STATEMENT

ADOPTED: 24 May 2019

doi: 10.2903/j.efsa.2019.5742

Assessment of the 2017 post-market environmental monitoring report on the cultivation of genetically modified maize MON 810

European Food Safety Authority (EFSA),
Fernando Álvarez, Ana M Camargo and Yann Devos

Abstract

Following a request from the European Commission, EFSA assessed the 2017 post-market environmental monitoring (PMEM) report on the cultivation of Cry1Ab-expressing maize event MON 810. Like previous years, partial compliance with refuge requirements is reported for Spain. European and Mediterranean corn borer populations collected from North-eastern Spain during the 2017 maize growing season and tested for Cry1Ab susceptibility show no symptoms of resistance to maize MON 810. No complaints about unexpected field damage caused by corn borers were received through the farmer complaint system. The assessment of farmer questionnaires and relevant scientific publications does not indicate any unanticipated adverse effects on human and animal health or the environment arising from the cultivation of maize MON 810. No information about the use of existing networks involved in environmental monitoring is provided. Overall, EFSA concludes that the evidence reported in the 2017 PMEM report does not invalidate previous EFSA and GMO Panel evaluations on the safety of maize MON 810. As in previous years, EFSA identifies methodological and reporting shortcomings pertaining to resistance monitoring that need revision in future PMEM reports. In particular, the monitoring plan, as implemented in 2017, is not sufficiently sensitive to detect the recommended 3% resistance allele frequency. Consequently, EFSA strongly recommends the consent holder to: (1) achieve full compliance with refuge requirements in areas where maize MON 810 adoption is high (i.e. North-eastern Spain); (2) increase the sensitivity of the resistance monitoring plan and address previously mentioned methodological, analytical and/or reporting limitations for resistance monitoring and farmer questionnaires; and (3) perform a F_2-screen on European and Mediterranean corn borer populations from North-eastern Spain. Moreover, relevant stakeholders should implement a methodological framework to enable making best use of existing networks involved in environmental monitoring for the general surveillance of genetically modified plants.

Keywords: *Bt*-maize, Cry1Ab, case-specific monitoring, farmer questionnaires, insect resistance management, *Ostrinia nubilalis*, *Sesamia nonagrioides*

Requestor: European Commission

Question number: EFSA-Q-2018-01001

Correspondence: gmo@efsa.europa.eu

Acknowledgements: EFSA wishes to thank Salvatore Arpaia and Antoine Méssean for peer reviewing this scientific output and Christoph Tebbe and EFSA staff members Michele Ardizzone, Antonio Fernandez-Dumont, Marios Georgiadis, Anna Lanzoni, and Irene Muñoz-Guajardo for the support provided to this scientific output.

Suggested citation: EFSA (European Food Safety Authority), Álvarez F, Camargo AM and Devos Y, 2019. Assessment of the 2017 post-market environmental monitoring report on the cultivation of genetically modified maize MON 810. EFSA Journal 2019;17(6):5742, 38 pp. https://doi.org/10.2903/j.efsa.2019.5742

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Summary

Following a request from the European Commission, the European Food Safety Authority (EFSA) assessed the 2017 post-market environmental monitoring (PMEM) report on the cultivation of the Cry1Ab-expressing maize event MON 810. This report presents the results of the 2017 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 *case-specific monitoring* data set comprises a farmer survey to assess the level of compliance with refuge requirements in areas in Spain and Portugal where maize MON 810 was grown in 2017; diagnostic bioassays conducted with European and Mediterranean corn borers collected from North-eastern Spain to monitor changes in susceptibility to the Cry1Ab protein; and potential farmer complaints about product performance collected through the farmer complaint system.

Like previous years, partial compliance with refuge requirements is observed in Spain where maize MON 810 adoption is high. In such areas, full compliance should be achieved to delay resistance evolution. EFSA, therefore, reiterates the need to ensure full compliance in North-eastern Spain and urges the consent holder, the Spanish National Competent Authorities and other relevant stakeholders to undertake actions for achieving this goal. In addition, EFSA recommends the consent holder and EU Member States where maize MON 810 is grown to develop proper information systems on genetically modified (GM) crop cultivation to ensure that structured refuges are planted in clustered areas greater than 5 ha.

The analysis of resistance monitoring data gathered through diagnostic bioassays with field-collected corn borers does not indicate a decrease in susceptibility to Cry1Ab in the European corn borer (ECB) populations sampled during the 2017 maize growing season. For the Mediterranean corn borer (MCB), moulting inhibition was lower than the expected $>$ 99% in the three populations tested. Additional studies with plant material indicated that none of the MCB larvae tested from any of the three populations were able to complete development on maize MON 810 leaves.

As in previous years, EFSA identifies methodological and reporting shortcomings pertaining to resistance monitoring that need revision in future PMEM reports. In particular, considering the estimated numbers of field-collected ECB and MCB larvae represented in the diagnostic concentration bioassays, the monitoring plan, as implemented in 2017, is not sufficiently sensitive to detect the recommended 3% resistance allele frequency for a timely detection of a surge of field resistance. Consequently, EFSA strongly recommends the consent holder to increase the sensitivity and precision of the monitoring strategy. In addition, the consent holder should: (1) optimise the rearing process of field-collected individuals and reduce the pre-imaginal mortality prior to susceptibility testing; (2) confirm the validity of the diagnostic concentration selected for the MCB; (3) harmonise the methodology of the diagnostic bioassays for both target pests; (4) conduct separate diagnostic bioassays with F_1-larvae from each sampling zone; (5) conduct standardised follow-up studies with suspected-resistant larvae to confirm and characterise Cry1Ab resistance alleles; (6) consider more sensitive testing methods (e.g. F_2-screen); and (7) consider EFSA's previous reporting recommendations for future resistance monitoring studies, especially those pertaining to including access to raw data.

EFSA considers that it is timely for the consent holder to perform a F_2-screen on MCB populations from the same area where the Cry1Ab resistance allele was detected in 2016 by Camargo et al. (2018)[1] as well as on ECB populations from North-eastern Spain, where the frequency of resistance alleles has never been estimated.

The consent holder and other companies marketing maize MON 810 seeds put in place a farmer complaint system allowing farmers to report complaints about product performance (including unexpected field plant damage caused by target pests). No farmer complaints related to unexpected damage by corn borers were received via this system during the 2017 growing season. However, EFSA considers that the consent holder should substantiate the usefulness of the farmer complaint system as a complementary resistance monitoring tool. In particular, more information should be provided to determine whether proper communication mechanisms and fit-for-purpose educational programs are implemented to ensure the timely and effective reporting of farmer complaints.

The *general surveillance* data set consists of a farmer survey based on 250 farmer questionnaires and relevant scientific publications published between June 2017 and May 2018 that were identified through an extensive/systematic literature search.

[1] Scientific Reports, 8, 3977. https://www.nature.com/articles/s41598-018-21943-4 (Accessed 28 May 2019).

The assessment of farmer questionnaires and relevant scientific publications does not indicate any unanticipated adverse effects on human and animal health or the environment arising from the cultivation of maize MON 810.

As in previous years, the consent holder did not consider several of EFSA's recommendations on the methodology and analysis of farmer questionnaires. EFSA notes that the 2017 PMEM report represents the twelfth reporting year and that a total of 3,127 questionnaires have been completed since 2006, exceeding the target sample size of 2,500 questionnaires deemed necessary for statistical analysis. Consequently, EFSA reiterates the need to pool the data obtained over this 12-year period and perform a proper analysis of the combined data sets.

EFSA advises that future literature searches on maize MON 810 performed in the context of annual PMEM reports comply with EFSA's updated explanatory note on literature searching.

Although general surveillance should make use of existing networks involved in environmental monitoring as an additional tool for the assessment of potential unanticipated effects arising from the cultivation of GM plants, no information about the use of such networks is provided in the 2017 PMEM report. In this respect, EFSA reiterates that relevant stakeholders should implement a methodological framework to enable making best use of such networks.

Overall, EFSA concludes that the evidence reported in the 2017 PMEM report does not invalidate previous EFSA and GMO Panel evaluations on the safety of maize MON 810, and notes the lack of sensitivity of the insect resistance monitoring put in place.

Table of contents

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* (Lefèbvre) (Lepidoptera: Noctuidae).

The cultivation of maize MON 810 was authorised under Directive 90/220/EEC in the European Union (EU) by the Commission Decision 98/294/EC of 22 April 1998.[2]

Since 2003, the transformation event MON 810 has been introduced into a wide range of maize varieties grown in the EU. In 2017, maize MON 810 was cultivated in Spain (124,227 ha) and Portugal (7,308 ha) over a total area of 131,535 ha (ISAAA, 2017).[3]

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) defined *a management strategy in order to minimise the development of insect resistance, and offered to inform the Commission and/or Competent Authorities of Member States of the results of monitoring of this aspect*.

Since 2003, the consent holder has followed the harmonised insect resistance management (IRM) plan developed by EuropaBio[4] for single lepidopteran-active *Bt*-maize events (Alcalde et al., 2007), which was updated in 2017 (EuropaBio, 2017), 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 individuals of the 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 (i.e. blocks or strips of non-*Bt*-maize that are located near (within 750 m), within or adjacent to the *Bt*-maize field) is required where the target insect pest does not encounter the *Bt*-protein, and which therefore acts as a reservoir of susceptible individuals.[5]

As part of the IRM plan, monitoring of resistance evolution and refuge compliance 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 field populations in laboratory bioassays; and (2) monitoring of unexpected field damage caused by ECB/MCB through a farmer complaint 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 refuge requirements and carry out the operational details of IRM plans is one of the contributing factors to the field-evolved resistance to certain *Bt*-crops (reviewed by Tabashnik et al., 2013; Tabashnik and Carrière, 2017). Grower education (training) and information programs are 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) for all market applications for deliberate release submitted under Directive 2001/18/EC and Regulation (EC) No 1829/2003 (including the pending application for the renewed market authorisation for cultivation of maize MON 810). This general surveillance aims at detecting unanticipated adverse effects associated with the commercial use of GM plants. General surveillance activities include surveys

[2] 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.

[3] 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; Castañera et al., 2016).

[4] http://www.europabio.org/ (Accessed 16 May 2019)

[5] 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 meters 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.

based on questionnaires from EU farmers growing maize MON 810 and extensive/systematic literature searches to find relevant scientific publications.

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 monitoring resistance evolution and refuge compliance), 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. Annual PMEM reports on maize MON 810 have been assessed by EFSA since 2010 (EFSA GMO Panel, 2011a, 2012a, 2013, 2014a, 2015a,b, 2016, 2017; EFSA, 2018). Based on the data provided in the previous 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, EFSA and its GMO Panel noted shortcomings in the methodology for case-specific monitoring and general surveillance and made several recommendations to improve future PMEM reports on maize MON 810. Some of the recommendations on insect resistance monitoring were incorporated by EuropaBio in the updated IRM plan (EFSA, 2018).

1.1. Terms of Reference as provided by the requestor

On 17 October 2018, the European Commission received from the consent holder the annual PMEM report for the 2017 growing season of maize MON 810 (hereafter referred to as 2017 PMEM report). The reporting period of the 2017 PMEM report is July 2017 till July 2018.

On 29 November 2018, the European Commission mandated EFSA *to assess the 2017 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 considered the information provided in the 2017 PMEM report[6] as well as additional information on insect resistance management and literature searching provided by the consent holder upon EFSA's request, comments submitted by the EU Member States and relevant scientific publications.

2.2. Methodologies

EFSA assessed the evidence contained in the 2017 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 2017 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 extensive/systematic literature search in accordance with the relevant principles and criteria outlined in EFSA (2010) and the recommendations given in EFSA (2017).

EFSA followed the principles and approach described in the guidance on the use of the weight of evidence approach in scientific assessments (EFSA Scientific Committee, 2017)

The comments raised by the EU Member States, which are addressed in the supporting information of this statement, were considered during the scientific assessment.

[6] The 2017 PMEM report is publicly available at https://ec.europa.eu/food/plant/gmo/reports_studies/report_2017_mon_810_en (Accessed 16 May 2019).

