safety
Osborne SL, Lehman RM and Rosentrater KA, 2016. Grain and biomass nutrient uptake of conventional corn and their genetically modified isolines. Journal of Plant Nutrition, 39, 2047–2055.	Primary	FF safety
Peterson JA, Obrycki JJ and Harwood JD, 2016. Spiders from multiple functional guilds are exposed to *Bt*-endotoxins in transgenic corn fields via prey and pollen consumption. Biocontrol Science and Technology, 1230–1248.	Primary	ENV safety
Schmidt K, Döhring J, Kohl C, Pla M, Kok EJ, Glandorf DCM, Custers R, van der Voet H, Sharbati J, Einspanier R, Zeljenková D, Tulinská J, Spök A, Alison C, Schrenk D, Pöting A, Wilhelm R, Schiemann J and Steinberg P, 2016. Proposed criteria for the evaluation of the scientific quality of mandatory rat and mouse feeding trials with whole food/feed derived from genetically modified plants. Archives of Toxicology, 90, 2287–2291.	Review	FF safety
Shu Y, Zhang Y, Zeng H, Zhang Y and Wang J, 2017. Effects of Cry1Ab *Bt* maize straw return on bacterial community of earthworm *Eisenia fetida*. Elsevier, 173, 1-13.	Primary	ENV safety
Sousa FF, Mendes SM, Santos-Amaya OF, Araujo OG, Oliveira EE and Pereira EJG, 2016. Life-history traits of *Spodoptera frugiperda* populations exposed to low-dose *Bt* maize. PLOs, 11, 1-18.	Primary	ENV safety
Stenekamp D, Pringle K and Addison M, 2016. Effect of genetically modified *Bt* maize in an artificial diet on the survival of *Cydia pomonella* (Lepidoptera: Tortricidae). Florida Entomologist, 99, 200-205.	Primary	ENV safety

Reference	Study type	Relevant area
Tefera T, Mugo S, Mwimali M, Anani B, Tende R, Beyene J, Gichuki S, Oikeh SO, Nang'ayo F, Okeno J, Njeru E, Pillay K, Meisel B and Prasanna BM, 2016. Resistance of *Bt*-maize (MON 810) against the stem borers *Busseola fusca* (Fuller) and *Chilo partellus* (Swinhoe) and its yield performance in Kenya. Crop Protection, 89, 202–208.	Primary	ENV safety
Waquil MS, Pereira EJG, De Sousa Carvalho SS, Pitta RM, Waquil JM and Mendes SM, 2016. Fitness index and lethal time of fall armyworm on *Bt* corn. Pesquisa Agropecukiria Brasileira, 5, 563–570.	Primary	ENV safety
Yang G, Niu Y, Head GP, Price PA and Huang F, 2016. Performance of Cry1Ab-susceptible and -heterozygous resistant populations of sugarcane borer in sequential feedings on non-*Bt* and *Bt* maize plant tissue. Entomologia Experimentalis et Applicata, 162, 51–59.	Primary	ENV safety
Yao J, Zhu Y, Lu N, Buschman LL and Zhu KY, 2017. Comparisons of transcriptional profiles of gut genes between Cry1Ab-resistant and susceptible strains of *Ostrinia nubilalis* revealed genes possibly related to the adaptation of resistant larvae to transgenic Cry1Ab corn. International Journal of Molecular Sciences, 18, 1–17.	Primary	ENV safety
Yinghua S, Yan D, Jin C, Jiaxi W, Wei J and Jianwu W, 2017. Responses of the cutworm *Spodoptera litura* (Lepidoptera: Noctuidae) to two *Bt* corn hybrids expressing Cry1Ab. Scientific Reports, 7, 41577.	Primary	ENV safety
Van den Berg J, 2016. Resistance of *Busseola fusca* Insect Resistance Management in *Bt* Maize: Wild host plants of stem borers do not serve as refuges in Africa. Journal of Economic Entomology, 110, 221–229.	Review	ENV safety
Venter HJ and Bøhn T, 2016. Interactions between *Bt* crops and aquatic ecosystems: a review. Environmental Toxicology and Chemistry, 35, 2891–2902.	Review	ENV safety
Zeljenková D, Alácová R, Ondrejková J, Ambrušová K, Bartušová M, Kebis A, Kovrižnych J, Rollerová E, Szabová E, Wimmerová S, Černák M, Krivošíková Z, Kuricová M, Líšková A, Spustová V, Tulinská J, Levkut M, Révajová V, Ševcíková3 Z, Schmidt K, Schmidtke J, Schmidt P, La Paz J, Pla M, Kleter G, Kok E, Sharbati J, Bohmer M, Bohmer N, Einspanier R, Adel-Patient K, Spök A, Pöting A, Kohl C, Wilhelm R, Schiemann J and Steinberg P, 2016. One-year oral toxicity study on a genetically modified maize MON 810 variety in Wistar Han RCC rats (EU 7th Framework Programme project GRACE). Archives of Toxicology, 90, 2531–2562.	Primary	FF safety

ENV: environmental; FF: food/feed.

