

APPROVED: 31 August 2020

doi: 10.2903/j.efsa.2020.6245

Assessment of the 2018 post-market environmental monitoring report on the cultivation of genetically modified maize MON 810 in the EU

European Food Safety Authority (EFSA),
Fernando Álvarez, Marios Georgiadis, Antoine Messéan and Franz Streissl

Abstract

Following a request from the European Commission, the EFSA assessed the 2018 post-market environmental monitoring (PMEM) report on the cultivation of Cry1Ab-expressing maize event MON 810. Like previous years, there was partial compliance with refuge requirements by Spanish farmers growing MON 810 varieties. European and Mediterranean corn borer populations collected from north-eastern Spain during the 2018 maize growing season and tested for Cry1Ab susceptibility show no symptoms of resistance to maize MON 810. 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. The report does not provide information about the use of existing networks involved in environmental monitoring. Overall, EFSA concludes that the evidence reported in the 2018 PMEM report does not invalidate previous EFSA evaluations on the safety of maize MON 810. However, as in previous years, EFSA identifies shortcomings on resistance monitoring that need revision in future reports. In particular, the monitoring plan, as implemented in 2018, 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 obligations in areas where adoption of maize MON 810 is high; (2) increase the sensitivity of the monitoring plan and address previously mentioned limitations for resistance monitoring; and (3) perform an F₂ screen on corn borer populations from north-eastern Spain. A fit-for-purpose farmer alert system may help to detect unexpected adverse effects associated with the cultivation of MON 810 varieties and be an alternative to the current farmer survey system. Moreover, relevant stakeholders should implement a methodological framework to enable making the best use of existing networks involved in environmental monitoring for the general surveillance of genetically modified plants.

© 2020 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

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

Requestor: European Commission

Question number: EFSA-Q-2020-00184

Correspondence: gmo@efsa.europa.eu

Acknowledgements: EFSA wishes to thank Christoph Tebbe and EFSA staff members Michele Ardizzone, Valeria Ercolano, Antonio Fernandez-Dumont, Silvia Federici, Arthur Healy, Anna Lanzoni and Irene Muñoz-Guajardo.

Suggested citation: EFSA (European Food Safety Authority), Álvarez F, Georgiadis M, Messéan A and Streissl F, 2020. Assessment of the 2018 post-market environmental monitoring report on the cultivation of genetically modified maize MON 810 in the EU. *EFSA Journal* 2020;18(10):6245, 42 pp. <https://doi.org/10.2903/j.efsa.2020.6245>

ISSN: 1831-4732

© 2020 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

This is an open access article under the terms of the [Creative Commons Attribution-NoDerivs](https://creativecommons.org/licenses/by/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited and no modifications or adaptations are made.



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



Technical summary

Following a request from the European Commission, the European Food Safety Authority (EFSA) assessed the 2018 post-market environmental monitoring (PMEM) report on the cultivation of the Cry1Ab-expressing maize event MON 810. This report presents the results of the 2018 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 of (i) 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 2018; and (ii) diagnostic bioassays conducted with European and Mediterranean corn borers collected from north-eastern Spain to monitor changes in susceptibility to the Cry1Ab protein.

Like previous years, partial compliance with refuge obligations is observed in Spain where maize MON 810 adoption is high. EFSA therefore reiterates the need to ensure full compliance in north-eastern Spain to delay resistance evolution and urges the consent holder, the Spanish National Competent Authorities and other relevant stakeholders to undertake actions for accomplishing 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 and 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 2018 maize growing season. For the Mediterranean corn borer (MCB), moulting inhibition was lower than the expected > 99% in one of the three populations tested. Additional studies using 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.

However, as in previous years, EFSA identifies methodological and reporting shortcomings on to resistance monitoring that need revision in future PMEM reports. Considering the estimated numbers of field-collected ECB and MCB larvae represented in the diagnostic concentration bioassays, the monitoring plan, as implemented in 2018, 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 by using a more sensitive testing method, like F₂ screening. Eventually, periodic estimations of resistance alleles through F₂ screening, together with a robust farmer complaint system, could replace annual diagnostic concentration assays. In addition, the consent holder should: (1) optimise the rearing process of field sampled 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 standardised follow-up studies with suspected resistant larvae to confirm and characterise Cry1Ab resistance alleles; and (5) consider EFSA's previous reporting recommendations for future resistance monitoring studies.

EFSA considers that it is timely for the consent holder to perform an F₂ screen on MCB populations from the same area where the Cry1Ab resistance allele was detected in 2016 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.

The consent holder and other companies marketing maize MON 810 seeds have in place a farmer complaint system that allows farmers to report complaints about product performance. Although this system is not targeting resistance monitoring, it might be used to report unexpected field plant damage caused by target pests. No farmer complaints related to unexpected damage by corn borers were reported during the 2018 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 programmes 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 2018 and May 2019 that were identified through a systematic literature search. Besides, the consent holder has published the results of the pooled analysis of the questionnaires completed between 2006 and 2015 in a peer-reviewed publication (Bertho et al., 2020).

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. Concerning the analysis of the pooled data (2006–2015 maize growing seasons) from Bertho et al. (2020), EFSA cannot comment on the results and conclusions presented in this publication since the statistical model used is not considered appropriate.

EFSA thinks that a fit-for-purpose farmer alert system may help to detect unexpected adverse effects associated with the cultivation of MON 810 varieties and be an alternative to the current farmer survey system. EFSA recommends all stakeholders, including EU Member States and relevant national Competent Authorities, to have a dialogue and agree on how farmers growing maize MON 810 could best identify and report unexpected adverse effects from the cultivation of *Bt* maize varieties and on how to take stock of existing environmental networks. In the meantime, EFSA is of the opinion that farmer questionnaires should remain in place, but they should integrate several recommendations to improve their efficiency and potential to detect unexpected adverse effects.

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

Overall, EFSA concludes that the evidence reported in the 2018 PMEM report does not invalidate previous EFSA and genetically modified organism (GMO) Panel evaluations on the safety of maize MON 810 but notes the lack of sensitivity of the insect resistance monitoring put in place.

Plain language summary

Background

The European corn borer (*Ostrinia nubilalis*) and the Mediterranean corn borer (*Sesamia nonagrioides*) are important insect pests of maize fields in Europe. Maize MON 810 is a genetically modified (GM) maize that produces a protein called Cry1Ab. This protein originates from the bacterium *Bacillus thuringiensis* (*Bt*). Caterpillars of both pests that feed on leaves of maize MON 810 plants die within a few hours. In the European Union, cultivation of maize MON 810 currently takes place mostly in Spain and, to a lesser extent, in Portugal. In 2018, the cultivated area exceeded 120,000 hectares.

Insect pests can develop resistance to *Bt* proteins and, because of that, an insect resistance management (IRM) plan is required. This IRM relies on two measures: first, planting GM crops that produce high concentrations of the *Bt* protein to kill almost all individuals sensitive to the Cry1Ab protein; and second, growing non-GM plants in the vicinity of the GM crop which serves as a refuge area where sensitive individuals can survive and reproduce. The idea is that resistant insects will mate with sensitive individuals coming from the refuge areas. The progeny of those insects will be susceptible to Cry1Ab and will not survive after feeding on GM plants, thus preventing the spread of resistance in the insect population. Every year, the authorisation holder (Bayer Agriculture BVBA) monitors the development of resistance. The monitoring programme serves to identify whether corn borer populations develop resistance to the *Bt* protein and, in that case, to undertake actions for mitigating or preventing the spread of resistant populations.

In addition, the authorisation holder carries out a general surveillance (GS) programme aimed at detecting unanticipated adverse effects associated with the cultivation of GM maize plants.

The results of the resistance monitoring and the GS activities are reported to the European Commission and the EU Member States on an annual basis. EFSA has evaluated these yearly reports since 2009.

Methods

In 2018, the authorisation holder monitored possible changes in the susceptibility of field-collected European and Mediterranean corn borer populations to the Cry1Ab protein.

Corn borer populations were collected from maize fields located in different areas of north-eastern Spain, where more than 60% of the maize grown is MON 810. The susceptibility to the Cry1Ab protein was tested in laboratory studies.

The GS activities comprised (i) surveys of Spanish and Portuguese farmers cultivating GM maize, and (ii) a literature search to find scientific publications relevant to the safety assessment of maize MON 810 and the Cry1Ab protein. The farmer surveys also provide information on whether farmers plant refuge areas.

Results

Insect resistance monitoring

The analysis of the laboratory studies does not indicate signs of resistance in the European and Mediterranean corn borer populations sampled during the 2018 maize growing season.

General surveillance

The data from the 2018 farmer surveys showed that all farmers in Portugal and 89% of Spanish farmers planted a refuge of the correct size. The assessment of the surveys does not indicate unanticipated adverse effects on human and animal health or the environment associated with the cultivation of maize MON 810. In addition, an analysis of the surveys completed between 2006 and 2015 was published in a scientific publication. The authors concluded that the analysis of the 10-year data does not reveal any unexpected adverse effects related to the cultivation of maize MON 810 in the EU. However, EFSA cannot confirm the results presented in the publication because the statistical model used to analyse the data is not appropriate.

The literature search, covering the period June 2018 to May 2019, identified 21 scientific publications relevant to the food, feed and environmental safety of maize MON 810 and Cry1Ab. EFSA identified two additional relevant articles published after May 2019, one of which included the analysis of the 10-year surveys mentioned above. EFSA evaluated all 23 articles and considered that none of

them contains information that would invalidate previous risk assessments by EFSA or risk management recommendations on maize MON 810.

Conclusion and recommendations

Overall, EFSA considers that the evidence from the 2018 monitoring report does not indicate adverse effects on human and animal health or the environment arising from the cultivation of maize MON 810 during the 2018 growing season. Therefore, EFSA concludes that previous evaluations on the safety of this GM maize remain valid.

However, EFSA believes that several aspects of the insect resistance management and monitoring strategy for maize MON 810 need improvement. Specifically, EFSA recommends increasing the precision of the monitoring strategy by using more sensitive testing methods. Given that the planting of refuge areas is crucial for resistance management, EFSA suggests implementing additional measures to ensure that all farmers comply with refuge requirements.

EFSA considers that a robust farmer alert system could help to detect unexpected adverse effects associated with the cultivation of MON 810 varieties and would be a more effective alternative to the current farmer survey system. EFSA recommends that all stakeholders reach agreement on how farmers growing maize MON 810 could best identify and report unexpected adverse effects from the cultivation of *Bt* maize varieties. In the meantime, EFSA is of the opinion that farmer surveys should remain in place.