3. Assessment

3.1. Case-specific monitoring

3.1.1. Implementation of non-*Bt*-maize refuges[7]

3.1.1.1. Consent holder's assessment

Compliance with non-*Bt*-maize refuge requirements was assessed through the farmer questionnaires supplied as part of the general surveillance (Section 3.2.1). In 2017, 236 farmers from Spain and 14 farmers from Portugal completed a questionnaire which included one question on compliance with the refuge strategy, i.e. *Did you plant a refuge in accordance to the technical guidelines?*

a) Spain

In Spain, 219 out of the 236 farmers growing maize MON 810 stated that they complied with refuge requirements, either because they did implement a refuge (200 farmers) or because they planted less than 5 ha of maize MON 810 and were thus required to plant a refuge (19 farmers, i.e. 8% of the farmers surveyed) (Appendix A).

The 17 farmers that did not plant a refuge despite cultivating 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 (8 farmers); (2) she/he had two or three plots smaller than 5 ha (5 farmers); and (3) the refuge was smaller than 20% of maize MON 810 area (4 farmers).

The locations of the *Bt*-maize fields and total number of farmers where no refuges were planted were provided by the consent holder upon EFSA's request: Huesca (11 farmers) and Lleida (2 farmers) – North-eastern Spain; Badajoz (3 farmers) and Sevilla (1 farmer) – South-eastern Spain.

b) Portugal

In Portugal, the 14 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 78 farms (out of the 213 *Bt*-maize cultivation notifications received in 2017) where maize MON 810 was grown to check compliance with refuge and coexistence requirements outlined in Portuguese law (DGAV, 2017). Based on these inspections, the Portuguese authorities concluded that there was full compliance with refuge and labelling requirements.

Based on the compliance monitoring data, the consent holder concluded that *the results from the presented surveys (. . .) during the 2017 season are consistent and do show a high level of compliance*.

3.1.1.2. EFSA's assessment

The 2017 compliance monitoring data show partial compliance (92%) with refuge requirements in Spain and full compliance in Portugal, as reported in previous years (see Appendix A). Ensuring compliance with refuge requirements is crucial to sustain the efficiency of the technology and delay resistance evolution, especially where adoption of *Bt*-maize is high (e.g. Tabashnik et al., 2013; Castañera et al.,2016). Consequently, EFSA considers that the consent holder should strive to increase the level of compliance in high adoption areas (North-eastern Spain, see Appendix B). Spanish National Competent Authorities and other relevant stakeholders, including farmers' associations, could contribute to reinforce farmers' awareness of refuge compliance.

EFSA reiterates that refuge requirements 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 can 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 (8%), the proportion of farmers planting less than 5 ha of maize MON 810 (8%) in Spain, and the recent

[7] 2017 PMEM report: Section 3.2.1.1, Appendix 1, additional information: 1/4/2019.

findings reported by Camargo et al. (2018) on the frequency of Cry1Ab resistance alleles in MCB populations in the Ebro Valley,[8] 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 the consent holder and EU Member States where maize MON 810 is grown to develop appropriate information systems on GM crop cultivation to ensure that structured refuges are planted in clustered areas greater than 5 ha.

3.1.2. Insect resistance monitoring[9]

3.1.2.1. Consent holder's assessment

In line with the updated IRM plan, the 2017 resistance monitoring activities targeted North-eastern Spain where the adoption rate of maize MON 810 exceeds 60% (Appendix B). The susceptibility of collected ECB and MCB populations to the Cry1Ab protein was tested in diagnostic bioassays.

European corn borer monitoring

a) Field sampling and laboratory rearing

In 2017, 1,111 ECB late-instars from the last generation were collected at the end of the maize growing season from eight sampling sites (refuges and non-*Bt*-maize fields) located in three zones across North-eastern Spain (for more details, see Appendix C). Ten additional sites were sampled, but the minimum number of larvae established in the study protocol could not be reached for these sites.

Field-collected larvae were shipped to the laboratory (BTL GmbH, Sagerheide, Germany), where their progeny was tested for susceptibility to Cry1Ab. Larvae were reared following a standardised protocol (Thieme et al., 2018). A total of 628 larvae reached the adult stage (57% 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 to evaluate potential changes in the biological activity of the test substance. Both strains have been reared in the laboratory since their establishment on non-*Bt*-diet, i.e. without any exposure to maize MON 810 or Cry1Ab, and have never been invigorated.

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 both reference strains.

Diagnostic bioassay: The bioassay was conducted using neonates obtained from the progeny of field-collected insects (F_1-larvae). Purified Cry1Ab protein[10] at a diagnostic concentration of 28.22 ng Cry1Ab/cm^2 of diet surface area was used in an artificial diet-overlay assay. The selected concentration corresponds to the mean 99% moulting inhibition concentration (MIC_{99}) estimated with data pooled from ECB populations collected in the Czech Republic, France, Germany, Hungary, Italy,

[8] Camargo et al. (2018) estimated the frequency of Cry1Ab resistance alleles in MCB populations collected from North–eastern Spain during the 2016 season, using a F_2-screen assay (Andow and Alstad, 1998). Three hundred and eighty-five lines were initially established, and one of the 137 lines screened was considered to carry a major resistance allele. 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 0–8.6×10^{-3}), calculated from MCB populations collected in 2004/2005 (Andreadis et al., 2007). However, both estimates are not significantly different because of the few numbers of lines screened between 2004 and 2005. 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 North-eastern Spain; 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.

[9] 2017 PMEM report: Sections 3.2.1.2 and 3.2.1.3, Appendices 6–8, additional information: 1/4/2019.

[10] Batch 2b was used: 1.64 mg Cry1Ab/mL in 50 mM bicarbonate buffer; pH 10.25; 91% purity.

Poland, Portugal, Romania and Spain between 2005 and 2012.[11] This diagnostic concentration was validated by testing several ECB populations collected in Spain in 2013, 2014 and 2015 since in all validation assays moult inhibition values were higher than the expected > 99% (EFSA, 2018).

In the 2017 bioassay, 1,488 neonates were tested against the diagnostic concentration. One hundred and ninety larvae 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 7 days. None of the reference strains were included in the diagnostic bioassay.

Moulting inhibition of ECB larvae tested against Cry1Ab was 99.19%, whereas moulting inhibition in the control group was 1.05% (see Table 1). The study authors indicated that *evidence for a decrease of Cry1Ab susceptibility of ECB during the monitoring duration could not be detected*. This value is similar to that reported in the 2016 growing season (Appendix D).

Table 1: Moulting inhibition (%) of *Ostrinia nubilalis* (ECB) larvae at a diagnostic concentration of Cry1Ab protein: 2017 field populations [Table based on data provided in the 2017 PMEM report]

Sampling area	Treatment % Moulting inhibition (No. of larvae tested)	
	Control	Cry1Ab[(b)]
North-eastern Spain[(a)]	1.05[(c)] (190)	99.19[(d)] (1,488)

ECB: European corn borer; PMEM: post-market environmental monitoring.
(a): Emerging adults from the different sampling zones were pooled for mating and a single bioassay was performed with their progeny.
(b): A diagnostic concentration of 28.22 ng Cry1Ab/cm^2 of diet surface area was used.
(c): Of the 190 larvae tested, one larva died, and one larva did not develop to second instar, whereas the rest (188) moulted to other instars.
(d): Of the 1,488 larvae tested, 138 larvae died, 1,338 larvae survived but did not moult to the second instar and 12 larvae moulted.

Bioassay with maize MON 810 leaves: A follow-up study using maize MON 810 leaves (variety DKC5277YG) was conducted with the 12 larvae that reached the second instar in the diagnostic bioassays to confirm that they were not potentially resistant to Cry1Ab. Surviving larvae were placed individually on maize MON 810 leaf discs and mortality was assessed after 7 days of exposure. The negative control group consisted of neonates from the North-eastern Spain field population fed non-*Bt*-maize leaves (variety Golden Bantam) for 7 days. Cry1Ab expression was not measured in the maize plants used in the bioassay.

All ECB larvae fed maize MON 810 leaves died within 5 days. From the 192 larvae fed non-GM maize leaves, three larvae died (1.56%), 11 larvae reached the second instar (5.73%) and 178 larvae moulted to third instar (92.71%) 7 days after the bioassay started.

Concentration–response assays: The susceptibility of the two reference strains 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 7 days of exposure. MIC_{50} and MIC_{90} values, with a 95% confidence interval (CI), were estimated by probit analysis (Robertson et al., 2007).

MIC_{50} and MIC_{90} values estimated in 2017 for one of the reference strains (ECB-G.04) were higher than those obtained in previous years, and a 49- and 38-fold difference has been observed between the values reported in 2014 and 2017 (Appendix E).

The consent holder provided no raw data from the bioassays conducted with ECB.

[11] The 99% moulting inhibition concentration (MIC_{99}) of these populations corresponds to 48.22 ng Cry1Ab/cm^2 of diet surface area. Due to a change in the protein batch in 2012, the diagnostic concentration was recalibrated, resulting in a MIC_{99} value of 28.22 ng Cry1Ab/cm^2 of diet surface area.

Mediterranean corn borer monitoring

a) Field sampling and laboratory rearing

In 2017, 1,452 MCB late-instars from the last generation were collected at the end of the maize growing season from 17 sampling sites (refuges and non-*Bt*-maize fields) in three zones across the Ebro Valley (for more details, see Appendix C). Attempts were made to collect larvae from two additional sites, but the minimum number of larvae established in the IRM study protocol could not be reached for these sites.

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 788 larvae reached the adult stage (54% of the field-collected larvae) and were placed in 62 oviposition cages for mating. Emerging adults from the different sampling zones were kept separately. Fifty-six cages, containing 749 adults were used to obtain F_1-progeny for the diagnostic bioassay (i.e. 52% of the field-collected larvae).

In addition, a reference (susceptible) strain established from approximately 3,000 larvae collected from Spain in 1998 was used as an additional negative control. The strain has been reared in the laboratory on non-*Bt* diet ever since its establishment. To preserve the reference strain from excessive inbreeding, it has been refreshed periodically with the addition of new field-collected individuals.[12] This strain was not invigorated in 2017.

A new population of MCB has been established with larvae collected in 2018 from Galicia (Spain), where *Bt*-maize has never been grown. The consent holder intends to use this MCB population, which has never been subjected to Cry1Ab selection pressure, as reference population in future monitoring bioassays.

b) Monitoring assays

The consent holder performed: (1) a diagnostic bioassay with the field-collected populations to detect potential increases in resistance allele frequency; (2) an additional bioassay with maize MON 810 leaves; and (3) concentration–response assays with the reference strain established in 1998.

Diagnostic bioassays: Independent diagnostic bioassays were performed with the progeny of field-collected larvae (from three sampling zones) exposed 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.[13] The selected diagnostic concentration corresponds to the upper limit of the 95% CI of the MIC_{99} estimated with data pooled from MCB populations collected in non-*Bt*-maize fields from North-eastern Spain over 2009, 2011, 2013 and 2015. The reference strain was tested against the diagnostic concentration.

In the 2017 assays, between 1,048 and 1,174 neonates per sampling zone 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 7 days.

For all three zones, moulting inhibition was lower than the expected 99%, whereas in the control treatments it ranged between 1.71% and 15.09%. Moult inhibition observed in the laboratory reference strain was 97.69% (see Table 2).

Average moulting inhibition of the progeny of field-collected larvae (94.14 $\pm$ 1.40%) was significantly lower than the expected 99% ($t = -3.4647$; degrees of freedom (df) = 2; $p = 0.037$). No statistically significant differences were observed in moulting inhibition between the reference strain (97.69%) and the field-collected larvae ($t = -2.5373$; df = 2; $p = 0.063$).

[12] 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). Each time the strain was refreshed, between 10% and 15% of new field-collected individuals were added to the reference strain. The similarity in susceptibility to Cry1Ab was verified before adding new individuals.

[13] Batch B2-4 was used: 1.8 mg Cry1Ab/ml in 50 mM sodium bicarbonate buffer; pH 10.25; purity 91%.

Table 2: Moulting inhibition (%) of *Sesamia nonagrioides* (MCB) larvae at a diagnostic concentration of Cry1Ab protein: 2017 field populations [Table based on data provided in the 2017 PMEM report]

Sampling area		Treatment % Moulting inhibition (No. of larvae tested)	
		Control	Cry1Ab[(a)]
North-eastern Spain	Zone 1	1.71 (175)	91.65 (1,048)
	Zone 2	15.09 (159)	96.50 (1,111)
	Zone 3	6.25 (160)	94.28 (1,174)
	Total	7.68 ± 3.9[(b)] (494)	94.14 ± 1.4[(b)] (3,333)
Reference strain		14.01 (157)	97.69 (654)

MCB: Mediterranean corn borer; PMEM: post-market environmental monitoring.
Statistically significant differences were observed between the North-eastern population and the expected value of 99% ($t = -3.4647$; $df = 2$; $p = 0.037$).
No statistically significant differences were observed between the north-eastern population and the reference strain ($t = -2.5373$; $df = 2$; $p = 0.063$).
(a): A diagnostic concentration of 1,091 ng Cry1Ab/cm^2 of diet surface area was used. Values have been corrected using Abbott's formula (Abbott, 1925).
(b): Mean ± standard error.