Appendix G – Reporting recommendations for insect resistance monitoring studies

The recommendations provided in the below table aim to assist the consent holder in the reporting of the insect resistance monitoring studies performed in the context of annual PMEM reports of maize MON 810, so that sufficient information is provided enabling a proper assessment of the relevance and reliability of such studies. These recommendations may be revised in the future.

Category	Specific reporting recommendations
General information	1) Scientific name of the lepidopteran species tested 2) Assay type (e.g. concentration-response, diagnostic concentration, follow-up study with plant material/survival assays on plants) 3) Purpose of the study
Field collection	4) Geographical area where the test organisms were collected[a] 5) Locations and number of fields per location where test organisms were collected (e.g. geographical coordinates, nearest municipality) 6) Sampling source (e.g. non-*Bt*–maize field, refuge) and distance to the nearest *Bt*-maize field 7) Adoption rate of *Bt*-maize (in the geographical area or in the sampling zone if relevant data are available)
Test organism	8) Number and life-stage of collected individuals (per sampling zone/field) 9) Sampling date(s) 10) Measures taken to avoid the collection of siblings 11) Diapause status of field-collected populations 12) Description of the laboratory rearing protocol (including environmental conditions during laboratory rearing of field-collected individuals) 13) Number of field-collected individuals reaching adulthood after laboratory rearing of field-collected individuals (pre-imaginal mortality) 14) Number, sex and location of adults placed in oviposition cages for obtaining F_1 larvae 15) Description of the use of susceptible/resistant laboratory reference strain, including information on how the strain was initiated and how it is maintained and invigorated
Test substance	16) Biochemical characterisation of the test substance (e.g. source, % purity, batch/lot used, nominal concentration, solvent/vehicle used) 17) Method used to quantify the concentration of the test substance (e.g. Bradford, ELISA, SDS-PAGE/densitometry) 18) Description of the storage conditions of the test substance 19) Biological activity (in case of new batch, comparison of biological activity to the former batch(es) 20) Equivalence to the plant-expressed protein[b]
Study design	21) Study performed according to standardised guideline/peer-reviewed protocol 22) Study performed according to GLP or other standards 23) Description of control(s) 24) Preparation of stock solutions, including solvent concentrations in control(s) 25) Nominal concentration(s) of test substance and rationale for their selection 26) Administration of test substance (e.g. diet-overlay, mixed with artificial diet) 27) Age and generation of individuals tested (e.g. < 24-h-old larvae from F_1 generation) 28) Duration of the assay(s) 29) Description of measurement endpoints (e.g. mortality, moult inhibition) 30) Environmentally-controlled conditions (e.g. temperature, humidity and light regime) 31) Validity criteria of the study (e.g. mortality in the control group < 20%) 32) Blinding of personnel

Category	Specific reporting recommendations
Statistical design	33) Number of replicates for control(s) and test concentration(s); set-up of replicates (to avoid pseudo-replication) 34) Number of individuals tested per replicate 35) Treatment design (e.g. block, randomised) 36) Statistical method used 37) Statistical software used
Results and discussion	38) Deviations from the protocol 39) Description of the response effects for each of the measurement endpoints followed 40) Control mortality and other observed endpoints, and comparison to validity criteria from protocol 41) Estimation of variability for measurement endpoints (if relevant, e.g. 95% confidence intervals for MIC_x values) 42) Comparison to laboratory reference population (e.g. use of resistance ratios in case of concentration/response assays) 43) Estimation of slope, chi-square (for Probit analysis) 44) Relevance of the results (in the context of baseline susceptibility and natural variability to the test substance) 45) Availability of raw data

GLP: Good laboratories practices; MIC_x: x% moult inhibition concentration.

(a): The term *geographical area* is defined as a zone where maize is typically grown following similar agronomic practices isolated from other maize areas by barriers that might impair an easy exchange of target pests between those areas.

(b): For further information, see Raybould et al. (2013): Characterising microbial protein test substances and establishing their equivalence with plant-produced proteins for use in risk assessments of transgenic crops. Transgenic Research, 22, 445–460.