Table of contents

Abstract.....	1
Technical summary.....	3
Plain language summary.....	5
1. Introduction.....	8
1.1. Terms of Reference as provided by the requestor.....	9
2. Data and methodologies.....	9
2.1. Data.....	9
2.2. Methodologies.....	9
3. Assessment.....	9
3.1. Case-specific monitoring.....	9
3.1.1. Implementation of non-Bt maize refuges.....	9
3.1.1.1. Consent holder's assessment.....	9
3.1.1.2. EFSA's assessment.....	10
3.1.2. Insect resistance monitoring.....	11
3.1.2.1. Consent holder's assessment.....	11
3.1.2.2. EFSA's assessment.....	16
3.1.2.3. Conclusions on farmer questionnaires.....	19
3.2. General surveillance.....	20
3.2.1. Farmer's questionnaires.....	20
3.2.1.1. Consent holder's assessment.....	20
3.2.1.2. EFSA's assessment.....	21
3.2.1.3. Conclusions on farmer questionnaires.....	23
3.2.2. Existing monitoring networks.....	24
3.2.2.1. Consent holder's assessment.....	24
3.2.2.2. EFSA's assessment.....	24
3.2.3. Literature searching.....	24
3.2.3.1. Consent holder's assessment.....	24
3.2.3.2. EFSA's assessment.....	25
3.2.3.3. Additional scientific publications assessed by EFSA.....	25
3.2.3.4. Conclusions on literature searching.....	26
3.3. Weight-of-evidence assessment.....	26
4. Conclusions.....	28
5. Recommendations.....	28
Documentation provided to EFSA.....	30
References.....	30
Appendix A – Farmer compliance with refuge requirements in Spain between 2004 and 2018.....	34
Appendix B – Growing area and adoption rate of maize MON 810 in north-eastern, Central and South-western Spain between 2014 and 2018.....	35
Appendix C – Field sampling of <i>Ostrinia nubilalis</i> (ECB) and <i>Sesamia nonagrioides</i> (MCB) larvae during the 2018 maize growing season in north-eastern Spain.....	36
Appendix D – Sites for <i>Ostrinia nubilalis</i> (A) and <i>Sesamia nonagrioides</i> (B) collection in north-eastern Spain between 2004 and 2018.....	37
Appendix E – Historical data on Cry1Ab susceptibility of <i>Ostrinia nubilalis</i> (ECB) and <i>Sesamia nonagrioides</i> (MCB) populations from north-eastern Spain.....	38
Appendix F – Cry1Ab susceptibility of reference susceptible populations of <i>Ostrinia nubilalis</i> (ECB) and <i>Sesamia nonagrioides</i> (MCB).....	39
Appendix G – Proposed step-wise approach for confirming resistance to Bt plants of suspected resistant populations.....	40
Appendix H – Recommended minimum reporting information for insect resistance monitoring studies.....	41

1. Introduction

Genetically modified (GM) maize MON 810 produces the insecticidal protein Cry1Ab from the naturally occurring bacterium *Bacillus thuringiensis* (*Bt*). Maize MON 810 varieties protect against certain lepidopteran pests, such as the European corn borer (ECB), *Ostrinia nubilalis* (Hübner) (Crambidae) and the Mediterranean corn borer (MCB), *Sesamia nonagrioides* (Lefèbvre) (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 1998.¹ Since 2003, the transformation event MON 810 has been introduced into a wide range of maize varieties grown in the EU. In 2018, maize MON 810 was cultivated in Spain (115,246 ha) and Portugal (5,733 ha) over a total area of 120,979 ha (ISAAA, 2018).²

According to the Commission Decision 98/294/EC of 22 April 1998 authorising the placing on the market of maize MON 810, Monsanto Europe S.A.³ (hereafter referred to as 'the consent holder') defined a management strategy to minimise the development of insect resistance and offered to inform the Commission and Competent Authorities of the 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 EuropaBio⁴ for single lepidopteran-active *Bt* maize events (Alcalde et al., 2007), which has been updated in 2019 (EuropaBio, 2019). The implemented resistance management measures are based on the high-dose/refuge strategy (e.g. Gould, 1998; Tabashnik et al., 2013). This strategy prescribes planting *Bt* crops that produce an extremely 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. Besides, a nearby structured refuge (i.e. blocks or strips of non-*Bt* maize that are located near, 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 showing increases in tolerance of target pests in the field. Timely detection of such signs enables actions to limit the survival of resistant insects, and slow or prevent their spread. In the case of maize MON 810, the consent holder follows a two-pronged approach for resistance monitoring. It consists 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 demands and carry out the operational details of IRM plans is a crucial factor⁶ contributing to the field-evolved resistance to certain *Bt* crops (see reviews by Tabashnik et al., 2013; and Tabashnik and Carrière, 2017). Grower education (training) and information programmes are an integral part of IRM plans. They aid farmers to understand the importance of adhering to IRM principles and are critical 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, voluntarily, a general surveillance monitoring programme in anticipation of the mandatory obligation 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 the 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

¹ Commission Decision of 22 April 1998 concerning the placing on the market of genetically modified maize (*Zea mays* L. line MON 810), pursuant to Council Directive 90/220/EEC (98/294/EC). OJ L 131, 5.5.1998, pp. 32–33.

² At present, maize MON 810 is the only GM maize event cultivated in the EU. Maize varieties derived from event *Bt*176, also producing the protein Cry1Ab, were cultivated in the EU between 1998 and 2005 (Ortego et al., 2009; Castañera et al., 2016).

³ Note that Monsanto has become a subsidiary of Bayer AG as of 21 August 2018.

⁴ Available online: <http://www.europabio.org/> (Accessed: 18 September 2020).

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.

⁶ Other factors contributing to the field-evolved resistance to *Bt* crops may include (1) limited modes of action of *Bt* proteins used in *Bt* crops; (2) cross-resistance among *Bt* proteins; (3) use of non-high dose *Bt* crop traits; (4) that the resistance is complete on *Bt* maize plants; (5) abundant in initial resistance alleles; and (6) lack of fitness costs/recessive fitness costs of the resistance (Huang, 2020).

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

Since 2005, the consent has reported to the European Commission and the EU Member States 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. EFSA has evaluated the annual PMEM reports on maize MON 810 corresponding to the 2009–2017 growing seasons (EFSA GMO Panel, 2011a, 2012a, 2013, 2014a, 2015a,b, 2016, 2017; EFSA et al., 2019a, 2018). Based on the data provided in those reports, EFSA and its 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 noted several shortcomings in the methodology for case-specific monitoring and general surveillance and made several recommendations to improve future PMEM reports on maize MON 810 (see also EFSA, 2015a for further recommendations on IRM). EuropaBio has incorporated some of the recommendations on insect resistance monitoring in the updated IRM plan.

1.1. Terms of Reference as provided by the requestor

On 18 December 2019, the European Commission received from the consent holder the annual PMEM report for the 2018 growing season of maize MON 810 (hereafter referred to as the '2018 PMEM report'). The reporting period of the 2018 PMEM report is from July 2018 till June 2019.

On 24 February 2020, the European Commission mandated EFSA *to assess the 2018 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 2018 PMEM report⁷ as well as additional information on insect resistance management, literature searching and farmer questionnaires provided by the consent holder upon EFSA's request, comments submitted by the EU Member States and relevant scientific publications.

2.2. Methodologies

Following Annex VII of Directive 2001/18/EC and the terms of reference of the mandate, EFSA assessed the evidence contained in the 2018 PMEM report and appraised the methods used for the monitoring activities.

EFSA considered the principles described in its guidelines for the PMEM of GM plants (EFSA GMO Panel, 2011b). EFSA also assessed the consent holder's systematic literature search following the relevant principles and criteria outlined in EFSA (2010) and the recommendations given in EFSA (2017).

EFSA implemented the weight-of-evidence (WoE) approach described in its guidance (EFSA Scientific Committee, 2017).

EFSA scrutinised the comments raised by the EU Member States during the scientific assessment and addressed them in Annex 1 of supporting information of this statement.

3. Assessment

3.1. Case-specific monitoring

3.1.1. Implementation of non-Bt maize refuges⁸

3.1.1.1. Consent holder's assessment

Compliance with non-Bt maize refuge requirements was available through the farmer questionnaires supplied as part of the general surveillance (Section 3.2.1). In 2018, 238 farmers from Spain and

⁷ The 2018 PMEM report is publicly available online: https://ec.europa.eu/food/plant/gmo/post_authorisation/plans_reports_opinions_en (Accessed: 18 September 2020).

⁸ 2018 PMEM report: Section 3.2.1.1; Appendix 1.

12 farmers from Portugal completed a questionnaire which included the following question on compliance with the refuge strategy: *Did you plant a refuge in accordance to the technical guidelines?*

a) Spain

In Spain, 216 out of the 238 maize MON 810-growing farmers surveyed stated that they complied with refuge obligations, either because they did implement a refuge (186 farmers) or because they planted less than 5 ha of maize MON 810 and were thus not required to plant a refuge (30 farmers) (Appendix A).

The 22 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): she/he felt it complicates the planting, did not have enough time and feared yield losses in conventional maize.

The locations of the *Bt* maize fields and total number of farmers where no refuges were planted were Lleida (10 farmers) and Huesca (5 farmers) – north-eastern Spain; Cáceres (5 farmers), Badajoz (1 farmer) and Sevilla (1 farmer) – south-eastern Spain.

b) Portugal

In Portugal, the 12 maize MON 810-growing farmers surveyed followed the refuge requisites. None of them were exempted since they cultivated more than 5 ha with maize MON 810 varieties. In addition to the farmer questionnaires, the Portuguese authorities performed inspections on 54 farms (out of the 172 *Bt* maize cultivation notifications received in 2018) where maize MON 810 was grown to check compliance with refuge and coexistence obligations outlined in Portuguese law (DGAV, 2019). 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 2018 season are consistent and do show a high level of compliance (...)*. Besides, the consent holder proposed to integrated refuge planting as requirement for direct payments under the Common Agricultural Policy or other national rules so that compliant farmers would be encouraged to continue implementing refuges, whereas those farmers reluctant to be compliant could be subjected to reductions or exclusions from direct support schemes.

3.1.1.2. EFSA's assessment

The data from farmer surveys show partial compliance (89%) with refuge obligations in Spain and full compliance in Portugal, as reported in previous years (see Appendix A).