Bioassay with maize MON 810 leaves: An additional bioassay was conducted with field populations using maize MON 810 leaves (variety DKC6451YG). To this end, 10,650 F_1-first instars not used in the diagnostic bioassays (approximately 200 larvae per oviposition cage) were fed maize MON 810 leaves. Larvae were placed in plastic boxes containing leaves of maize MON 810 (variety DKC6451YG). Larvae were fed *ad libitum* for 10 days and moulting to the second instar was recorded. A negative control group, consisting of 426 larvae fed non-*Bt*-maize leaves (variety DKC6450) (approximately 10 larvae per cage), was included in the study. Cry1Ab expression was not measured in the maize plants used in the bioassay.

Ten (0.09%) of the MCB larvae fed maize MON 810 leaves moulted to second instar (moulting in the control group resulted in 95.3%). To confirm that these larvae were not resistant to maize MON 810, siblings from the same oviposition cage were reared on artificial diet during the F_1 generation, and 1,000 F_2 neonates were fed maize MON 810 leaves. No F_2-larvae moulted to second instar or survived after 10 days. As a negative control, > 200 larvae from different oviposition cages were fed conventional maize for 7 days. The consent holder reported that at the end of the bioassay no signs of mortality were observed and that *most of the larvae* had moulted to the second or third instar.

Concentration–response assays: Concentration–response assays were performed with the reference strain. Six concentrations, ranging from 4 to 128 ng Cry1Ab/cm^2 of diet surface area, 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 per concentration including the control. Each replicate consisted of 32 larvae (64 for the controls), giving a total of 96 larvae tested for each concentration (192 for the controls). Moulting inhibition was assessed after 7 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 2017 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 E.

The consent holder provided no raw data from the bioassays conducted with MCB.

Farmer complaint system

The consent holder and other companies marketing maize MON 810 seeds have implemented a farmer complaint system allowing farmers to report complaints about product performance (including unexpected crop damage caused by or failure in protection against target pests in maize MON 810 varieties).

The consent holder states that, during the 2017 growing season, no complaints related to corn borer infestation of maize MON 810 were received.

The consent holder also refers to a survey conducted by member companies of the National Breeder Association in Spain[14] marketing maize MON 810 to have an overview of the farmer complaint schemes. None of the 1,703 complaints received by companies marketing maize MON 810 in 2017 were attributed to loss of efficacy of the GM maize.

3.1.2.2. EFSA's assessment

European and Mediterranean corn borer resistance monitoring

a) Field sampling and laboratory rearing

For the second consecutive growing season, the consent holder implemented the harmonised IRM plan updated in 2017 (EuropaBio, 2017). The IRM plan was revised in regard to the sampling strategy and monitoring protocol of ECB and MCB populations, accounting for some of the previously recommendations made by EFSA (e.g. EFSA, 2015a; EFSA GMO Panel, 2017), the experience gained with the implementation of the initial IRM plan (Farinós et al., 2018; Thieme et al., 2018), and relevant scientific publications.

The sampling scheme of the updated IRM plan establishes that ECB and MCB populations should be monitored 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. Consequently, in 2017, ECB and MCB populations were collected exclusively from three sampling zones in North-eastern Spain. Currently, this area is the only hotspot area for resistance evolution in the EU where more than 60% of the total maize acreage corresponds to maize MON 810 hybrids (Appendix B; Castañera et al., 2016; Farinós et al., 2018) and where ECB and MCB populations are multivoltine as they complete two generations annually (Alfaro, 1972).

ECB and MCB populations were collected from refuges and non-*Bt*-maize fields. In 74% and 53% of the sampling sites that were inspected, none or very few numbers of ECB and MCB larvae were found, respectively. EFSA notes that the numbers of ECB (1,111) and MCB (1,452) larvae collected reached the target sampling size of 1,000 larvae (corresponding to 2,000 genomes) established in the 2017 IRM plan. EFSA acknowledges the efforts made by the consent holder to achieve the target sampling size, and recognises that this might not always be possible due to several factors such as natural fluctuation in pest density, environmental conditions and regional pest suppression (Dively et al., 2018). Although the consent holder underlines the increasing difficulties to find different fields infested with ECB larvae for sampling, EFSA is not aware of any evidence of area-wide corn borer suppression in North-eastern Spain.

EFSA notes that overall the pre-imaginal mortality values reported for both target pests during laboratory rearing of field-collected individuals are high; only 56.5% of and 54% of the ECB and MCB larvae collected in the fields reached adulthood. This means that over half of the field-collected larvae were represented in the diagnostic concentration assays, which prevents from reaching the recommended detection level of 3% (recessive) resistance allele frequency to timely detect a possible insurgence of field resistance. While mortality values reported for ECB in 2017 were similar to those recorded in previous reports, mortality values for MCB were much higher in 2017, i.e. 30% in 2016 and 16% in 2015. The consent holder did not explain the reasons for this increase in MCB mortality. Because field-collected individuals are not adapted to environmentally controlled conditions and to feeding on meridic diets, some mortality is unavoidable under laboratory rearing conditions. Nevertheless, EFSA encourages the consent holder to undertake measures to optimise the rearing process of collected individuals and reduce the pre-imaginal mortality prior to susceptibility testing, so that as many field-collected individuals as possible are represented in the bioassays as F_1-larvae.

b) Monitoring assays

Since the 2016 growing season, susceptibility of target pests to the Cry1Ab protein is assessed by conducting diagnostic bioassays with F_1-progeny larvae from the field-collected individuals, instead of concentration–response assays.[15] EFSA already agreed with the principles driving the revision of the testing approach previously proposed by the consent holder but expressed reservations on the actual implementation and made considerations regarding the design of the diagnostic bioassays, the

[14] Asociación Nacional de Obtentores Vegetales (ANOVE): http://web.anove.es/ (Accessed 16 May 2019)

[15] Concentration–response bioassays were conducted with the progeny of ECB and MCB larvae collected in North-eastern, Central and South-western Spain between 2004 and 2015. The analyses of data sets did not indicate a decrease in susceptibility to the Cry1Ab protein in the ECB and MCB populations tested. Historical results of the bioassays with the larvae collected in North-eastern Spain are shown in Appendix E.

selection of the diagnostic concentrations and the follow-up studies performed with suspected-resistant individuals (EFSA, 2018). Also, EFSA encouraged the consent holder to continuously improve the IRM plan and consider alternative testing methods. EFSA notes that most of these recommendations have not been implemented by the consent holder in the 2017 PMEM report.

Design of diagnostic bioassays: Like in the previous PMEM report, EFSA notes that the methodology for the diagnostic bioassays differs between ECB and MCB. Whereas ECB individuals from the different sampling zones are pooled and a single diagnostic bioassay is conducted with F_1-larvae, MCB larvae from each zone are kept separately and independent bioassays are performed. Moreover, the diagnostic bioassays with MCB include a reference strain serving as an negative control and additional point of comparison. For ECB, EFSA notes that a Cry1Ab concentration corresponding to the diagnostic concentration was tested in both reference strains in the concentration–response bioassays; yet, moult inhibition at that concentration is not reported by the consent holder.

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 because, in case of suspected or confirmed resistance of larvae from a particular zone, this approach would allow collecting individuals from the same zone in the following seasons. EFSA also recommends that the consent holder either includes a reference susceptible strain in future diagnostic bioassays with ECB, or reports moult inhibition at the diagnostic concentration tested in concentration–response assays. For both target pests, reference strains should be used as a quality control instead as an additional point of comparison for field populations. In this regard, moulting inhibition observed in diagnostic bioassays in field-collected ECB and MCB populations should not be compared with the reference strain but only with the expected 99% (see *Testing approach*, below).

Selection of diagnostic concentrations: During the assessment of the 2016 PMEM report, EFSA noted that the concentrations selected for discriminating between susceptible and resistant individuals in diagnostic bioassays were estimated using data that included ECB and MCB populations exposed to *Bt*-maize hybrids and thus subjected to selection pressure. For MCB, the diagnostic concentration was recalculated in 2016 using only data of larvae collected from North-eastern Spain over recent years that were subjected to very high selection pressure (for further details, see EFSA, 2018). Although requested by EFSA, the consent holder did not provide additional evidence to underpin the appropriateness of the diagnostic concentration selected for MCB. Consequently, EFSA reiterates its recommendation that the validity of the concentration should be confirmed by comparing it with data generated with MCB larvae collected from areas with low or no selection pressure.

Testing approach: In the diagnostic concentration assays with F_1-larvae of MCB populations collected from zones 1, 2 and 3 of North-eastern Spain, corrected moult inhibition values are 91.65%, 96.50% and 94.28% and the mean (94.14%) is statistically significantly lower than the expected 99%. EFSA considers that moulting inhibition values lower than the expected >99% in the diagnostic bioassay should be regarded as statistically (although not necessarily biologically) significant, and that any population showing unusually low sensitivity to the Cry1Ab protein should be further investigated to determine if the population has field-relevant resistance to the trait. EFSA recommends that the consent holder standardises the testing approach for confirming resistance of suspected populations and adapts the harmonised IRM plan accordingly. In this respect, the step-wise approach recommended by the US Environmental Protection Agency for confirming resistance of lepidopteran pests of *Bt*-plants (US EPA, 2010, 2018) could be applied by the consent holder to the ECB and MCB resistance monitoring program (Figure 1).

EFSA notes that the detection limit for resistance allele frequency achieved in the diagnostic bioassays was higher than the recommended 3% for both target pests. Consequently, EFSA strongly recommends the consent holder to increase the sensitivity and precision of the monitoring strategy so that alternative management measures can be implemented timely to delay resistance evolution. This could be achieved by (1) increasing the sampling size of field populations and/or reducing the mortality during the laboratory rearing of field-collected populations or (2) replacing diagnostic bioassays by more sensitive testing methods (see *Alternative testing methods*, below).