Ensuring compliance with the requirements for structured refuge areas is crucial to sustain the efficiency of the technology and to delay resistance evolution of maize MON 810. This is especially in high adoption areas, like north-eastern Spain where selection pressure is the highest and resistance is, therefore, most likely to occur (Castañera et al., 2016). Low levels of refuge compliance have led to several cases of practical resistance to *Bt* crops by different lepidopteran pests (reviewed by Tabashnik et al., 2013, Tabashnik and Carrière, 2017). Insufficient refuge areas might have also been the cause of the first case of practical resistance to a *Bt* protein by ECB (Smith et al., 2019).

Other strategies have been proposed to delay resistance to *Bt* crops. These are deploying refuges together with *Bt* crops 'pyramids' producing two or more *Bt* proteins with different modes of action targeting the same pest or planting seed mixtures (also referred to as 'refuge-in-a-bag') yielding random distributions of *Bt* and non-*Bt* plants within fields (Carrière et al., 2016). However, these alternative strategies are not implemented in the EU. Given the dispersal of ECB and MCB larvae, the use of refuge in the bag seed mixtures serving as unstructured refuges are not suitable for managing resistance development of both corn borers (Davis and Onstad, 2000; Camargo et al., 2020). Besides, pyramided *Bt* maize varieties are currently not authorised for cultivation in the EU.

EFSA acknowledges the efforts made by the consent holder to develop communication tools and education programmes for raising farmers' awareness of the importance of implementing IRM measures. However, EFSA considers that the consent holder should strive to increase the level of compliance in areas of high adoption. Spanish National Competent Authorities and other relevant stakeholders, including farmers' associations, could also contribute to reinforcing farmers' awareness of refuge compliance. EFSA acknowledges the consent holder's proposal to integrate refuge planting as a prerequisite for direct payments under the Common Agricultural Policy or other national rules but considers that this should not prevent the consent holder from pursuing higher levels of compliance.

EFSA reiterates that refuge requirements also apply to clusters of small maize MON 810 fields in which the aggregated area planted with *Bt* maize 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 before planting and of those farmers that will handle planting the refuge area). However, based on the proportion of non-compliant farmers (11%) and of those growing less than 5 ha of maize MON 810 (13%) in Spain, and the findings on the frequency of Cry1Ab resistance alleles in MCB populations in the Ebro basin (Camargo et al., 2018), it is paramount ensuring full compliance in high-adoption rate 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 cultivated developing adequate information systems on GM crop cultivation to ensure that growers plant structured refuges in clustered areas larger than 5 ha.

3.1.2. Insect resistance monitoring⁹

3.1.2.1. Consent holder's assessment

Following the IRM plan, the 2018 resistance monitoring activities targeted north-eastern Spain, around the Ebro basin, where the adoption rate of maize MON 810 exceeds 60% (Appendix B). The susceptibility of sampled ECB and MCB populations to the Cry1Ab protein was tested in diagnostic and plant bioassays. An overview of the bioassays conducted for the 2018 PMEM report is presented in Table 1.

Table 1: Overview of bioassays conducted with the European corn borer (*Ostrinia nubilalis*, ECB) and the Mediterranean corn borer (*Sesamia nonagrioides*, MCB) as documented in the 2018 PMEM report

Assay	Population (generation)	ECB	MCB
Susceptibility assay – Diagnostic	NE Spain (F ₁ larvae)	<ul style="list-style-type: none"> Diet-overlay assay with purified Cry1Ab at a diagnostic concentration Progeny of field-collected larvae 1,768 neonates exposed to 28.22 ng Cry1Ab/cm² for 7 days Separate bioassays performed for each sampling site^(a) Endpoint: Mortality and moult inhibition 	<ul style="list-style-type: none"> Diet-overlay assay with purified Cry1Ab at a diagnostic concentration Progeny of field-collected larvae 3,449 neonates exposed to 1,091 ng Cry1Ab/cm² for 7 days Separate bioassays performed for each sampling zone^(a) Susceptible reference population tested for comparison Endpoint: Moult inhibition
Susceptibility assay – Plant tissue	NE Spain (F ₁ larvae)	<ul style="list-style-type: none"> Assay using maize leaves Larvae not used in the diagnostic assays (N = 10,267) Neonates fed maize MON 810 leaves for 5 days Endpoint: Moult to L₂ and L₃ 	<ul style="list-style-type: none"> Assay using maize leaves Larvae not used in the diagnostic assays (N = 10,294) Neonates fed maize MON 810 leaves for 10 days Susceptible reference population tested for comparison Endpoint: Moult to L₂
Confirmatory assay Step I – Plant tissue	NE Spain (F ₁ larvae)	<ul style="list-style-type: none"> Assay using maize leaves Larvae that survived the diagnostic concentration and moulted to L₂ (N = 3) L₂ fed maize MON 810 leaves for 5 days Endpoint: Mortality 	<ul style="list-style-type: none"> Assay using maize leaves Larvae that survived the diagnostic concentration and moulted to L₂ (N = 40) L₂ fed maize MON 810 leaves for 10 days Susceptible reference population tested for comparison Endpoint: % Moult to L₃

⁹ 2018 PMEM report: Sections 3.2.1.2 and 3.2.1.3 and Appendixes 7 and 8; additional information 27/5/2020.

Assay	Population (generation)	ECB	MCB
Confirmatory assay Step II – Diagnostic	NE Spain (F ₂ larvae)	<ul style="list-style-type: none"> Not conducted^(b) 	<ul style="list-style-type: none"> Diet-overlay assay with purified Cry1Ab Siblings of larvae that reached L₃ in confirmatory plant assay Step I 128 neonates exposed to diagnostic concentration for 7 days Endpoint: Moulting inhibition
Confirmatory assay Step II – Plant tissue	NE Spain (F ₂ larvae)	<ul style="list-style-type: none"> Not conducted^(b) 	<ul style="list-style-type: none"> Assay using maize leaves Siblings of larvae that reached L₃ in confirmatory plant assay Step I 1,200 neonates fed maize MON 810 leaves for 10 days Endpoint: Moulting to L₃
Concentration-response	Laboratory	<ul style="list-style-type: none"> Diet-overlay assay with purified Cry1Ab Susceptible reference populations (Spain & Germany) Nine concentrations (0.2–28.22 ng Cry1Ab/cm²) Duration: 7 days Endpoint: MIC_{50,95} 	<ul style="list-style-type: none"> Diet-overlay assay with purified Cry1Ab Susceptible reference population (Spain) Seven concentrations (2–128 ng Cry1Ab/cm²) Duration: 7 days Endpoint: MIC_{50,95}

L₂: second instar; L₃: third instar; MIC_{50,95}: concentration causing 50% or 95% moulting inhibition; NE: north-eastern.

(a): Details on sampling zones and sites are provided in Appendix C.

(b): The consent holder did not conduct further confirmatory assays as none of the larvae fed maize MON 810 leaves in the confirmatory plant assay (Step I) survived.

European corn borer monitoring

a) Field sampling and laboratory rearing

In 2018, 1,144 ECB late-instars from the last generation were collected at the end of the maize growing season from 11 sampling sites (refuge areas or non-*Bt* maize fields) located in three zones across north-eastern Spain (for more details, see Appendixes C and D). Six 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 (hereafter referred to as 'F₁ larvae') was tested for susceptibility to Cry1Ab. Larvae were reared following a standardised protocol (Thieme et al., 2018). A total of 534 larvae reached the adult stage (47% of the field-sampled larvae) and were placed in 61 oviposition cages for mating. Emerging adults from the different sampling zones were kept separately.

In addition, two laboratory populations were tested to evaluate potential changes in the biological activity of the test substance. Both populations had been reared in the laboratory since their establishment on non-*Bt* diet, i.e., without any exposure to maize MON 810 or Cry1Ab.

b) Monitoring assays

The following bioassays were performed: (1) a diagnostic bioassay with F₁ larvae to detect potential increases in resistance allele frequency; (2) an additional bioassay with F₁ larvae using maize MON 810 leaves; (3) a follow-up study to the diagnostic bioassay with exposure to maize MON 810 leaves; and (4) concentration-response assays with both susceptible reference populations (Table 1).

Diagnostic bioassay: The bioassay was conducted by exposing F₁ neonates to purified Cry1Ab protein at a diagnostic concentration of 28.22 ng Cry1Ab/cm² of diet surface area in an artificial diet overlay assay.¹⁰

¹⁰ The selected diagnostic concentration corresponds to the mean 99% moulting inhibition concentration (MIC₉₉) estimated with data pooled from ECB populations collected in the Czech Republic, France, Germany, Hungary, Italy, Poland, Portugal, Romania and Spain between 2005 and 2012. This concentration was considered validated after moulting inhibition values in all validation assays with ECB populations collected in Spain between 2013 and 2015 were higher than the expected > 99% (EFSA et al., 2019a, 2018). Batch 2b was used for the bioassays: 1.64 mg Cry1Ab/ml in 50 mM bicarbonate buffer; pH 10.25; 91% purity.

In the 2018 bioassays, 1,768 neonates were tested against the diagnostic concentration. Three hundred and sixty-eight larvae treated with the same buffer solution used to dissolve the Cry1Ab protein were used as a negative control. Larval mortality and moulting inhibition, corresponding to dead larvae and larvae not reaching the second instar, was determined after 7 days. None of the reference populations were included in the diagnostic bioassay.

Moulting inhibition of ECB larvae tested against Cry1Ab was 99.87%, whereas all larvae in the control group moulted to the third or fourth instar (see Table 2). This value is similar to those reported in the 2016 and 2017 growing seasons (Appendix E). The study authors indicated that *no decrease in Cry1Ab susceptibility of ECB has been observed during the monitoring duration*.

Table 2: Moulting inhibition of European corn borer (*Ostrinia nubilalis*) larvae at a diagnostic concentration of Cry1Ab protein: 2018 field populations [Table based on data provided in the 2018 PMEM report]

Population	Sampling area ^(a)	Treatment % Moulting inhibition (No. of larvae tested) ^(b)	
		Control	Cry1Ab ^(c)
North-eastern Spain	Huesca – 1	0.00 (160)	99.97 (744)
	Huesca – 2	0.00 (112)	99.63 (544)
	Navarra	0.00 (96)	100 (480)
	Total	0.00 ^(d) (368)	99.87 ± 0.12 ^(e) (1,768)

(a): Details on sampling sites are provided in Appendix C.

(b): Data from the different fields within a sampling area have been pooled.

(c): A diagnostic concentration of 28.22 ng Cry1Ab/cm² of diet surface area was used.

(d): Of the 368 larvae tested, 7, 194 and 167 larvae moulted to the second, third and fourth instar respectively.

(e): Of the 1,768 larvae tested, 76 larvae died, 1,689 larvae survived but did not moult to the second instar and 3 larvae moulted to the third instar.

Bioassay with maize MON 810 leaves: To complement the diagnostic bioassay, an additional assay was conducted with F₁ larvae from the field-collected populations using maize MON 810 leaves. To this end, 10,267 of the first instars not used in the diagnostic bioassays (between 495 and 1,518 larvae per sampling site) were fed maize MON 810 leaves. Larvae were placed in plastic boxes containing detached leaves of maize. Larvae were fed ad libitum for 7 days and mortality and the number of larvae moulting to the second instar were recorded. A negative control group, consisting of 368 larvae fed non-*Bt* maize leaves (between 32 and 48 larvae per site), was included in the study. Larvae from the control group were placed individually onto leaf discs. Cry1Ab protein levels were not measured in the maize plants used in the bioassay.

All ECB larvae fed maize MON 810 leaves died. In the control group, 0.8% of the larvae died or did not reach the second instar, whereas 43.5% and 55.7% of the larvae moulted to the second and third instar, respectively.

Confirmatory bioassay with maize MON 810 leaves: A follow-up study using maize MON 810 leaves was conducted with the three larvae that reached the second instar in the diagnostic bioassays to confirm that they were not potentially resistant to Cry1Ab. The surviving larvae were placed individually on maize MON 810 leaf discs. All three larvae died within 7 days.

Concentration-response assays: The susceptibility of the two reference populations was assessed in concentration-response assays. For each assay, nine concentrations, ranging from 0.2 to 28.22 ng Cry1Ab/cm² of diet surface area, 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. MIC₅₀ and MIC₉₀ values, with a 95% confidence interval (CI), were estimated by probit analysis (Robertson et al., 2007).

MIC₅₀ and MIC₉₀ values estimated in 2018 for both reference populations were similar to those obtained in previous years (Appendix F).

Mediterranean corn borer monitoring

a) Field sampling and laboratory rearing

In 2018, 1,490 MCB late-instars from the last generation were sampled at the end of the maize growing season from 10 sampling sites (refuge areas or non-*Bt* maize fields) in three zones across north-eastern Spain (for more details, see Appendixes C and D). Attempts were made to collect larvae from seven additional sites, but the minimum number of larvae established in the IRM study protocol could not be reached for these sites.

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 584 larvae reached the adult stage (39% of the field-collected larvae) and were placed in 60 oviposition cages for mating. Emerging adults from the different sampling zones were kept separately. Fifty-five cages, containing 554 adults (245 males and 309 females), were used to obtain F₁-progeny for the diagnostic bioassay (i.e. 37% of the field-collected larvae).

In addition, a population initiated from larvae collected in 2018 from Galicia (north-western Spain), where *Bt* maize has never been grown, and reared in the laboratory since then without any exposure to maize MON 810 or the Cry1Ab protein, was used as an additional comparator in the diagnostic concentration and plant bioassays. The consent holder informed that this susceptible reference population will be used from 2018 onwards, replacing the previous one.

b) Monitoring assays

The following bioassays were performed: 1) a diagnostic bioassay with F₁ larvae to detect potential increases in resistance allele frequency; 2) an additional bioassay with F₁ larvae using maize MON 810 leaves; 3) a follow-up study to the diagnostic bioassay with exposure to maize MON 810 leaves; and (4) concentration-response assays with the reference population (Table 1).

Diagnostic bioassay: Independent diagnostic bioassays were performed with F₁ larvae from each of the three sampling zones. Neonates were exposed to purified Cry1Ab protein at a diagnostic concentration of 1,091 ng Cry1Ab/cm² of diet surface area in an artificial diet-overlay assay.¹¹ The reference population was tested against the diagnostic concentration.

In the 2018 assays, between 1,120 and 1,181 larvae 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 negative control. Moulting inhibition was recorded after 7 days.

In one of the zones, moulting inhibition was lower than the expected 99%, whereas in the control treatments, it ranged between 11.19% and 16.78%. Moulting inhibition observed in the reference population was 97.75% (see Table 3).

Average moulting inhibition of the progeny of field-collected larvae (98.65 ± 0.40%) was not significantly lower than the expected 99%. No statistically significant differences were observed between larvae from the reference population and the field-collected larvae.

¹¹ The selected diagnostic concentration corresponds to the upper limit of the 95% confidence interval of the MIC₉₉ estimated with data pooled from MCB populations collected in non-*Bt* maize fields from North-eastern Spain over 2009, 2011, 2013 and 2015. Batch B2-4 was used for the bioassays: 1.8 mg Cry1Ab/ml in 50 mM sodium bicarbonate buffer; pH 10.25; purity 91%.

Table 3: Moulting inhibition of Mediterranean corn borer (*Sesamia nonagrioides*) larvae at a diagnostic concentration of Cry1Ab protein: 2018 field populations [Table based on data provided in the 2018 PMEM report]

Population	Sampling area ^(a)	Treatment % Moulting inhibition (No. of larvae tested)	
		Control	Cry1Ab ^(b)
North-eastern Spain	Huesca 1	12.68 (142)	97.85 (1,120)
	Huesca 2	16.78 (143)	99.06 (1,148)
	Navarra	11.19 (143)	99.05 (1,181)
	Total	13.55 ± 1.36 ^(c) (428)	98.65 ± 0.40 ^(c) (3,449)
Laboratory reference population		13.50 (163)	97.75 (924)

No statistically significant differences were observed between the north-eastern population and the expected value of 99% ($t = -0.8038$; $df = 2$; $p = 0.253$).

No statistically significant differences were observed between the north-eastern population and the reference population ($t = -2.1759$; $df = 2$; $p = 0.081$).

(a): Details on sampling sites are provided in Appendix C.

(b): A diagnostic concentration of 1,091 ng Cry1Ab/cm² of diet surface area was used. Values have been corrected using Abbott's formula (Abbott, 1925).

(c): Mean ± standard error.

Bioassay with maize MON 810 leaves: An additional bioassay was conducted with F₁ larvae from the collected field populations using maize MON 810 leaves. To this end, 10,294 first instars not used in the diagnostic bioassays (approximately 200 larvae per oviposition cage) were fed maize MON 810 leaves. A negative control group, consisting of 543 larvae fed non-*Bt* maize leaves (approximately 10 larvae per cage), was included in the study. Neonates from the laboratory reference population were also fed on maize MON 810 leaves (3,430 larvae) and conventional maize leaves. All larvae were placed in plastic boxes containing leaves of maize MON 810. Larvae were fed ad libitum for 10 days and numbers of larvae moulting to the second instar were recorded. Expression of Cry1Ab in maize MON 810 leaves used in the bioassay was verified using immunostrips.

None of the MCB larvae from the field-collected populations or the reference population feeding on maize MON 810 leaves reached to second instar, whereas moulting in the control groups of the field-collected populations ranged between 88.41% and 93.81% and resulted in 97.53% in the reference population (see Table 4).

Table 4: Moulting to second instar of Mediterranean corn borer (*Sesamia nonagrioides*) neonates feeding on *Bt* (MON 810) or non-*Bt* maize leaves: 2018 field populations [Table based on data provided in the 2018 PMEM report]

Population	Sampling area ^(a)	Treatment % Moulting (No. of larvae tested)	
		Non- <i>Bt</i>	<i>Bt</i>
North-eastern Spain	Huesca – 1	88.41 (164)	0.00 (3,187)
	Huesca – 2	87.57 (185)	0.00 (3,427)
	Navarra	93.81 (192)	0.00 (3,680)
Laboratory reference population		97.50 (160)	0.00 (3,430)

(a): Details on sampling sites are provided in Appendix C.

Confirmatory bioassays: A follow-up study using maize MON 810 leaves was conducted with the 40 larvae that reached the second instar in the diagnostic bioassays to confirm that they were not potentially resistant to Cry1Ab. Larvae were individually placed on experimental arenas and fed maize MON 810 leaves. Five larvae reached the third instar. Siblings of these larvae were then reared on artificial diet and additional diagnostic concentration and maize leaf bioassays were conducted with their progeny (F_2 larvae):

- in the diagnostic concentration bioassay, 128 F_2 larvae were tested and four larvae reached the second instar (96.88% moulting inhibition). These larvae were subsequently fed maize MON 810 and none of them moulted to the third instar after 10 days;
- in the maize leaf bioassays, none of the 1,200 F_2 first instars moulted after feeding on maize MON 810 leaves for 10 days, while 95% of the larvae from the control group (non-*Bt* maize leaves) moulted to second or third instar.

Concentration-response assays with the reference population: Seven concentrations, ranging from 2 to 128 ng Cry1Ab/cm² 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. MIC₅₀ and MIC₉₀ values, with a 95% CI, were estimated by probit analysis.

Both MIC₅₀ and MIC₉₀ values estimated in 2018 fell within the range of those estimated in previous years. The historical results of the concentration assays with the reference population are given in Appendix F.

Farmer complaint system

The consent holder and other companies marketing maize MON 810 seeds have implemented a farmer complaint system. This system allows farmers to report complaints to seed suppliers about product-related topics via the local sales representatives or customer service routes about product performance-related issues. Such a system may help farmers reporting unexpected crop damage caused by or failure in protection against target pests in maize MON 810 varieties. The consent holder states that, during the 2018 growing season, no complaints related to corn borer infestation of maize MON 810 were received via the farmer complaint system. The consent holder reports the outcome of a survey conducted by member companies of the National Breeder Association in Spain¹² selling maize MON 810 varieties to have an overview of the farmer complaint schemes. None of the 1,249 complaints received by these companies in 2018 were attributed to loss of efficacy of the *Bt* maize.

The consent holder also refers to regional monitoring networks that Spanish regional authorities have implemented for integrated pest management (IPM) (e.g. @redfaragon in Aragón,¹³ north-eastern Spain; @RAIF_noticias in Andalucía,¹⁴ southern Spain). These networks monitor and alert on incidence/outbreaks of agricultural pests and plant health issues and inform about IPM practices.