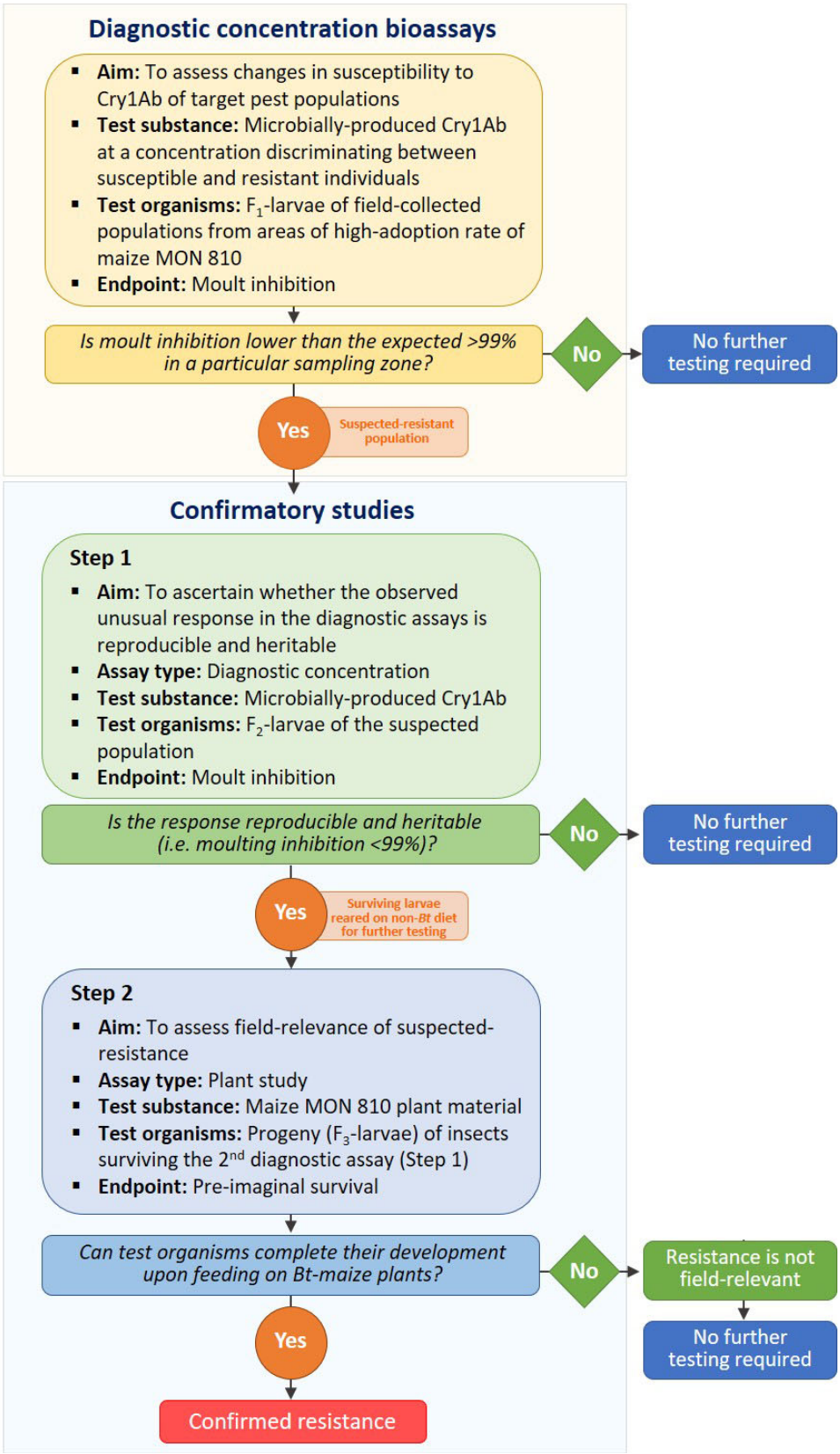

Figure 1: Proposed step-wise approach for confirming resistance to *Bt*-plants of suspected resistant populations. Adapted from US EPA (2010, 2018). Once resistance is confirmed, the insect resistance management plan foresees the implementation of a remedial action plan (EuropaBio, 2017)

Bioassays with plant material: Additional bioassays using maize MON 810 leaves were performed with those ECB larvae that survived the diagnostic concentration as well as with the spare MCB larvae that were not used in the bioassays *to confirm that resistant individuals were not present in the field-collected populations*. EFSA recognises the value of conducting such studies with plant material but considers that they should rather be performed in cases of suspected resistance with progeny of larvae surviving the diagnostic bioassays, following the step-wise approach presented in Figure 1.

EFSA identifies methodological differences between the additional studies conducted with the two species (e.g. experimental arenas, test material, test duration) and advocates harmonising their methodology.

EFSA acknowledges that some of its previous recommendations made to increase the reliability of the studies with plant material, including the use of a negative control (non-*Bt*-maize leaves) (EFSA, 2018), have been implemented by the consent holder. Yet, EFSA notes that the negative control used in the follow-up plant bioassay with ECB is not the most suitable because it consisted of neonates from the field-collected population and not of surviving larvae from the control group of the diagnostic bioassay (see Section 3.1.2.1).

EFSA also recommends the consent holder to confirm the expression of the Cry1Ab protein (e.g. by using commercial test strips) in the *Bt*-maize leaves used in future studies with plant material.

Alternative testing methods – F_2-screen: EFSA recommends the consent holder to consider 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). In Europe, F_2-screen has been used to estimate the upper 95% CI for Cry1Ab-resistance allele frequencies in several ECB (Bourguet et al., 2003; Engels et al., 2010) and MCB populations (Andreadis et al., 2007) when establishing baseline susceptibility data. More recently, Camargo et al. (2018) re-estimated the resistance allele frequency of MCB populations from North-eastern Spain 11 years after the initial estimation by Andreadis et al. (2007), and after almost 20 years of continuous cultivation of *Bt*-maize hybrids in that area. A Cry1Ab-resistance allele was identified in one of the 137 F_2-lines tested (see discussion in Section 3.2.3.2).

EFSA is aware that the F_2-screen is resource intensive (Andow and Alstad, 1998; Huang et al., 2012), presents technical limitations (Siegfried et al., 2007; Siegfried and Spencer, 2012) and is only being implemented routinely in the resistance management plan for *Bt*-cotton in Australia (Downes and Mahon, 2012; Downes et al., 2016). Yet, as recommended previously, EFSA still considers that a F_2-screen should be performed periodically with ECB and MCB populations to confirm the results of the diagnostic bioassays; to assess whether the frequency of the Cry1Ab resistance allele is $< 10^{-3}$ in order to confirm one of the key assumptions of the 'high dose/refuge' strategy; and to monitor whether the frequency of the Cry1Ab resistance allele is evolving as predicted by resistance evolution models. Ultimately, periodic estimations of resistance alleles through F_2-screening could replace annual diagnostic concentration assays. To obtain sufficient sensitivity for detecting resistance alleles before they become common enough to cause measurable field damage, the target for testing should be at least 100 lines.

Considering the time elapsed since the last estimation of the frequency of resistance alleles in MCB populations from North-eastern Spain by Andreadis et al. (2007) and given the findings reported by Camargo et al. (2018), EFSA considers that it is timely to perform a F_2-screen on MCB populations from the same area where the Cry1Ab resistance allele was detected by Camargo et al. (2018) as well as on ECB populations from North-eastern Spain, where the frequency of resistance alleles has never been estimated.

In case that *Bt*-resistant laboratory strains of ECB and MCB were available or could be obtained by laboratory selection, an alternative would be to perform a F_1-screen. This technique consists of crossing field-collected individuals (of unknown genotype) with homozygous resistant individuals in single pairs and subsequently screening the F_1 offspring for resistance using *Bt*-plant material or a diagnostic concentration (Gould et al., 1997). The F_1-screen is considered more efficient and less resource intensive than the F_2-screen for detecting and monitoring rare *Bt*-resistance alleles in field populations of target pests (Liu et al., 2008).

Reference strains: EFSA acknowledges the establishment of a new MCB population originated from individuals collected in Galicia, where *Bt*-maize has never been grown and thus target pests are subjected to a low selection pressure, to use it as a reference strain for future bioassays.

Reporting of monitoring data: To ease the appraisal of the quality of resistance monitoring assays, sufficient information should be reported. In this respect, EFSA developed a list of recommendations (presented as a checklist in Appendix F of this statement) that aim at improving the reporting of future

resistance monitoring assays. The checklist focuses on several elements relevant for the evaluation of study design and the interpretation of results, and it was first published as part of EFSA's assessment of the 2016 PMEM report (EFSA, 2018). Study authors should consider these recommendations when preparing the reports of resistance monitoring assays; they are encouraged to provide a rationale whenever a reporting recommendation cannot be met. This checklist has been updated in this statement to address recurrent reporting issues identified by EFSA.

The consent holder did not provide raw data of the different bioassays conducted with both target pests, even though the submission of raw data has been previously requested by EFSA. The consent holder argued that *the data provided with 2017 ECB and MCB bioassays report (See Appendix 7 and Appendix 8 of the 2017 monitoring report) are considered sufficient to assess the quality and accuracy of the bioassays*. EFSA does not accept this rationale and considers that raw data are necessary to further evaluate and verify data quality.

Farmer complaint system

EFSA considers that the farmer complaint system can be 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 product performance, and thus provides an additional source of first-hand information. However, at present, EFSA is not in the position to evaluate the usefulness of this farmer complaint system as complementary resistance monitoring tool. In particular, the consent holder should provide more information to determine whether proper communication mechanisms and fit-for-purpose educational programs 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 does not indicate a decrease in susceptibility to the Cry1Ab protein in the ECB populations collected from North-eastern Spain during the 2017 maize growing season. For MCB, moulting inhibition observed in the diagnostic concentration bioassays is lower than the expected > 99% in the three populations tested. Additional studies with plant material indicate that none of the MCB larvae tested from any of the three populations could complete development on maize MON 810 leaves; however, EFSA considers that a proper follow-up study should have been conducted to ascertain whether the observed unusual response in the diagnostic assays was reproducible and heritable and, if so, to assess the field-relevance of the suspected-resistant populations following the approach presented in Section 3.1.2.2.

Considering the estimated numbers of ECB and MCB field-collected larvae represented in the diagnostic concentration bioassays, the monitoring strategy implemented in the 2017 growing season is not sensitive enough to detect the recommended 3% resistance allele frequency. Consequently, EFSA strongly recommends the consent holder to increase the sensitivity and precision of the monitoring strategy so that alternative management measures can be implemented timely to delay resistance evolution.

EFSA considers that it is timely to perform a F_2-screen on MCB populations from the same area where the Cry1Ab resistance allele was detected by Camargo et al. (2018) as well as on ECB populations from North-eastern Spain, where the frequency of resistance alleles has never been estimated. EFSA also notes that several other recommendations to resolve previously identified shortcomings and to improve the monitoring plan have not been followed by the consent holder. These are summarised in Section 5.

3.2. General surveillance

3.2.1. Farmer questionnaires[16]

3.2.1.1. Consent holder's assessment

In the annual 2017 PMEM report, the consent holder submitted a survey based on 250 farmer questionnaires completed by farmers in Spain and Portugal (Table 3). Both countries accounted for 100% of the maize MON 810 grown in the EU in 2017.

The 2017 PMEM report represents the twelfth reporting year, with the completion of a total of 3,127 questionnaires since 2006.

[16] 2017 PMEM report: Sections 3.1.2.1 and 3.1.4.1, Appendixes 1 and 2.

The surveys were performed in each country by an external company and were completed between January and March 2018. The response rate was approximately 47% in Spain,[17] and 100% in Portugal. Out of 250 farmers, 103 (41%) were interviewed for the first time.

Table 3: Farmers surveyed and maize MON 810 areas monitored in 2017 through questionnaires [Table based on data provided in the 2017 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	236[(a)]	33.8	7,977	124,227	6.4
Portugal	14[(b)]	75.0	1,050	7,308	14.4
Total	**250**	**36.1**	**9,027**	**131,535**	**6.9**

PMEM: post-market environmental monitoring.
(a): One hundred and sixty-nine farmers were from Aragón/Cataluña, 27 from Extremadura, 15 from Andalucía, 15 from Comunidad Foral de Navarra and 10 from Castilla la Mancha. Out of 236 farmers, 97 were interviewed for the first time.
(b): Six farmers were from Alentejo, 5 from Lisbon and Vale do Tejo and 3 from Center. Out of 14 farmers, 6 were interviewed for the first time.

The questionnaire was designed to collect information 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. Overall, the questionnaire aimed at identifying unintended effects caused by the cultivation of maize MON 810.

The consent holder concluded that *the results of the analysis of the 2017 farmer questionnaires on maize MON 810 did not identify potential adverse effects that might be related to MON 810 plants and their cultivation*.

3.2.1.2. EFSA's assessment

The farmer questionnaires and the approach followed to identify unanticipated adverse effects potentially caused by the cultivation of maize MON 810 are similar to those from previous annual PMEM reports. EFSA therefore reiterates previous observations on the methodology (e.g. sampling, comparator (non-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 2017 PMEM report, EFSA considers that the data from the farmer questionnaires should be pooled for statistical analysis when the target sample size of 2,500 questionnaires is obtained. EFSA has made this consideration since the assessment 2015 PMEM report (EFSA GMO Panel, 2016).

The 2017 PMEM report represents the 12th reporting year, with the completion of a total of 3,127 questionnaires since 2006. However, a pooled analysis of all the data has not yet been provided by the consent holder or reported in the scientific literature. The statistical analysis of the pooled data 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 only manifest following repeated cultivation of a GM crop, and so amendments to the study design and the analysis plan should be considered to assess potential effects due to successive years of GM crop cultivation.

EFSA recommends that the data obtained over this 12-year period are pooled and appropriately analysed. 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 1 year in the survey (and the enumeration of these farmers in the report) and the interim analyses performed for the annual reports.

[17] The questionnaire was completed by 236 out of the 502 farmers that were contacted in Spain. The 266 farmers that did not respond gave the following reasons: (1) because they did not grow maize MON 810 in 2017 (86 farmers); (2) they did not grow maize in 2017 (61 farmers); (3) they grew maize MON 810 in 2017 but refused to sign the consent form (45 farmers); (4) they grew MON810 in 2017 but refused to answer the interview (43 farmers); (5) they were absent or could not be localized (18 farmers); (6) they were retired (13 farmers).