3.1.2.2. EFSA's assessment

European and Mediterranean corn borer resistance monitoring

a) Field sampling and laboratory rearing

The sampling scheme of the IRM plan establishes that target pest 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 these populations are multivoltine. Following this scheme, in 2018, the consent holder collected ECB and MCB larvae exclusively from three sampling zones in north-eastern Spain. Currently, this area is the only hotspot for resistance evolution in the EU, where more than 60% of the total maize acreage corresponds to maize MON 810 hybrids (Appendix B) and ECB and MCB populations complete two generations annually (Alfaro, 1972).

ECB and MCB populations were collected from refuges and non-*Bt* maize fields. In 6 out of 17 and 8 out of 18 of the sampling sites inspected in 2018, none or very few numbers of ECB and MCB larvae

¹² Asociación Nacional de Obtentores Vegetales (ANOVE): <http://anove.es/> (Accessed: 18 September 2020).

¹³ Red de avisos Fitosanitarios de Aragón: <http://web.redfara.es/> (Accessed: 18 September 2020).

¹⁴ Noticias de la Red de Alerta e Información Fitosanitaria de Andalucía: <http://www.juntadeandalucia.es/agriculturapescaydesarrollorural/raif> (Accessed: 18 September 2020).

were found, respectively. These numbers are lower to those reported for the 2017 growing season (EFSA, 2019a). EFSA notes that the numbers of ECB (1,144) and MCB (1,490) individuals collected in 2018 reached the target sampling size of 1,000 larvae (corresponding to 2,000 genomes) established in the current IRM plan. EFSA acknowledges the efforts made by the consent holder to achieve the target sampling size and recognises that it 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 fields infested with ECB and MCB larvae for sampling, EFSA is not aware of any evidence of area-wide corn borer suppression in north-eastern Spain.

Overall pre-imaginal mortality values during the laboratory rearing of field-collected individuals were high for both target pests; only 47% and 39% of the ECB and MCB larvae collected in the fields reached adulthood. Therefore, less than half of the field-collected larvae were represented in the diagnostic concentration assays. This prevented from reaching the recommended detection level of 3% (recessive) resistance allele frequency to detect a possible insurgence of field resistance timely. While mortality values reported for ECB in 2018 were similar to those recorded in previous reports, mortality values for MCB were much higher in 2018 (54% in 2017, 30% in 2016 and 16% in 2015). The consent holder indicated that the laboratories performing the bioassays have extensive experience working with larvae populations of ECB and MCB, and that both apply good experimental practices. EFSA agrees that many factors can cause mortality during insect rearing before susceptibility testing, and that it is not possible to control some of those (e.g. parasitism of corn borer larvae by hymenopteran species). Nevertheless, EFSA encourages the consent holder to explore whether the rearing process can be further optimised and reduce the pre-imaginal mortality so that as many field-collected individuals as possible are represented in the bioassays.

b) Monitoring assays

Since the 2016 growing season, the consent holder conducts diagnostic bioassays with F_1 larvae from the field-collected individuals to assess the susceptibility of target pests to the Cry1Ab protein, instead of concentration-response assays. 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 selection of the diagnostic concentrations and the confirmatory studies performed with suspected-resistant individuals (EFSA, 2019a, 2018). EFSA has also encouraged the consent holder to improve the IRM plan and consider alternative testing methods continuously. However, the consent holder has not yet implemented all of its recommendations.

Design of diagnostic assays: EFSA notes that the consent holder has implemented some of the previous recommendations to harmonise the methodology of the diagnostic bioassays used for both target pests. For the 2018 PMEM report, field-collected ECB larvae from each sampling site were kept separately in the laboratory, and independent bioassays were conducted with F_1 larvae. This approach would allow collecting individuals from the same zone in the following seasons should be suspected or confirmed resistance from a particular zone or site to occur.

The diagnostic bioassays with MCB included a reference population serving as negative control and as an additional comparator. For ECB, EFSA notes that a Cry1Ab concentration corresponding to the diagnostic concentration was tested in both reference populations in the concentration-response bioassays; yet, moult inhibition at that concentration was not reported by the consent holder. Therefore, EFSA reiterates the recommendation to include a susceptible reference population in future diagnostic bioassays with ECB. For both target pests, reference populations should be used as a quality control instead of as an additional comparator 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 population but only with the expected 99% (see proposed testing approach in Appendix G).

Selection of diagnostic concentrations: The concentrations selected for discriminating between susceptible and homozygous 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 consent holder re-calculated the diagnostic concentration in 2016 using only data of larvae collected from north-eastern Spain over recent years. These larvae came from high selection pressure areas (for further details, see EFSA, 2019a, 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 consent

holder should confirm the validity of the concentration 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 zone 1, 2 and 3 of north-eastern Spain, corrected moult inhibition values were 97.85%, 99.06% and 99.05%, and the mean (98.65%) was lower than the expected > 99%. EFSA considers that moulting inhibition values lower than the expected > 99% in the diagnostic bioassays should trigger further investigations any population showing unusually low sensitivity to the Cry1Ab protein to determine if the population has field-relevant resistance to the trait. EFSA recommends that the consent holder standardises and harmonises the testing approach for confirming resistance of suspected target pest populations and adapts the IRM plan accordingly. In this respect, the consent holder could apply the step-wise approach recommended by the US Environmental Protection Agency for confirming resistance of lepidopteran pests of *Bt* plants (US EPA, 2010, 2018) to the corn borer monitoring programme (Appendix G).

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 reiterates the recommendation to increase the sensitivity and precision of the monitoring strategy so that the consent holder can implement alternative management measures timely to delay resistance evolution. As indicated in EFSA (2019a), this could be achieved by (1) increasing the sampling size of field populations and reducing the mortality during the laboratory rearing of field-collected populations; or (2) replacing diagnostic bioassays by more sensitive testing methods. The consent holder has conveyed the difficulties to find sampling sites with sufficient numbers of corn borer larvae and to reduce the mortality of field-collected individuals before laboratory testing. Therefore, using a more sensitive method appears as the most adequate alternative to increase the sensitivity of the monitoring strategy.

Bioassays with plant tissue: The consent holder conducted supplementary bioassays using maize MON 810 leaves with those ECB and MCB larvae surviving the diagnostic concentration as well as with spare larvae not used in the bioassays. These assays with plant material aim to verify whether resistant individuals were present in the field-collected populations. EFSA recognises the value of conducting such studies with plant material but considers that the consent holder should perform them in cases of suspected resistance with the progeny of larvae surviving the diagnostic bioassays, following the step-wise approach presented in Appendix G.

EFSA identifies methodological differences between the additional studies conducted with the two corn borer 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 an acceptable negative control (non-*Bt* maize leaves) (EFSA, 2019a, 2018) have been implemented. The consent holder confirmed the expression of Cry1Ab in all *Bt* plants used in the assays used with MCB larvae using commercial immunostrips. EFSA encourages the consent holder to follow a similar approach in future plant bioassays with ECB.

Alternative testing methods

EFSA recommends the consent holder to consider alternative testing methods for increasing 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, the F_2 screen has been used to estimate the upper 95% confidence interval for Cry1Ab-resistance allele frequencies in several ECB (Bourguet et al., 2003; Engels et al., 2010) and MCB populations (Andreadis et al., 2007) for the establishment of 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. Camargo et al. (2018) found a Cry1Ab resistance allele in one of the 137 F_2 lines tested.

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 has only been implemented routinely in the resistance management plan for *Bt* cotton in Australia (Downes and Mahon, 2012; Downes et al., 2016). Nevertheless, as recommended previously, EFSA still considers that an F_2 screen should be performed periodically with ECB and MCB populations to confirm the results of the diagnostic bioassays and to monitor whether the frequency of the Cry1Ab resistance allele is evolving as predicted by the resistance evolution models. Eventually, periodic estimations of

resistance alleles through F_2 screening, together with a robust farmer complaint system (see Section 3.2.3.3 for further insights), could replace annual diagnostic concentration assays. To obtain sufficient sensitivity for detecting Cry1Ab resistance alleles before they become common enough and resistant individuals cause measurable field damage, the target for testing should be at least 100 isolines. After each F_2 screen, the consent holder should run new simulations with resistance evolution models using the latest resistance frequency estimations and accounting for changes in the parameters of the model (e.g. proportion of maize MON 810, refuge compliance). The outcome of these simulations would help to decide when to conduct the next F_2 screen.

Considering that 4 years have passed since the last estimation of the frequency of resistance alleles and that Camargo et al. (2018) identified a Cry1Ab resistance allele in an MCB population from north-eastern Spain, EFSA considers that it is time to perform an F_2 screen on MCB populations from that area. The consent holder should also estimate the frequency of Cry1Ab resistance alleles in ECB populations from north-eastern Spain as there are no previous calculations.

In case that *Bt*-resistant laboratory populations of ECB and MCB would be available (e.g. after laboratory selection), an alternative could be performing an 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).

Reporting of monitoring data: Insect resistance monitoring assays should report sufficient information to facilitate the appraisal of their validity. In this respect, EFSA has developed a list of recommended reporting information (presented as a checklist in Appendix H of this statement) that aim at facilitating open data reporting of future monitoring assays. The checklist focuses on several elements relevant to the evaluation of study design and the interpretation of results. Study authors should consider these recommendations when preparing the reports of resistance monitoring assays, and they are encouraged to justify whenever it is not possible to meet any of the recommendations. EFSA has updated this checklist to address identified reporting issues.

Farmer complaint system

EFSA considers that a farmer complaint system could complement the other strategies used for managing insect resistance as it allows those managing crops to comment on pest infestation levels and product performance and report possible damages. Therefore, it may provide an additional source of first-hand information to field sampling and laboratory monitoring assays. However, at present, EFSA is not in the position to evaluate the usefulness of the existing farmer complaint system as a complementary resistance monitoring tool. In particular, the consent holder has not provided evidence that adequate communication mechanisms and educational programmes (e.g. field scouting techniques and characterisation of the damage caused by corn borers) exist ensuring the prompt and effective reporting of farmer complaints. As for the regional monitoring networks mentioned, although they might help warning farmers about a possible outbreak, they currently do not address this issue.

3.1.2.3. Conclusions on insect resistance monitoring

The analysis of the resistance monitoring data does not show a decrease in susceptibility to the Cry1Ab protein in the ECB populations collected from north-eastern Spain during the 2018 maize growing season. For MCB, moulting inhibition observed in the diagnostic concentration bioassays was lower than the expected > 99% in one of the three populations tested. Additional studies with plant material indicate that none of the MCB larvae tested from that population could complete development on maize MON 810 leaves. EFSA recommends the consent holder standardising the testing approach to ascertain whether the observed unusual response in the diagnostic assays is reproducible and heritable and if so, to assess the field relevance of the suspected-resistant populations and adapting the harmonised IRM plan accordingly.