3.2.1.3. Conclusions on farmer questionnaires

EFSA considers that an assessment of the pooled data is needed to confirm that no unintended effects caused by the cultivation of maize MON 810 have been observed and to appraise the reliability/ methodological quality of the farmer questionnaires.

3.2.2. Existing monitoring networks[18]

Directive 2001/18/EC and Council Decision 2002/811/EC propose to make use of existing networks involved in environmental monitoring 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. The consent holder stated that *the value of using the reports of existing environmental networks to confirm the safety of GM crops in general and MON 810 in particular was assessed, but were considered of less additional value than the other approaches*.

3.2.2.2. EFSA's assessment

In an external report commissioned by EFSA (Centre for Ecology and Hydrology et al., 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 considers that relevant stakeholders should implement a methodological framework that enables to make best use of existing networks involved in environmental monitoring.

3.2.3. Literature searching[19]

3.2.3.1. Consent holder's assessment

The consent holder performed a systematic literature search to find publications relevant to the food and feed and environmental safety assessment of maize MON 810 and the Cry1Ab protein published between June 2017 and May 2018.

As information sources, the electronic bibliographic databases Web of Science (WoS) Core Collection and CAB Abstracts, hosted under the Web of Science (Clarivate Analytics) and the EBSCOhost (EBSCO Information Services) platforms, respectively, were searched and, altogether, 209 publications were identified (including duplicates). After applying the predefined eligibility/inclusion criteria, 29 publications were identified as relevant for the assessment of food and feed safety (six publications) or environmental safety (23 publications). The list of relevant publications identified by the consent holder through the extensive/systematic literature search described above is listed in Appendix G. In addition to the electronic bibliographic databases, the websites of nine relevant key organisations involved in risk assessment of single GM maize products were searched. None of the 70 retrieved records in those searches were considered relevant.

The consent holder evaluated the relevant publications identified and concluded that *the literature search (…) identified no relevant publications that would invalidate the initial conclusions of the MON 810 risk assessment*.

3.2.3.2. EFSA's assessment

Systematic literature search

EFSA assessed the extensive/systematic literature search provided by the consent holder using a critical appraisal tool (EFSA, 2015b) that was developed following the relevant principles and criteria outlined in EFSA (2010) and the recommendations given in EFSA (2017).

The overall quality of the literature search is acceptable. However, EFSA considers that future searches on maize MON 810 could be improved further.

[18] 2017 PMEM report: Sections 3.1.2.3 and 3.1.4.3.
[19] 2017 PMEM report: Section 3.1.6, Appendices 5.1–5.3, additional information: 1/4/2019.

For future searches, EFSA recommends the consent holder to:

- 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, and where subject headings are available, used both free-text terms and controlled vocabulary in the searches;
- ensure that enough truncation is used and used consistently;
- increase the proximity operator distance (NEAR/5 (or greater) instead of NEAR/3);
- adapt the search to the size of the retrieved publications (and thus not combine search sets when one of the search sets already yields only few publications).
- follow the guidelines given in EFSA's updated explanatory note on literature searching (EFSA, 2019).

Relevant scientific publications

EFSA assessed the 29 publications identified by the consent holder as being relevant to the food and feed and environmental safety assessment of maize MON 810 and the Cry1Ab protein. The results reported in these publications do not provide any 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.

3.2.3.3. Conclusions on literature searching

Although the overall quality of the literature search performed by the consent holder is acceptable, EFSA considers that the methodology and reporting of the literature search can be improved. Future searches should comply with EFSA's updated explanatory note on literature searching (EFSA, 2019).

The assessment of the relevant publications identified does not point to new hazards, modified exposure, or new scientific uncertainties that would change former risk assessment conclusions on and risk management recommendations for maize MON 810.

3.3. Weight-of-evidence assessment

EFSA assembled, weighed and integrated the evidence provided in the 2017 PMEM report, additional information provided by the consent holder on insect resistance management and literature searching, comments provided by EU Member States and relevant scientific publications, following a weight of evidence approach (EFSA Scientific Committee, 2017).

Table 4 presents EFS A's weight of evidence assessment as comprising three basic steps: (1) assembling the evidence into lines of evidence of similar type; (2) weighing the evidence; and (3) integrating the evidence (EFSA Scientific Committee, 2017).

Table 4: Weight-of-evidence approach followed to assess the evidence provided in the 2017 PMEM report on maize MON 810

Question:	*Do the findings of the insect resistance monitoring and general surveillance activities indicate any adverse effects on human and animal health or the environment arising from the cultivation of maize MON 810 during the 2017 growing season that would invalidate previous GMO Panel evaluations on the safety of this GM maize?*	
Assemble the evidence	Select the evidence	The evidence was obtained from: – The 2017 PMEM report submitted by the consent holder – Additional information on insect resistance management and literature searching provided by the consent holder following EFSA's requests – Scientific comments submitted by EU Member States – Relevant scientific publications
	Lines of evidence (LoE)	A summary of the evidence provided is as follows: **Case-specific monitoring** – **LoE 1:** Farmer compliance with refuge requirements. Survey of 214 Spanish and 36 Portuguese farmers growing maize MON 810 *(Section 3.1.1)* – **LoE 2:** ECB and MCB resistance monitoring *(Section 3.1.2)*: • Sampling of 1,111 ECB and 1,452 MCB larvae from three zones in North-eastern Spain • DC bioassays conducted with progeny of field-collected individuals • Bioassays with maize MON 810 leaves with spare MCB larvae (10,650) and ECB larvae surviving the DC – **LoE 3:** Farmer complaint system: 1,703 complaints were received during the 2017 growing season. None of them were attributed to loss of efficacy of maize MON 810 *(Section 3.1.2)* **General surveillance** – **LoE 4:** Extensive/systematic literature search (June 2017–May 2018). Twenty-nine food and feed- and environmental-safety relevant publications were identified and assessed *(Section 3.2.1)* – **LoE 5:** Existing monitoring networks – **LoE 6:** Farmer survey based on 250 questionnaires received from farmers in Spain and (236) and Portugal (14) *(Section 3.2.3)*
Weigh the evidence	Methods	– **LoE 1:** Best professional judgement – **LoE 2:** The methodological and reporting quality (reliability) was assessed by best professional judgement considering EFSA's previous recommendations. In the DC bioassays, MI values of the field populations were compared with the expected > 99% MI and with the results reported for the susceptible reference populations (MCB only) – **LoE 3:** The methodology of the search was assessed by best professional judgement considering the principles for literature searching laid down in EFSA (2010) and the recommendations given in EFSA et al. (2017). A critical appraisal tool was used (EFSA, 2015b). The implications of each of the publications identified in the search were assessed by best professional judgement – **LoE 4:** Best professional judgement – **LoE 5:** Best professional judgement – **LoE 6:** The methodology of the farmer questionnaire was assessed by best professional judgement based on an evaluation grid for surveys used for general surveillance on GM plants (see Appendix 1 of EFSA GMO Panel, 2011a,b)

	Results	**Case-specific monitoring** – **LoE 1:** Partial compliance (92%) with refuge requirements in Spain and full compliance in Portugal was reported in the farmer's questionnaires – **LoE 2:** 1) ECB: Moulting inhibition of larvae tested against the DC was 99.19%. The 12 larvae that moulted to the second instar in the DC assay died within 5 days of feeding on maize MON 810 leaves 2) MCB: Moulting inhibition was lower than the expected 99% in all three sampling zones. No resistant larvae were observed in the bioassays with maize MON 810 leaves – **LoE 3:** None of the 1,703 complaints received in 2017 were attributed to loss of efficacy of maize MON 810 **General surveillance** – **LoE 4:** The information reported in the four food and feed- and 21 environmental-safety relevant publications identified through the systematic literature search do not point to new hazards, modified exposure or new scientific uncertainties that would invalidate the risk assessment conclusions on and risk management recommendations for maize MON 810 – **LoE 5:** The consent holder did not report information gathered through existing networks involved in environmental monitoring in the EU – **LoE 6:** No adverse effects that might be caused by the cultivation of maize MON 810 were reported in the analysis of the farmer questionnaires. The results of the pooled analysis were not included in the 2017 PMEM report
Integrate the evidence	Methods	– The different LoE were integrated by best professional judgement (i.e. no formal method was used) 1) LoE 1–LoE 3 were integrated to conclude on resistance management strategies and insect resistance monitoring 2) LoE 4–LoE 6 were integrated to conclude on unexpected adverse effects due to the cultivation of maize MON 810 in the EU during the 2017 growing season
	Results	**Conclusions** *(Section 4)* – The monitoring strategy implemented in 2017 is not sensitive enough to detect the recommended 3% resistance allele frequency – The information reported in the 2017 PMEM report does not show any adverse effects on human and animal health or the environment arising from the cultivation of maize MON 810 during the 2017 growing season – EFSA concludes that no new evidence has been reported in the context of the 2017 PMEM report that would invalidate previous GMO Panel evaluations on the safety of maize MON 810 **Recommendations** – EFSA strongly recommends the consent holder to 1) Achieve full compliance with refuge requirements in areas where maize MON 810 adoption is high (i.e. North-eastern Spain) 2) Increase the sensitivity of the resistance monitoring plan 3) Perform a F_2-screen on European and Mediterranean corn borer populations from North-eastern Spain – EFSA gives other practical recommendations on insect resistance monitoring, farmer questionnaires, existing environmental networks and literature searching that should be implemented by the consent holder in future reports *(Section 5)*

DC: Diagnostic concentration; ECB: European corn borer; GM: genetically modified; MCB: Mediterranean corn borer; MI: moult inhibition; PMEM: post-market environmental monitoring.

4. Conclusions

The evidence from the 2017 PMEM report and the additional information provided by the consent holder upon EFSA's request do not indicate any adverse effects on human and animal health or the environment arising from the cultivation of maize MON 810 during the 2017 growing season. Consequently, EFSA concludes that no new evidence has been reported that would invalidate previous EFSA/GMO Panel evaluations on the safety of maize MON 810 (EFSA, 2009; EFSA GMO Panel, 2012b,c).

EFSA identifies methodological and reporting limitations pertaining to insect resistance monitoring, farmer questionnaires and literature searching that should be resolved by the consent holder in future PMEM reports. In particular, EFSA notes that the monitoring strategy implemented in the 2017 growing season is not sufficiently sensitive to detect the recommended 3% resistance allele frequency necessary for a timely detection of a surge of field resistance. Recommendations to resolve these limitations are listed in Section 5.

5. Recommendations

EFSA notes that several of its recommendations to resolve previously identified shortcomings for case-specific monitoring and general surveillance have not been implemented by the consent holder (summarised in Table 5). Consequently, EFSA strongly recommends the consent holder to: (1) achieve full compliance with refuge requirements in areas where maize MON 810 adoption is high (i.e. North-eastern Spain); (2) increase the sensitivity of the resistance monitoring plan and address previously mentioned methodological, analytical and/or reporting limitations for resistance monitoring and farmer questionnaires; and (3) perform a F_2-screen on European and Mediterranean corn borer populations from North-eastern Spain. Moreover, relevant stakeholders should implement a methodological framework to enable making best use of existing networks involved in environmental monitoring for the general surveillance of GM plants.