Based the estimated numbers of ECB and MCB field-collected larvae represented in the diagnostic concentration bioassays, the monitoring strategy implemented in the 2018 growing season was not sensitive enough to detect the recommended 3% resistance allele frequency (EFSA, 2015a). Consequently, EFSA strongly recommends the consent holder increasing the sensitivity and precision of the monitoring strategy so that alternative management measures can be implemented timely to delay resistance evolution. The consent holder has acknowledged some difficulties to locate maize fields with sufficient infestation levels of corn borers and to reduce their mortality in the laboratory before testing.

Therefore, the most adequate way to increase the precision of the monitoring strategy is by using a more sensitive testing method, i.e. F₂ screening.

EFSA considers that it is timely to perform an F₂ 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 the consent holder has not followed several other recommendations to resolve previously identified shortcomings and to improve the monitoring plan (for a summary of these, see Section 5).

3.2. General surveillance

3.2.1. Farmer's questionnaires¹⁵

3.2.1.1. Consent holder's assessment

2018 Questionnaires

In the annual 2018 PMEM report, the consent holder submitted a survey based on 250 farmer questionnaires completed by farmers in Spain and Portugal (Table 5). Both Member States accounted for all the maize MON 810 grown in the EU in that year.

The 2018 PMEM report represented the 13th reporting year, with the completion of a total of 3,377 questionnaires since 2006.

The surveys were performed in each country by an external company and were completed between February and March 2019. The response rate was 49.7% in Spain,¹⁶ and 100% in Portugal. Eighty-two of the 250 farmers (32.8%) were interviewed for the first time.

Table 5: Farmers surveyed and maize MON 810 areas monitored in 2018 through questionnaires [Table based on data provided in the 2018 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	238 ^(a)	16.0	3,735	115,246	3.2
Portugal	12 ^(b)	53.0	641	5,735	11.2
Total	250	17.5	4,376	120,979	3.6

(a): One-hundred and seventy-three farmers were from Aragón/Cataluña, 29 from Extremadura, 11 from Andalucía, 17 from Comunidad Foral de Navarra and 8 from Castilla la Mancha. Eighty-two out of the 238 farmers were interviewed for the first time.

(b): Seven farmers were from Alentejo, two from Lisbon and Vale do Tejo and three from Center. Nine out of the 12 farmers were interviewed for the first time.

The questionnaire collected information on four specific areas: (1) maize cropping area; (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 2018 farmer questionnaires on maize MON 810 did not identify potential adverse effects that might be related to MON 810 plants and their cultivation.*

Pooled analysis 2006–2015 questionnaires

The consent holder has recently published the results of the pooled analysis of the questionnaires completed between 2006 and 2015 in Bertho et al. (2020) (for further details, refer to Section 3.2.3.3). Over that period, 2,627 EU farmers growing maize MON 810 varieties were surveyed. Data on 25 monitoring characteristics covering agronomic practices, characteristics in the

¹⁵ 2018 PMEM report: Sections 3.1.2.1 and 3.1.4.1 and Appendixes 1 and 2; additional information 5/6/2020.

¹⁶ The questionnaire was completed by 238 out of the 479 farmers that were contacted in Spain. The 241 farmers that did not respond gave the following reasons: 1) because they did not grow maize MON 810 in 2018 (74 farmers); 2) they did not grow maize in 2018 (63 farmers); 3) they grew maize MON 810 in 2018 but refused to sign the consent form (42 farmers); 4) they grew MON 810 in 2018 but refused to answer the interview (39 farmers); 5) they were absent or could not be localized (12 farmers); (6) they were retired (11 farmers).

field and environmental and wildlife aspects were collected and analysed, including an analysis of trends within the data. For 10 characteristics, the estimated 99% confidence intervals (CIs) for the model proportions for the 'As usual', 'Plus or changed' or 'Minus' responses (for more details, see Bertho et al., 2020) excluded the predefined threshold values (10% or 10%) for an effect (Table 6).

Table 6: Summary of the results of the 2006–2015 analysis for the monitoring characteristics (model proportions, p^{mo}) for which the estimated 99% confidence intervals (CI) did not include the predefined threshold values [Table based on data provided in Bertho et al. (2020)]

Subject	Monitoring characteristic ^(a)	Observations for maize MON 810 vs. conventional maize ^(b)
Agronomic practices	Insect control practices	20.04% less (20.03–20.06)
	Time of harvest	16.70% later (16.69–16.71)
	Maize borer control practice	20.79% less (20.77–20.81)
Characteristics in the field	Germination vigour	10.83% more (10.82–10.85)
	Incidence of stalk/root lodging	34.03% less (34.01–34.05)
	Time to maturity	19.30% delayed (19.28–19.32)
	Yield	53.74% more (53.71–53.76)
	Occurrence of maize MON 810 volunteers	11.14% less (11.12–11.16)
Environment and wildlife	Disease susceptibility	32.25% less (32.24–32.27)
	Pest susceptibility	18.20% less (18.19–18.21)

(a): The following monitoring characteristics were not changed: *Agronomic practices*: Crop rotation; time of planting, tillage and planting technique, fertiliser application, weed and fungal control practices, irrigation practices; *Characteristics in the field*: time to emergence and to male flowering; *Environment and wildlife*: Weed pressure, performance of fed animals, occurrence of insects, birds and mammals.

(b): Figures correspond to proportions estimated from the statistical model (p^{mo}) on 10 years pooled data of different answers (i.e. 'Plus' or 'Minus'). Values in brackets correspond to the 99% CIs. For additional details on the statistical analysis, please refer to the supporting information of Bertho et al. (2020).

Bertho et al. (2020) concluded that the analysis of the 2006–2015 data did not reveal any unexpected adverse effects associated with the cultivation of maize MON 810 in the EU. Besides, they proposed replacing annual surveys of farmers by the farmer complaint systems implemented by companies marketing maize MON 810 seeds (see Section 3.1.2.1).

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 in the 2018 growing season are similar to those from previous annual PMEM reports.

EFSA makes the following observations and comments on the methodology of the 2018 farmer questionnaire survey:

- The initial sampling frame for the farmer questionnaires survey aimed at considering a population of all maize fields. However, it is stated that *the sampling frame for this survey cannot be based on the total population of fields with MON 810 cultivation in Europe*, and so farmers are sampled instead of fields. Survey design methodology requires the use of a sampling frame which is representative for the sampled target populations and that the random selection process is applied to the sample units in the sampling frame prior to proceeding with the interviews. In the annual 2018 PMEM report, it is not clear whether such sampling frames

were used since it is indicated that *farmers therefore are selected from customer lists of the seed selling companies or interviewer companies, plus experience from previous surveys or search in the region*. Therefore, it cannot be ascertained that the selected farmers (from Portugal and Spain) are representative of the target farmer population. Therefore, the claim *The whole sampling procedure ensured that the monitoring area was proportional to and representative of the total regional area under GM cultivation* cannot be substantiated based on the information provided in the report.

- The questionnaire relies on a comparison between a representative GM maize field and a representative conventional field to detect unanticipated adverse effects. Consequently, the choice of representative fields and the recollection of similarities and differences are crucial to the success of the survey. The questionnaire provides a list of the GM and non-GM varieties grown by each farmer, but it is unclear which conventional and GM fields have been actually compared. The farmer questionnaire should be more specific on the comparison that has been made. If no comparators are being grown spatially or temporally close to the GM crop, then the rationale for selecting another comparator (e.g. maize grown in previous years) should be fully described. The specific comparators selected by the farmers for the survey should also be summarised in the monitoring report.
- Farmers completed the questionnaires between February and March 2019, that is, several months after the harvest of maize cultivated in 2018, when maize MON 810 growers might not recorded everything that occurred in the field or is required in the questionnaire. It would be advisable to send the questionnaire to the selected farmers at the beginning of the growing season, so that they know which questions are included and which observations they have to pay attention to all along the growing season.;
- Farmer questionnaires should focus on changes that would be recognised by the farmer during the daily management of the farm. However, additional questions could be included to gain a better understanding of the intensity of GM maize cultivation on the farm (number of years of maize MON 810 cultivation and frequency of maize MON 810 in crop rotations), and further information on plant protection product usage (in particular, in the comparator field) should be obtained to facilitate a full understanding of any observed changes. Moreover, qualitative responses may sometimes relate to a subjective assessment on the part of the farmer. An effort should be made to use objective measurable outcomes, whenever this is possible.
- The choice of statistical test should be based on the number of possible outcomes, since the use of a series of binomial tests for multinomial distributions would increase the experiment-wise Type I error rate (i.e. failure to detect a true adverse effect). In the current analysis, a closed principle test procedure is proposed to be used in order to address the issue of the overall type I error. This approach is acceptable, and can be effective for this purpose, if applied correctly, with the relevant not 'as usual' effects being assessed only when the null hypothesis 'proportion As usual' ($p_{AS\ usual} \leq 0.9$) is not rejected. However, in practice, in the comparison of the monitoring characteristics presented in the 2018 PMEM report (Table 8 of Appendix 1), the results of all possible tests are presented, when in the first hypothesis test, the null hypothesis $p_{AS\ usual} \leq 0.9$ is not rejected. Therefore, in the current report whenever the $p_{AS\ usual} \leq 0.9$ hypothesis is rejected, the decision is based only on this outcome. When this hypothesis is not rejected then, in the case of questions with three possible responses, two additional tests (for the probabilities of Plus- and Minus-answers) are being conducted, and therefore, some correction for the overall type-I error rate is still necessary.

In the assessment that follows, EFSA makes several observations and comments on the pooled analysis of the farmer questionnaires data published in Bertho et al. (2020). For the full explanations, see the analysis report in Annex 2 of supporting information of this statement.

- The software code used for the statistical analysis was not made available by the consent holder and, therefore, several aspects and details of the statistical analysis are unclear. EFSA attempted to replicate the results autonomously, using Software R, [R Core Team (2017)], but it has not been possible, on various occasions, to match the results reported by Bertho et al. (2020).
- Regarding the model chosen to estimate the proportions, not all the possible options have been taken into consideration during model selection. After the rejection of the full model (three-factorial model with all possible interactions among all independent variables), the authors considered a two-factorial model with no interactions, while models with different combinations

of interaction terms have not been evaluated. Furthermore, after choosing the final model (a two-factorial model with country and year as fixed terms), no regression diagnostics were reported for confirming that the model would be suitable to analyse the data.

- The dependent variable of the proposed regression model is the response of a farmer to a specific question each time, coded as a 0-1 variable. Even though the software code used for the statistical analysis was not made available by the consent holder, Bertho et al. (2020) states that a linear mixed model was used for this analysis. As the outcome variable is binary, a linear model is not appropriate, since it assumes a continuous outcome and errors which are normally distributed. In such a case, this assumption would be violated, and so in general, one might expect inferences to be invalid. Instead, since the dependent variable is dichotomous, it would require using an appropriate regression model, e.g. logistic.
- The description of the statistical model should have been made clearer. The model proportion (term p^{mo}), the variable that is estimated from the model, appears in the model as an independent variable (like the covariate variables 'year' and 'country'), instead of as a dependent variable.
- In the statistical analysis, a least square means procedure has been applied to estimate the yearly marginal model proportions. Since the explanatory variables used in the model are 'year' and 'country', it would have been preferable to report the marginal model proportions by both year and country.
- It is not clear how the CIs of the total model proportions have been calculated. In general, the CIs reported by Bertho et al. (2020) seem to be too narrow. It would be important to clarify how they were estimated, since the inferences about the endpoints are based on the CIs [in the sense of examining the position of the CIs in relation to the respective threshold values (0.9 and 0.1)].

3.2.1.3. Conclusions on farmer questionnaires

From the data provided by the 2018 farmer survey, EFSA could not identify any unintended effects associated with the cultivation of maize MON 810 varieties. Concerning the analysis of the pooled data (2006–2015 maize growing seasons) from Bertho et al. (2020), EFSA cannot confirm or comment on the results and conclusions presented in the publication since the statistical model used is not considered appropriate.

EFSA acknowledges that the current design and implementation of the farmer questionnaires have a rather low cost–benefit ratio. Besides, the farmer questionnaires present several limitations associated with the sampling frame, the time of the surveys, the selection of comparators and the adequacy of some of the questions (see Section 3.2.1.2). EFSA agrees with the applicant that a fit-for-purpose farmer alert system might help to detect unexpected adverse effects caused by the cultivation of maize MON 810 and be an alternative. However, EFSA does not support the proposal of Bertho et al. (2020) to replace farmer questionnaires by the farmer complaint system that the consent holder and other companies marketing maize MON 810 seeds are currently implementing. The main reasons are that the existing system is not dedicated to monitoring and lacks adequate communication mechanisms and educational programmes (e.g. field scouting techniques and characterisation of the damage caused by corn borers).

EFSA believes that a robust and fit-for-purpose farmer alert system should be linked or integrated into existing pest monitoring systems as established to support the implementation of Integrated Pest Management across Member States (See Directive on sustainable use of pesticides 2009/128¹⁷), and that farmers growing maize MON 810 varieties could be encouraged to report any unusual observations through the Common Agricultural Policy cross-compliance or additional incentives. In the meantime, EFSA is of the opinion that farmer questionnaires should remain in place and their implementation should integrate the above-mentioned recommendations to improve their efficiency and potential to detect unexpected adverse effects.

Together with the use of existing environmental monitoring networks (see following Section 3.2.2), this farmer alert system would be part of a general framework on general surveillance as suggested by EFSA GMO Panel (2011b).

¹⁷ Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides.

EFSA strongly recommends all stakeholders, including EU Member States and relevant national Competent Authorities, to have a dialogue and agree on how farmers growing maize MON 810 could best identify and report unexpected adverse effects from the cultivation of *Bt* maize varieties.

3.2.2. Existing monitoring networks¹⁸

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

An external report commissioned by EFSA (Centre for Ecology and Hydrology et al., 2014) and associated publications (e.g. Smets et al., 2014) have identified several existing environmental monitoring networks on the evolution of environment-related endpoints. Such networks may provide useful information on how agricultural practices at large impact the environment and, as such, may be useful for the general surveillance of GM plants. EFSA acknowledges that the use of such systems raises a major methodological challenge, namely the feasibility of linking a given agricultural practice, such as GM cultivation, with global impacts while many other stressors may explain the observed changes. Other challenges include data heterogeneity, incompleteness, accessibility to data, exploitation methodologies, data reporting format and data connectivity with GMO registers (EFSA GMO Panel, 2014b). Also, the lack of a clear definition of the protection goals in each EU Member State or region is a significant obstacle. However, there exist networks adapted to such an exercise (e.g. monitoring of butterflies). These systems would equally inform the potential effect of other agricultural practices (e.g. pesticides).

Therefore, as part of the general framework on general surveillance that would also include a robust farmer alert system, EFSA encourages the EU Member States and relevant stakeholders to engage in the pooling of networks and the development of a methodological framework that enables making the best use of existing ones involved in environmental monitoring of agricultural practices.

3.2.3. Literature searching¹⁹

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 2018 and May 2019.

The consent holder searched the electronic bibliographic databases Web of Science Core Collection and CAB Abstracts, hosted under the Web of Science (Clarivate Analytics) and the EBSCOhost (EBSCO Information Services) platforms, respectively. Altogether, 209 publications were retrieved (including duplicates). After applying the predefined eligibility/inclusion criteria, the consent holder identified 21 publications as relevant for the assessment of food and feed (eight publications) or environmental safety (13 publications). Besides, the consent holder searched the websites of nine organisations involved in risk assessment of single GM maize products. None of the 76 records obtained in those searches was considered relevant.

The consent holder evaluated the reliability and implications for the risk assessment of all relevant publications and indicated that none of them would invalidate the initial conclusions of the maize MON 810 risk assessment.

¹⁸ 2018 PMEM report: Sections 3.1.2.3 and 3.1.4.3.

¹⁹ 2018 PMEM report: Section 3.1.6 and Appendix 5; additional information 27/5/2020.

3.2.3.2. EFSA's assessment

Systematic literature search

EFSA evaluated the systematic literature search using a modified version of the EFSA critical appraisal tool for assessing quality of extensive literature searches (EFSA, 2015b). To develop this tool, EFSA followed the relevant principles and criteria outlined in EFSA (2010) and the recommendations provided in EFSA (2017).

The overall quality of the literature search is acceptable (for further details on the assessment, see Annex 3 of supporting information). However, EFSA considers that the consent holder could fine-tune future searches on maize MON 810. Specifically, EFSA recommends the consent holder to:

- Use enough search term variation (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, use both free-text terms and controlled vocabulary in the searches;
- Use enough truncation and use it consistently;
- Increase the proximity operator distance (NEAR/5 (or higher) 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 a few publications);
- Follow the guidelines given in EFSA's updated explanatory note on literature searching (EFSA, 2019b).

Relevant scientific publications

EFSA assessed the 21 publications identified as being relevant to the food and feed and environmental safety assessment of maize MON 810 and the Cry1Ab protein (Annex 4 of supporting information). 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. Additional scientific publications assessed by EFSA

EFSA identified two additional relevant publications, Bertho et al. (2020) and Pott et al. (2020), which were published after the period covered by the literature search performed by the consent holder.

Publication by Bertho et al. (2020)

This publication presents the results of the general surveillance activities for maize MON 810 in the EU conducted by the consent holder between 2006 and 2015. These consist of i) assessment of relevant publications found in annual literature searches and ii) annual surveys of farmers growing maize MON 810. The publication by Bertho et al. (2020) includes the results of the pooled analysis of the data from the farmer questionnaires. An assessment of the analysis has been provided in Annex 2 of supporting information.

In their publication, Bertho et al. (2020) conclude that the analysis of the pooled data did not reveal any unexpected adverse effects associated with the cultivation of maize MON 810 and that none of 375 relevant peer-reviewed publications led to a change in the conclusions of the initial risk assessment that demonstrated the food and feed and environmental safety of the GM maize MON 810. EFSA cannot confirm or comment on the results and conclusions presented in the publication since the statistical model used is not considered appropriate.

The authors consider that annual surveys of farmers could be replaced by farmer complaint systems that are already put in place by companies marketing maize MON 810 seeds and propose that future general surveillance to detect unanticipated adverse effects of maize MON 810 focuses on annual literature searches and farmer complaint systems. EFSA has commented on such proposal in Section 3.2.1.3.

Publication by Pott et al. (2020)

This publication addresses the effect of the Cry1Ab protein on two freshwater caddisfly (Trichoptera) shredders under environmentally controlled (laboratory) conditions. The results reported by the authors do not provide any new information that would invalidate the previous safety

assessment conclusions and risk management recommendations on maize MON 810 made by EFSA or its GMO Panel (for further details on the assessment, see Annex 4 of supporting information).

3.2.3.4. Conclusions on literature searching

The overall quality of the literature search performed by the consent holder is acceptable; however, EFSA considers that the consent holder can fine-tune the methodology and reporting of the literature search. Besides, in future searches, the consent holder should comply with EFSA's updated explanatory note on literature searching (EFSA, 2019b).

EFSA assessed the relevant publications identified by the consent holder through the performed literature search and two other publications which were published after the period covered by the literature search. The assessment of these publications 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 2018 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 7 presents EFSA'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 7: Weight of evidence approach followed to assess the evidence provided in the 2018 PMEM report on maize MON 810

Question:	<i>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 2018 growing season that would invalidate previous GMO Panel evaluations on the safety of this GM maize?</i>	
Assemble the evidence	Select the evidence	The evidence was obtained from: <ul style="list-style-type: none"> – The 2018 PMEM report submitted by the consent holder – Additional information on insect resistance management, literature searching and farmer questionnaires 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: <p><u>Case-specific monitoring</u></p> <ul style="list-style-type: none"> – LoE 1: Farmer compliance with refuge requirements. Survey of 238 Spanish and 12 Portuguese farmers growing maize MON 810 (<i>Section 3.1.1</i>) – LoE 2: ECB and MCB resistance monitoring (<i>Section 3.1.2</i>): <ul style="list-style-type: none"> • Sampling of 1,144 ECB and 1,490 MCB larvae from three zones in North-eastern Spain <ul style="list-style-type: none"> • DC and plant bioassays conducted with the progeny of field-collected individuals • Confirmatory/Follow-up studies with larvae surviving the DC assay – LoE 3: Farmer complaint system: complaints received from farmers growing maize MON 810 varieties during the 2018 growing season (<i>Section 3.1.2</i>) <p><u>General surveillance</u></p> <ul style="list-style-type: none"> – LoE 4: Systematic literature search (June 2018–May 2019). Twenty-nine food and feed- and environmental safety relevant publications were