Table 5: Summary of EFSA's recommendations for future PMEM reports on maize MON 810. Further details are provided in the respective sections of this Statement

Area (Section)		Recommendation	Responsible for implementation
Case-specific monitoring	Implementation of non-*Bt*-maize refuges (Section 3.1.1.2)	– To take relevant actions, in order to reinforce the adoption of sufficient refuge areas, especially in regions of high maize MON 810 adoption	– Consent holder – Spanish National Competent Authorities – Other relevant stakeholders (e.g. farmer associations)
		– To develop appropriate information systems on GM crop cultivation to ensure that structured refuges are planted in clustered areas greater than 5 ha	– Consent holder – EU Member States
	ECB/MCB resistance monitoring (Section 3.1.2.2)	Monitoring strategy – To increase the sensitivity of the monitoring strategy so that it achieves a detection level of 3% resistance allele frequency in target pest populations	– Consent holder
		Laboratory rearing – To optimise the rearing process of field-collected individuals and reduce the pre-imaginal mortality prior to susceptibility testing	

Area (Section)		Recommendation	Responsible for implementation
		Testing – To confirm the validity of the diagnostic concentration by comparing it with data generated with larvae collected from areas of low or no selection pressure (Mediterranean corn borer) – To harmonise the methodology of the bioassays between both target pests – To standardise the testing approach for confirming resistance of suspected resistant populations – To consider more sensitive testing methods (e.g. F_2-screen) – To perform F_2-screening on European and Mediterranean corn borer populations in North-eastern Spain	
		Reporting – To consider recommendations outlined in Appendix F of this statement when preparing the reports of bioassays – To supply the raw data of the different resistance monitoring bioassays conducted with both target pests	
	Farmer complaint system (Section 3.1.2.2)	– To provide more information on the farmer complaint system complementary resistance monitoring tool to determine whether proper communication mechanisms and fit-for-purpose educational programmes	– Consent holder
General surveillance	Farmer questionnaires (Section 3.2.1.2)	– To provide the analysis of the pooled data from the surveys obtained over the last 12 years	– Consent holder
	Existing environmental networks (Section 3.2.2.2)	– To implement a methodological framework enabling the use of environmental networks in the broader context of environmental monitoring	– Relevant stakeholders
	Literature searching (Section 3.2.3.2)	– To follow the guidelines given in EFSA's updated explanatory note on literature searching (EFSA, 2019)	– Consent holder

Documentation provided to EFSA

1) Letter from the European Commission, dated 29 November 2018, requesting EFSA to assess the annual PMEM report on the cultivation of maize MON 810 during the 2017 season provided by the consent holder.
2) Letter from the European Commission, dated 20 December 2018, requesting EFSA to consider the revised version of the 2017 PMEM report.
3) Comments from the EU Member States on the 2017 PMEM report.
4) Letters from the consent holder, dated 29 March 2019, providing EFSA supplementary information on insect resistance management and literature searching.

Supporting information

EU Member States' comments.

References

Abbott WS, 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18, 265–267.

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

Alfaro A, 1972. Notas sobre *Ostrinia nubilalis* (Hub.) y *Sesamia nonagriodes* (Lef.). Anales INIA Serie Protección Vegetal, 2, 145–170.

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

Andow DA and Alstad DN, 1998. The F_2 screen for rare resistance alleles. Journal of Economic Entomology, 91, 572–578.

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

Bates SL, Zhao JZ, Roush RT and Shelton AM, 2005. Insect resistance management in GM crops: past, present and future. Nature Biotechnology, 23, 57–62.

Bourguet D, Chaufaux J, Séguin M, Buisson C, Hinton JL, Stodola TJ, Porter P, Cronholm G, Buschman LL and Andow DA, 2003. Frequency of alleles conferring resistance to Bt maize in French and US corn belt populations of the European corn borer, *Ostrinia nubilalis*. Theoretical and Applied Genetics, 106, 1225–1233.

Camargo AM, Andow DA, Castañera P and Farinós GP, 2018. First detection of a *Sesamia nonagrioides* resistance allele to Bt maize in Europe. Scientific Reports, 8, 3977.

Castañera P, Farinós GP, Ortego F and Andow DA, 2016. Sixteen years of Bt maize in the EU hotspot: Why has resistance not evolved? PLoS ONE, 11, e0154200.

Centre for Ecology and Hydrology, Perseus and Rijksinstituut voor Volksgezondheid en Milieu, 2014. Review of statistical methods and data requirements to support post market environmental monitoring of agro ecosystems. EFSA supporting publications 2014:EN-582, 151 pp. https://doi.org/10.2903/sp.efsa.2014.en-582

DGAV (Direção-Geral de Alimentação e Veterinária), 2017. Coexistência entre culturas geneticamente modificadas e outros modos de produção agrícola relatório de acompanhamento - 2017. Relatório de acompanhamento-2017, 1–27.

Dively GP, Venugopal PD, Whalen J, Holmstrom K, Kuhar TP, Doughty HB, Patton T, Cissel W and Hutchison WD, 2018. Regional pest suppression associated with widespread Bt maize adoption benefits vegetable growers. Proceedings of the National Academy of Sciences, 115, 3320–3325. https://doi.org/10.1073/pnas.1720692115

Downes S and Mahon R, 2012. Successes and challenges of managing resistance in *Helicoverpa armigera* to Bt cotton in Australia. GM Crops & Food, 3, 228–234.

Downes S, Walsh T and Tay WT, 2016. Bt resistance in Australian insect pest species. Current Opinion in Insect Science, 15, 78–83.

EFSA (European Food Safety Authority), 2009. Scientific Opinion of the Panel on Genetically Modified Organisms on applications (EFSA-GMO-RX-MON 810) for the renewal of authorisation for the continued marketing of (1) existing food and food ingredients produced from genetically modified insect resistant maize MON 810; (2) feed consisting of and/or containing maize MON 810, including the use of seed for cultivation; and of (3) food additives, and feed materials produced from maize MON 810, all under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal 2009;7(6):1149, 85 pp. https://doi.org/10.2903/j.efsa.2009.1149

EFSA (European Food Safety Authority), 2010. Application of systematic review methodology to food and feed safety assessments to support decision making. EFSA Journal 2010;8(6):1637, 90 pp. https://doi.org/10.2903/j.efsa.2010.1637

EFSA (European Food Safety Authority), 2015a. Clarifications on EFSA GMO Panel recommendations on the Insect Resistance Management plan for genetically modified maize MON 810. EFSA Supporting Publication 2015;12(7):EN-842, 14 pp. https://doi.org/10.2903/sp.efsa.2015.en-842

EFSA (European Food Safety Authority), 2015b. Tools for critically appraising different study designs, systematic review and literature searches. EFSA Supporting Publication 2015;12(7):EN-836, 65 pp. https://doi.org/10.2903/sp.efsa.2015.en-836

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2011a. Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2009. EFSA Journal 2011;9(10):2376, 66 pp. https://doi.org/10.2903/j.efsa.2011.2376

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2011b. Scientific Opinion providing guidance on the Post-Market Environmental Monitoring (PMEM) of genetically modified plants. EFSA Journal 2011;9(8):2316, 40 pp. https://doi.org/10.2903/j.efsa.2011.2316

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2012a. Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2010. EFSA Journal 2012;10(3):2610, 35 pp. https://doi.org/10.2903/j.efsa.2012.2610

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2012b. Scientific Opinion updating the risk assessment conclusions and risk management recommendations on the genetically modified insect resistant maize MON 810. EFSA Journal 2012;10(12):3017, 98 pp. https://doi.org/10.2903/j.efsa.2012.3017

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2012c. Scientific Opinion supplementing the conclusions of the environmental risk assessment and risk management recommendations for the cultivation of the genetically modified insect resistant maize Bt11 and MON 810. EFSA Journal 2012;10(12):3016, 32 pp. https://doi.org/10.2903/j.efsa.2012.3016

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2013. Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2011. EFSA Journal 2013;11(12):3500, 38 pp. https://doi.org/10.2903/j.efsa.2013.3500

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2014a. Scientific Opinion on the annual post-market environmental monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2012. EFSA Journal 2014;12(6):3704, 29 pp. https://doi.org/10.2903/j.efsa.2014.3704

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2014b. Scientific Opinion on the use of existing environmental surveillance networks to support the post-market environmental monitoring of genetically modified plants. EFSA Journal 2014;12(11):3883, 24 pp. https://doi.org/10.2903/j.efsa.2014.3883

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2015a. Scientific Opinion on the annual post-market environmental monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2013. EFSA Journal 2015;13(3):4039, 11 pp. https://doi.org/10.2903/j.efsa.2015.4039

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2015b. Scientific opinion on the revised annual post-market environmental monitoring (PMEM) report on the cultivation of genetically modified maize MON 810 in 2013 from Monsanto Europe S.A. EFSA Journal 2015;13(11):4295, 37 pp. https://doi.org/10.2903/j.efsa.2015.4295

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2016. Scientific Opinion on the annual post-market environmental monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2014. EFSA Journal 2016;14(4):4446, 26 pp. https://doi.org/10.2903/j.efsa.2016.4446

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H, Birch AN, Casacuberta J, De Schrijver A, Gralak MA, Guerche P, Jones H, Manachini B, Messéan A, Nielsen EE, Nogué F, Robaglia C, Rostoks N, Sweet J, Tebbe C, Visioli F, Wal J-M, Álvarez F, Ardizzone M, Devos Y and Fernández-Dumont A, 2017. Scientific Opinion on the annual post-market environmental monitoring (PMEM) report on the cultivation of genetically modified maize MON 810 in 2015 from Monsanto Europe S.A. EFSA Journal 2017;15(5):4805, 27 pp. https://doi.org/10.2903/j.efsa.2017.4805

EFSA Scientific Committee, Hardy A, Benford D, Halldorsson T, Jeger MJ, Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Schlatter JR, Silano V, Solecki R, Turck D, Benfenati E, Chaudhry QM, Craig P, Frampton G, Greiner M, Hart A, Hogstrand C, Lambre C, Luttik R, Makowski D, Siani A, Wahlstroem H, Aguilera J, Dorne J-L, Fernandez Dumont A, Hempen M, Valtueña Martínez S, Martino L, Smeraldi C, Terron A, Georgiadis N and Younes M, 2017. Scientific Opinion on the guidance on the use of the weight of evidence approach in scientific assessments. EFSA Journal 2017;15(8):4971, 69 pp. https://doi.org/10.2903/j.efsa.2017.4971

EFSA (European Food Safety Authority), Devos Y, Guajardo IM, Glanville J and Waigmann E, 2017. Explanatory note on literature searching conducted in the context of GMO applications for (renewed) market authorisation and annual post-market environmental monitoring reports on GMOs authorised in the EU market. EFSA supporting publications 2017:EN-1207, 48 pp. https://doi.org/10.2903/sp.efsa.2017.en-1207

EFSA (European Food Safety Authority), Álvarez F, Devos Y, Georgiadis M, Messéan A and Waigmann E, 2018. Statement on annual post-market environmental monitoring report on the cultivation of genetically modified maize MON 810 in 2016. EFSA Journal 2018;16(5):5287, 34 pp. https://doi.org/10.2903/j.efsa.2018.5287

EFSA (European Food Safety Authority), Devos Y, Guajardo IM, Álvarez F and Glanville J, 2019. Explanatory note on literature searching conducted in the context of GMO applications for (renewed) market authorisation and annual post-market environmental monitoring reports on GMOs authorised in the EU market. EFSA supporting publications 2019:EN-1614, 62 pp. https://doi.org/10.2903/sp.efsa.2019.en-1614

Engels H, Bourguet D, Cagáň L, Manachini B, Schuphan I, Stodola TJ, Micoud A, Brazier C, Mottet C and Andow DA, 2010. Evaluating resistance to Bt toxin Cry1Ab by F_2 screen in European populations of *Ostrinia nubilalis* (Lepidoptera: Crambidae). Journal of Economic Entomology, 103, 1803–1809.

EuropaBio, 2017. Harmonised insect resistance management (IRM) plan for cultivation of Bt maize (single insecticidal trait) in the EU. Available online: http://ec.europa.eu/food/plant/gmo/reports_studies_en

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

Farinós GP, Hernández-Crespo P, Ortego F and Castañera P, 2018. Monitoring of *Sesamia nonagrioides* resistance to MON 810 maize in the European Union: lessons from a long-term harmonized plan. Pest Management Science, 74, 557–568.

Glaser JA and Matten SR, 2003. Sustainability of insect resistance management strategies for transgenic Bt corn. Biotechnology Advances, 22, 45–69.

González-Núñez M, Ortego F and Castañera P, 2000. Susceptibility of Spanish populations of the corn borers *Sesamia nonagrioides* (Lepidoptera: Noctuidae) and *Ostrinia nubilalis* (Lepidoptera: Crambidae) to a *Bacillus thuringiensis* endotoxin. Journal of Economic Entomology, 93, 459–463.

Gould F, 1998. Sustainability of transgenic insecticidal cultivars: Integrating pest genetics and ecology. Annual Review of Entomology, 43, 701–726.

Gould F, Anderson A, Jones A, Sumerford D, Heckel DG, Lopez J, Micinski S, Leonard R and Laster M, 1997. Initial frequency of alleles for resistance to *Bacillus thuringiensis* toxins in field populations of *Heliothis virescens*. Proceedings of the National Academy of Sciences, 94, 3519–3523.

Head GP and Greenplate J, 2012. The design and implementation of insect resistance management programs for Bt crops. GM Crops & Food, 3, 144–153.

Huang F, Ghimire MN, Leonard BR, Daves C, Levy R and Baldwin J, 2012. Extended monitoring of resistance to *Bacillus thuringiensis* Cry1Ab maize in *Diatraea saccharalis* (Lepidoptera: Crambidae). GM Crops & Food, 3, 245–254.

ISAAA, 2017. Global Status of Commercialized Biotech/GM Crops in 2017: Biotech crop adoption surges as economic benefits accumulate in 22 years. ISAAA Brief No. 53. ISAAA: Ithaca, NY.

Liu F, Xu Z, Chang J, Chen J, Meng F, Zhu YC and Shen Y, 2008. Resistance allele frequency to Bt cotton in field populations of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in China. Journal of Economic Entomology, 101, 933–943.

Ortego F, Pons X, Albajes R and Castañera P, 2009. European commercial genetically modified plantings and field trials. In: Ferry N and Gatehouse AMR (eds.). Environmental impact of genetically modified crops. CAB International, Wallingford, UK. pp. 327–343.

Raybould A, Kilby P and Graser G, 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.

Robertson JL, Russell RM, Preisler HK and Savin NE, 2007. Pesticide bioassays with arthropods, 2nd edition. CRC Press, Boca Raton, FL.

Siegfried BD and Spencer T, 2012. Bt resistance monitoring in European corn borers and Western corn rootworms. In: Oliver MJ and Li Y (eds.). Plant gene containment. Wiley, New York. pp. 43–55.

Siegfried BD, Spencer T, Crespo AL, Storer NP, Head GP, Owens ED and Guyer D, 2007. Ten years of Bt resistance monitoring in the European corn borer: What we know, what we don't know and what we can do better. American Entomologist, 53, 208–214.

Smets G, Alcalde E, Andres D, Carron D, Delzenne P, Heise A, Legris G, Martinez Parrilla M, Verhaert J, Wandelt C, Ilegems M and Rüdelsheim P, 2014. The use of existing environmental networks for the post-market monitoring of GM crop cultivation in the EU. Environmental Science: Processes & Impacts, 16, 1754–1763.

Tabashnik BE and Carrière Y, 2017. Surge in insect resistance to transgenic crops and prospects for sustainability. Nature Biotechnology, 35, 926–935.

Tabashnik BE, Brévault T and Carrière Y, 2013. Insect resistance to Bt crops: lessons from the first billion acres. Nature Biotechnology, 31, 510–521.

Thieme TGM, Buuk C, Gloyna K, Ortego F and Farinos GP, 2018. Ten years of MON 810 resistance monitoring of field populations of *Ostrinia nubilalis* in Europe. Journal of Applied Entomology, 142, 192–200.

US EPA (United States Environmental Protection Agency), 2010. Biopesticide Registration Action Document: Cry1Ab and Cry1F *Bacillus thuringiensis* (Bt) corn plant-incorporated protectants. Available online: https://www3.epa.gov/pesticides/chem_search/reg_actions/pip/cry1f-cry1ab-brad.pdf [Accessed 16 May 2019]

US EPA (United States Environmental Protection Agency), 2018. White paper on resistance in lepidopteran pests of *Bacillus thuringiensis* (Bt) plant-incorporated protectants in the United States. Available online: https://www.epa.gov/sites/production/files/2018-07/documents/position_paper_07132018.pdf [Accessed 16 May 2019]

Abbreviations

Bt	*Bacillus thuringiensis*
CI	confidence interval
DC	diagnostic concentration
Df	degrees of freedom
ECB	European corn borer
ENV	environmental safety
FF	food and feed safety
FQ	farmer questionnaires
GLP	Good laboratory practice
GM	genetically modified
GMO	EFSA Panel on Genetically Modified Organisms
IRM	insect resistance management
LoE	lines of evidence
MCB	Mediterranean corn borer
MI	moult inhibition

MIC	moult inhibition concentration
PMEM	post-market environmental monitoring
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis

Appendix A – Farmer compliance with refuge requirements in Spain between 2004 and 2017

[Table based on data provided in 2004–2017 PMEM reports on maize MON 810]

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
2017	236	200	19	17	92	FQ

NR: not reported; PMEM: post-market environmental monitoring.
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 North-eastern Spain.

Appendix B – Growing area and adoption rate of maize MON 810 in North-eastern, Central and South-western Spain between 2013 and 2017

Season	Growing area 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)					
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	149,843	64.2	145,661	66.0
2017	96,748	148,962[e]	64.9[e]	119,182	81.2
Mean 2013–2017	–	–	**61.9**	–	**62.2**
Central Spain (Albacete)					
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	45.7	10,221[d]	42.9
2017	3,903	8,700[e]	44.9[e]	9,257[d]	42.2
Mean 2013–2017	–	–	**40.4**	–	**35.6**
South-western Spain (Extremadura and Andalucía)					
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	72,257	35.9	81,611	31.8
2017	21,989	62,584[e]	35.1[e]	72,930[d]	30.2
Mean 2013–2017	–	–	**29.6**	–	**26.1**

(a): Source: https://www.miteco.gob.es/es/calidad-y-evaluacion-ambiental/temas/biotecnologia/2017corregida_tcm30-429386.pdf (Accessed 16 May 2019).
(b): Avances de superficies y producciones de cultivos: http://www.mapa.gob.es/es/estadistica/temas/estadisticas-agrarias/agricultura/avances-superficies-producciones-agricolas/ (Accessed 16 May 2019).
(c): Encuesta sobre superficies y rendimiento de cultivos (ESYRCE): http://www.mapa.gob.es/es/estadistica/temas/estadisticas-agrarias/agricultura/esyrce/ (Accessed 16 May 2019).
(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 during the 2017 maize growing season in North-eastern Spain

[Table based on data provided in the 2017 PMEM report on maize MON 810]

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)	237	109 (46)
		Lanaja – 2 (Huesca)	215	128 (56)
		Total	**452**	**237 (52)**
	2	Candasnos – 1 (Huesca)	26	9 (35)
		Candasnos – 3 (Huesca)	280	181 (65)
		Ontiñena (Huesca)	13	13 (100)
		Total	**319**	**203 (64)**
	3	Artajona – 1 (Navarra)	239	122 (51)
		Artajona – 2 (Navarra)	4	8 (100)
		Artajona – 4 (Navarra)	4	
		Mendigorría – 3 (Navarra)	93	58 (62)
		Total	**340**	**188 (55)**
	Total		**1,111**	**628 (57)**
MCB	1	Lanaja – 1 (Huesca)	171	NR
		Lanaja – 2 (Huesca)	152	NR
		Lanaja – 3 (Huesca)	181	NR
		Total	**504**	**302 (60)**
	2	Candasnos – 1 (Huesca)	126	NR
		Candasnos – 2 (Huesca)	22	NR
		Candasnos – 3 (Huesca)	179	NR
		Candasnos – 4 (Huesca)	12	NR
		Candasnos – 4 (Huesca)	9	NR
		Ontiñena (Huesca)	145	NR
		Total	**493**	**238 (48)**
	3	Artajona – 1 (Navarra)	163	NR
		Artajona – 2 (Navarra)	25	NR
		Artajona – 3 (Navarra)	98	NR
		Artajona – 4 (Navarra)	3	NR
		Falces – 1 (Navarra)	5	NR
		Mendigorría – 1 (Navarra)	8	NR
		Mendigorría – 2 (Navarra)	12	NR
		Mendigorría – 3 (Navarra)	141	NR
		Total	**455**	**248 (55)**
	Total		**1,452**	**788 (54)**

Late-instars were collected from refuges and non-*Bt*-maize fields between 12 September and 5 October 2017. No geographical coordinates were provided for the sampling sites. All ECB and MCB larvae collected were in diapause.
NR: not reported; ECB: European corn borer; MCB: Mediterranean corn borer; PMEM: post-market environmental monitoring.
(a): Two and ten additional sites were inspected for ECB and MCB, respectively, but the minimum number of larvae established in the harmonised insect resistance management (EuropaBio, 2017) plan could not be reached for these sites.

Appendix D – Historical data on Cry1Ab susceptibility of *Ostrinia nubilalis* and *Sesamia nonagrioides* populations from North-eastern Spain

[Table based on data provided in 2008–2017 PMEM reports on maize MON 810]

Target pest	Season	Larvae collected	Protein batch[(a)]	Concentration response				Diagnostic concentration
				MIC_{50} (95% CI)[(b)]	MIC_{90} (95% CI)[(b)]	RR MIC_{50} (95% CI)[(c)]	RR MIC_{90} (95% CI)[(c)]	% Moult inhibition
ECB	2008	401	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)	NP
	2009	509	1	6.40 (5.32–7.75)	13.68 (10.77–20.02)	1.75* (NR)	1.43 (NR)	NP
	2011	382	2	1.79 (1.54–2.07)	4.19 (3.45–5.48)	0.61* (NR)	0.67 (NR)	NP
	2013	452	2a	2.48 (2.03–3.02)	5.41 (4.27–7.61)	1.26 (NR)	0.82 (NR)	NP
	2015	376	2a	2.12 (1.75–2.55)	5.43 (4.36–7.29)	0.53* (NR)	0.77 (NR)	NP
	2016	1,111	2b	NP	NP	NP	NP	99.23
	2017	1,111	2b	NP	NP	NP	NP	99.19
MCB	2004	424	B1	63 (34–99)	570 (333–1318)	3.5 (NR)	5.8 (NR)	NP
	2005	400	B1	9 (3–15)	76 (54–117)	0.5 (NR)[(e)]	0.8 (NR)[(e)]	NP
	2007	457	B1	14 (8–20)	99 (71–158)	0.9 (NR)	1.0 (NR)	NP
	2009†	489	B1	22 (16–28)	188 (138–277)	1.1 (0.8–1.7)	1.6 (NR)	NP
	2011†	564	B2-1	20 (14–27)	135 (91–232)	2.2 (1.6–3.0)*	2.0 (1.3–2.9)*	NP
	2013†	742	B2-2	19 (14–25)	163 (108–287)	2.6 (2.0–3.4)*	3.4 (2.2–5.2)*	NP
	2015†	529	B2-2	17 (13–21)	84 (63–124)	0.6 (0.5–0.8)*	1.3 (0.9–1.8)	NP
	2016	1,364	B2-3	NP	NP	NP	NP	97.96 ± 0.71[(f)]
	2017	1,452	B2-4	NP	NP	NP	NP	94.14 ± 1.40[(f)]

PMEM: post-market environmental monitoring; ECB: European corn borer; MCB: Mediterranean corn borer.
Shaded rows correspond to values from the annual PMEM report under assessment. NP: not performed; NR: not reported.
*: Significant difference ($p < 0.05$) between the field population and the reference strain was identified for that season.
†: Susceptibility data from these populations were used to estimate the diagnostic concentration (1,091 ng Cry1Ab/cm2 of diet surface area).
(a): Data provided by the consent holder confirmed 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 (95% CI) 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 growing 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.
(f): Mean ± standard error of three independent assays corresponding to the different sampling zones.

Appendix E – Cry1Ab susceptibility of reference susceptible strains of *Ostrinia nubilalis* (ECB) and *Sesamia nonagrioides* (MCB)

[Table based on data provided in 2004–2017 PMEM reports on maize MON 810]

Target pest (strain)	Season	Protein batch	Concentration response		Diagnostic concentration
			MIC_{50} (95% CI)[(a)]	MIC_{90} (95% CI)[(a)]	%Moult inhibition
ECB (G.04)[(b)]	2006	1	1.20 (0.50–2.21)	4.78 (2.57–14.38)	NP
	2007	1	1.44 (0.86–2.06)	3.94 (2.68–8.28)	NP
	2008	1	2.21 (1.89–2.55)	4.47 (3.70–6.00)	NP
	2008	1	2.26 (1.49–3.01)	8.16 (5.95–13.50)	NP
	2009	1	3.65 (2.77–4.90)	9.56 (6.72–17.75)	NP
	2010	1	2.77 (2.22–3.27)	6.03 (4.93–8.41)	NP
	2011	1	4.01 (2.58–6.12)	10.07 (6.50–28.96)	NP
	2011	2	2.94 (2.33–3.60)	6.27 (4.97–8.91)	NP
	2012	2	0.37 (0.14–0.62)	1.13 (0.67–6.39)	NP
	2013	2	1.97 (0.78–5.59)	5.66 (2.67–95.34)	NP
	2013	2a	1.96 (0.84–4.60)	6.57 (3.13–50.53)	NP
	2014	2a	0.28 (0.24–0.33)	0.46 (0.38–0.62)	NP
	2015	2a	4.03 (2.85–4.86)	7.03 (5.83–9.91)	NP
	2016	2b	6.07 (5.09–7.02)	11.10 (9.45–13.94)	NP
	2017	2b	13.63 (12.32–14.65)	17.67 (16.12–21.14)	NP
ECB (ES.ref)[(c)]	2015	2a	1.82 (1.53–2.16)	2.95 (2.43–4.54)	NP
	2016	2b	5.02 (3.61–6.33)	14.25 (11.29–19.87)	NP
	2017	2b	5.15 (4.20–6.05)	9.68 (8.15–12.37)	NP
MCB[(d)]	2004	B1	18 (11–25)	99 (66–208)	NP
	2007	B1	16 (11–22)	94 (69–147)	NP
	2008	B1	19 (10–30)	120 (76–255)	NP
	2010	B1	8 (5–11)	74 (51–117)	NP
	2011	B2-1	9 (6–13)	68 (45–127)	NP
	2012	B2-1	7 (5–10)	62 (41–107)	NP
	2013	B2-1	7 (5–10)	48 (31–88)	NP
	2013	B2-2	5 (3–9)	42 (26–87)	NP
	2014	B2-2	17 (11–25)	91 (57–209)	NP
	2015	B2-2	28 (21–36)	67 (50–110)	NP
	2016	B2-3	30 (24–38)	83 (62–132)	99.23
	2017	B2-4	24 (16–35)	162 (100–363)	97.69

ECB: European corn borer; MCB: Mediterranean corn borer; PMEM: post-market environmental monitoring.
Shaded rows correspond to values from the 2017 PMEM report. NP: not performed.

(a): 50% and 90% moulting inhibition concentration (MIC_{50} and MIC_{90}) and their 95% confidence intervals (95% CI) 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.

(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 F – 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-hour-old larvae from F_1 generation) 28. Duration of the assay(s) 29. Description of measurement endpoints (e.g. mortality, moult inhibition) Environmental-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[†]
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

Category	Specific reporting recommendations
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 (i.e. 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 laboratory practice; MIC_x: x % moult inhibition concentration.
†: Information not reported in the 2017 PMEM report for any of the two target pests.
‡: Information not reported in the 2017 PMEM report for ECB.
§: Information not reported in the 2017 PMEM report for MCB.
(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.

Appendix G – Scientific publications relevant to the food/feed and environmental safety of maize MON 810 identified through extensive/ systematic literature search performed as part of the 2017 PMEM report

Reference	Relevant area
Arias-Martín M, García M, Castañera P, Ortego F and Farinós GP, 2018. Farm-scale evaluation of the impact of Cry1Ab Bt maize on canopy nontarget arthropods: a 3-year study. Insect Science, 25, 87-98.	Non-target organisms (ENV)
Barbosa de Assis VC, Guedes Chagas P, Santos Marinho CG, Matiello Fadini MA, Delabie JHC and Martins Mendes S, 2018. Transgenic Bt maize does not affect the soil ant community. Pesquisa Agropecuária Brasileira., 53, 152-162.	Non-target organisms (ENV)
Bernillon S, Maucourt M, Deborde C, Chéreau S, Jacob D, Priymenko N, Laporte B, Coumoul X, Salles B, Rogowsky PM, Richard-Forget F and Moing A, 2018. Characterization of GMO or glyphosate effects on the composition of maize grain and maize-based diet for rat feeding. Metabolomics, 14, 1-12.	Crop composition (FF)
Camargo AM, Andow DA, Castañera P and Farinós GP, 2018. First detection of a *Sesamia nonagrioides* resistance allele to Bt maize in Europe. Scientific Reports, 8, 3977.[(a)]	Insect resistance management (ENV)
Cerevková A, Miklisová D, Szoboszlay M, Tebbe CC and Cagán L, 2018. The responses of soil nematode communities to Bt maize cultivation at four field sites across Europe. Soil Biology and Biochemistry, 119, 194-202.	Non-target organisms (ENV)
Czerwinski J, Slizewska K, Korwin-Kossakowska A, Bachanek I and Smulikowska S, 2017. Effects of genetically modified maize and soybean meal on the diversity and activity of gut microbiota in broiler chicken. Animal Science Papers and Reports, 35, 279-299.	Toxicology (FF)
Fahse L, Papastefanou P and Otto M, 2018. Estimating acute mortality of Lepidoptera caused by the cultivation of insect-resistant Bt maize – The LepiX model. Ecological Modelling, 371, 50-59.	Non-target organisms (ENV)
Farinós GP, Hernández-Crespo P, Ortego F and Castañera P, 2017. Monitoring of *Sesamia nonagrioides* resistance to MON 810 maize in the European Union: lessons from a long-term harmonized plan. Pest Management Science, 74, 557-568.[(b)]	Insect resistance management (ENV)
Ferreira TE, Matiello Fadini MA, Martins Mendes S, Santos Marinho CG and Cruz I, 2017. Phytophagous mites on genetically modified maize with *Bacillus thuringiensis* genes. Ciência Rural, 47, 1-7.	Non-target organisms (ENV)
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.	Protein fate (ENV)
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.	Insect resistance management (ENV)
Leclerc M, Walker E, Messéan A and Soubeyrand S, 2018. Spatial exposure-hazard and landscape models for assessing the impact of GM crops on non-target organisms. Science of the Total Environment, 624, 470-479.	Non-target organisms (ENV)
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.	Biogeochemical processes (ENV)
Nicodemo D, De Jong D, Garcia Reis L, Volpini de Almeida JM, Dos Santos AA and Manzani Lisboa LA, 2018. Transgenic corn decreased total and key storage and lipid transport protein levels in honey bee hemolymph while seed treatment with imidacloprid reduced lipophorin levels. Journal of Apicultural Research, 57, 321-328.	Non-target organisms (ENV)

Reference	Relevant area
Oliveira MR, Iank Bueno AV, Mattos Leao GF, Neumann M and Cabreira Jobim C, 2018. Nutritional composition and aerobic stability of wheat and corn silages stored under different environmental conditions. Semina: Ciências Agrárias, 39, 253-260.	Nutrition (FF)
Ondrejková J, Alacová R and Lakatos Hanicová D, 2017. Genetically modified MON810 maize: Wistar rats biochemical serum analysis. Elsevier, 280, 1.	Toxicology (FF)
Schmidt K, Schmidtke J, Schmidt P, Kohl C, Wilhelm R, Schiemann J, Van der Voet H and Steinberg P, 2017. Variability of control data and relevance of observed group differences in five oral toxicity studies with genetically modified maize MON810 in rats. Archives of Toxicology, 91, 1977-2006.	Toxicology (FF)
Sharbati J, Bohmer M, Bohmer N, Keller A, Backes C, Franke A, Steinberg P, Zeljenkova D and Einspanier R, 2017. Transcriptomic analysis of intestinal tissues from two 90-day feeding studies in rats using genetically modified MON810 maize varieties. Frontiers in Genetics, 8, 1-10.	Toxicology (FF)
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*. Chemosphere, 173, 1-13.	Non-target organisms (ENV)
Szabó B, Seres A and Bakonyi G, 2017. Long-Term consumption and food replacement of near-isogenic by Bt-maize alter life-history traits of *Folsomia candida* Willem 1902 (Collembola). Applied Ecology and Environmental Research, 15, 1275-1286.	Non-target organisms (ENV)
Thieme TGM, Buuk C, Gloyna K, Ortego F and Farinós GP, 2018. Ten years of MON 810 resistance monitoring of field populations of *Ostrinia nubilalis* in Europe. Journal of Applied Entomology, 34, 192-200.[(b)]	Insect resistance management (ENV)
Twardowski JP, Beres P, Hurej M, Klukowski Z and Warzecha R, 2017. Effects of maize expressing the insecticidal protein Cry1ab on non-target ground beetle assemblages (Coleoptera, Carabidae). Romanian Agricultural Research, 34, 352-361.	Non-target organisms (ENV)
Urechan V and Bonea D, 2017. Coexistence in cultivation of genetically modified maize (MON810) with conventional maize. Romanian Agricultural Research, 34, 51-58.	Vertical gene flow (ENV)
Van den Berg J, Warren JF and Du Plessis H, 2017. The potential effect of Bt maize on *Chrysoperla pudica* (Neuroptera: Chrysopidae). Environmental Entomology, 46, 413-417.	Non-target organisms (ENV)
Van Wyk DAB, Adeleke RA, Rhode OHJ, Bezuidenhout CC and Mienie C, 2017. Ecological guild and enzyme activities of rhizosphere soil microbial communities associated with Bt-maize cultivation under field conditions in North West Province of South Africa. Journal of Basic Microbiology, 57, 781-792.	Biogeochemical processes (ENV)
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.	Insect resistance management (ENV)
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.	Non-target organisms (ENV)
Yuan H, Li S, Liu J, Song C and Chen G, 2017. Cry1Ab Adsorption and transport in humic acid-coated geological formation of alumino-silica clays. Water, Air & Soil Pollution, 228, 1-8.	Protein fate (ENV)
Zhong W, Zeng H and Wang J, 2017. Effect of Bt gene insertion on growth, physiology and gene expression of phosphorus transporter gene of corn after arbuscular mycorrhizal fungi colonization. Chinese Journal of Eco-Agriculture, 25, 1198-1205.	Non-target organisms (ENV)

ENV: environmental safety; FF: food and feed safety.
(a): Assessed in EFSA (2018).

(b): Discussed by the EFSA GMO Panel Working Group on Application Environment on 24 October 2017. https://www.efsa.europa.eu/en/gmo/working-groups (Accessed 16 May 2019)
Thieme et al. (2018) and Farinós et al. (2018) summarise the results of the ECB and MCB resistance monitoring plan implemented for maize MON 810 in the EU between 2004 (ECB)/2005 (MCB) and 2015. The results reported in these two publications had been submitted to the European Commission as part of the annual PMEM reports that EFSA have assessed since the 2009 growing season. For the ECB resistance monitoring plan, 145 field populations of this pest were collected from maize growing regions in the Czech Republic, France, Germany, Italy, Hungary, Slovakia, Poland, Romania, Portugal and Spain. For the MCB resistance monitoring plan, larvae were collected every 2 years from North-eastern, Central and South-western Iberia (Spain and Portugal). For both target pests, susceptibility to Cry1Ab was estimated by means of concentration response diet overlay assays. There is no evidence in the results reported by Thieme et al. (2018) of changes in susceptibility to Cry1Ab in ECB populations between regions or over time. In addition, susceptibility values of the field collected populations were in line with those observed in a reference susceptible strain. ECB populations collected in Iberia, the only area in the EU where maize MON 810 hybrids have been commercialised continuously on a large scale remained susceptible to Cry1Ab. The results reported by Farinós et al. (2018) indicate no clear shifts in the susceptibility to Cry1Ab between 2004 and 2015 in any of the studied areas, and the fluctuations observed are in the range of those reported for MCB (González-Núñez et al., 2000; Farinós et al., 2004; Castañera et al., 2016) and other lepidopteran species that have not developed resistance to *Bt* crops. Additionally, no survival was observed among the nearly 15,000 larvae (> 4,800 survivors of concentration–response assays and > 10,000 spare neonates) that were fed maize MON 810 foliar tissue, indicating that maize MON 810 maize is high dose for MCB and that it still effectively controls this pest. In their publication, Farinós et al. (2018) suggest focusing monitoring in North-eastern Spain, where resistance is more likely to evolve owing to the high adoption of *Bt*-maize and the high pest pressure and moving from biennial to annual sampling. The authors, however, note the difficulties of having predefined sampling zones owing to the high rates of crop rotation in this area and the fluctuation in the success of insect collection, due to both biotic and abiotic factors. In the publication, Farinós et al. (2018) also highlight the importance of including a susceptible reference population in the bioassays to overcome differences/changes in the biological activity of different batches or formulations of microbially produced *Bt* proteins used in those bioassays. Both Thieme et al. (2018) and Farinós et al. (2018) advocate replacing concentration response by diagnostic concentration bioassays to assess changes in Cry1Ab susceptibility due to the latter sensitivity of the latter to detect shifts in susceptibility when the frequency of resistance alleles is low. Diagnostic concentrations were estimated for ECB and MCB using data obtained from the respective monitoring programs. The recommendations to focus insect collection on high adoption rate areas and to develop more sensitive testing methods are in line with those made by EFSA and its GMO Panel (e.g. EFSA, 2018) and have been included in the EuropaBio's IRM plan implemented since the 2016 growing season.