		<p>identified and assessed. Two additional publications were identified by EFSA and assessed (<i>Section 3.2.3</i>)</p> <ul style="list-style-type: none"> – LoE 5: Existing monitoring networks – LoE 6: Farmer survey based on 250 questionnaires received from farmers in Spain and (238) and Portugal (12) (<i>Section 3.2.1</i>) – LoE 7: Results of the pooled analysis of the questionnaires conducted over 2006–2015 from Bertho et al. (2020) (<i>Section 3.2.1</i>)
Weigh the evidence	Methods	<ul style="list-style-type: none"> – LoE 1: Best professional judgement – LoE 2: The relevance and validity of the bioassays 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 (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 and LoE7: 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	<p><u>Case-specific monitoring</u></p> <ul style="list-style-type: none"> – LoE 1: Partial compliance (89%) with refuge requirements in Spain and full compliance in Portugal was reported in the farmer's questionnaires – LoE 2: <ol style="list-style-type: none"> 1) ECB: MI inhibition of larvae tested against the DC was 99.19%. The three larvae that moulted to the second instar in the DC assay died within 5 days of feeding on maize MON 810 leaves 2) MCB: MI inhibition was lower than the expected 99% in one of the three sampling zones. No resistant larvae were found in the follow-up/confirmatory bioassays with maize MON 810 leaves. – LoE 3: None of the 1,249 complaints received in 2018 were attributed to loss of efficacy of maize MON 810 <p><u>General surveillance</u></p> <ul style="list-style-type: none"> – LoE 4: The information reported in the eight food and feed and 15 environmental-safety relevant publications identified through the systematic literature search and the two additional publications identified by EFSA 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. – LoE7: The results and conclusions presented in the publication cannot be confirmed because the statistical model used by Bertho et al. (2020) was not considered appropriate.
Integrate the evidence	Methods	<ul style="list-style-type: none"> – The different LoE were integrated by best professional judgement (i.e. no formal method was used) <ol style="list-style-type: none"> 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 2018 growing season

	Results	<p>Conclusions (<i>Section 4</i>)</p> <ul style="list-style-type: none"> – The monitoring strategy implemented in 2018 is not sensitive enough to detect the recommended 3% resistance allele frequency – The information reported in the 2018 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 2018 growing season – EFSA concludes that no new evidence has been reported in the context of the 2018 PMEM report that would invalidate previous GMO Panel evaluations on the safety of maize MON 810
		<p>Recommendations</p> <ul style="list-style-type: none"> – EFSA strongly recommends the consent holder to <ol style="list-style-type: none"> 1) Achieve full compliance with refuge obligations 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 an F₂ 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 (<i>Section 5</i>)

DC: Diagnostic concentration; ECB: European corn borer; MCB: Mediterranean corn borer; MI: moult inhibition.

4. Conclusions

The evidence from the 2018 PMEM report and the additional information provided by the consent holder upon EFSA's request does not indicate any adverse effects on human and animal health or the environment arising from the cultivation of maize MON 810 during the 2018 growing season. Consequently, previous evaluations on the safety of maize MON 810 (EFSA, 2009; EFSA GMO Panel, 2012b,c) remain valid.

EFSA identifies methodological and reporting limitations on insect resistance monitoring, farmer questionnaires and literature searching that the consent holder should resolve in future PMEM reports. In particular, EFSA notes that the monitoring strategy implemented in the 2018 growing season is not sufficiently sensitive to detect the recommended 3% resistance allele frequency necessary for timely detection of a surge of field resistance. EFSA advocates for using a more sensitive method, like F₂ screening.

EFSA believes that a robust and fit-for-purpose farmer alert system may help to detect unexpected adverse effects caused by the cultivation of maize MON 810 and be an alternative to the current farmer survey system. EFSA recommends all stakeholders, including EU Member States and relevant national Competent Authorities, to have a dialogue and agree on how farmers growing maize MON 810 could best identify and report unexpected adverse effects from the cultivation of *Bt* maize varieties and on how to take stock of existing environmental networks. In the meantime, EFSA is of the opinion that farmer questionnaires should remain in place and that their implementation should integrate the above-mentioned recommendations to improve their efficiency and potential to detect unexpected adverse effects.

Section 5 summarises EFSA's recommendations to resolve the shortcomings identified in the 2018 PMEM report.

5. Recommendations

EFSA notes that the consent holder has not yet implemented several recommendations to resolve previously identified shortcomings for case-specific monitoring and general surveillance. Consequently, EFSA strongly recommends the consent holder to: 1) achieve full compliance with refuge requirements in areas of high adoption of maize MON 810 (i.e. north-eastern Spain); 2) increase the sensitivity of the resistance monitoring plan and address previously mentioned methodological, analytical and reporting limitations for resistance monitoring and farmer questionnaires; and 3) perform an F₂ screen on European and Mediterranean corn borer populations from north-eastern Spain. Moreover, relevant

stakeholders should implement a methodological framework to enable making the best use of existing networks involved in environmental monitoring for the general surveillance of GM plants (see Table 8 for further details).

Table 8: Summary of EFSA's recommendations for future PMEM reports on maize MON 810

Area (Section)		Recommendation ^(a)	Responsible for implementation
Case-specific monitoring	Implementation of non- <i>Bt</i> 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 – Relevant 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)	<u>Monitoring strategy</u> – 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
		<u>Laboratory rearing</u> – To optimise the rearing process of field-collected individuals and reduce the pre-imaginal mortality before susceptibility testing	
<u>Testing</u> – 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 include a reference laboratory population in the bioassays with ECB – 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			
	<u>Reporting</u> – To consider recommendations outlined in Appendix H of this statement when preparing the reports of bioassays		
	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 exist ensuring the prompt and effective reporting of farmer complaints.	– Consent holder

Area (Section)		Recommendation ^(a)	Responsible for implementation
General surveillance	Farmer questionnaires (Section 3.2.1.2)	– To consider whether questionnaires could be replaced by a proper complaint system for identifying and reporting unexpected adverse effects from the cultivation of <i>Bt</i> maize varieties.	– Consent holder – EU Member States – Other relevant stakeholders
	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, 2019b)	– Consent holder

(a): Further details are provided in the respective sections of this Statement.

Documentation provided to EFSA

- 1) Letter from the European Commission, dated 24 February 2020, requesting EFSA to assess the annual PMEM report on the cultivation of maize MON 810 during the 2018 season provided by the consent holder.
- 2) Comments from the EU Member States on the 2018 PMEM report.
- 3) Additional information, dated 27 May 2020 and 5 June 2020, provided by the consent holder upon EFSA's request.

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 nonagrioides* (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 F₂ 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.
- Bertho L, Schmidt K, Schmidtke J, Brants I, Cantón RF, Novillo C and Head G, 2020. Results from ten years of post-market environmental monitoring of genetically modified MON 810 maize in the European Union. *PLoS ONE*, 15, e0217272. <https://doi.org/10.1371/journal.pone.0217272>
- 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.
- Camargo AM, Arias-Martín M, Castañera P and Farinós GP, 2020. Performance of *Sesamia nonagrioides* on cultivated and wild host plants: Implications for Bt maize resistance management. *Pest Management Science*. <https://doi.org/10.1002/ps.5913>
- Carrière Y, Fabrick JA and Tabashnik BE, 2016. Can pyramids and seed mixtures delay resistance to bt crops? *Trends in Biotechnology*, 34, 291–302.
- 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>
- Davis PM and Onstad DW, 2000. Seed mixtures as a resistance management strategy for european corn borers (Lepidoptera: Crambidae) infesting transgenic corn expressing Cry1Ab protein. *Journal of Economical Entomology*, 93, 937–948.
- DGAV (Direção-Geral de Alimentação e Veterinária), 2019. Coexistência entre culturas geneticamente modificadas e outros modos de produção agrícola. Relatório de acompanhamento-2018, 1–25.
- 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, Luttk R, Makowski D, Siani A, Wahlstroem H, Aguilera J, Dorne J-L, Fernandez Dumont A, Hempten 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), Álvarez F, Camargo AM and Devos Y, 2019a. 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>
- EFSA (European Food Safety Authority), Devos Y, Guajardo IM, Álvarez F and Glanville J, 2019b. 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, Cagán L, Manachini B, Schuphan I, Stodola TJ, Micoud A, Brazier C, Mottet C and Andow DA, 2010. Evaluating resistance to Bt toxin Cry1Ab by F2 screen in European populations of *Ostrinia nubilalis* (Lepidoptera: Crambidae). Journal of Economic Entomology, 103, 1803–1809.
- EuropaBio, 2019. 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.
- 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, 2020. Resistance of the fall armyworm, *Spodoptera frugiperda*, to transgenic *Bacillus thuringiensis* Cry1F corn in the America: lessons and implications for Bt corn IRM in China. Insect Science. <https://doi.org/10.1111/1744-7917.12826>
- 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. 2018. Global Status of Commercialized Biotech/GM Crops in 2017: Biotech crops continue to help meet the challenges of increased population and climate change. ISAAA Brief No. 54. 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.
- Pott A, Bundschuh M, Bundschuh R, Otto M and Schulz R, 2020. Effect of Bt toxin Cry1Ab on two freshwater caddisfly shredders – an attempt to establish dose-effect relationships through food-spiking. *Scientific Reports*, 10, 5262.
- 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.
- R Core Team, 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available online: <http://www.R-project.org/>
- 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.
- Smith JL, Farhan Y and Schaafsma AW, 2019. Practical Resistance of *Ostrinia nubilalis* (Lepidoptera: Crambidae) to Cry1F *Bacillus thuringiensis* maize discovered in Nova Scotia, Canada. *Scientific Reports*, 9, 18247. <https://doi.org/10.1038/s41598-019-54263-2>
- 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.
- 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.

Abbreviations

CI	confidence interval
ECB	European corn borer
ELISA	Enzyme-linked immunosorbent assay
GLP	Good laboratory practice
GMO	Genetically modified organism
GS	general surveillance
IRM	Insect resistance management
MCB	Mediterranean corn borer
PMEM	post-market environmental monitoring

Appendix A – Farmer compliance with refuge requirements in Spain between 2004 and 2018

[Table based on data provided in 2004–2018 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
2018	238	186	30	22	89	FQ

NR: not reported.

Shaded row corresponds to the annual PMEM report under assessment.

(a): Farmers planting < 5 ha of maize MON 810 in the farm are not required to plant a refuge. For the FQ, only farmers who are required to plant a refuge were considered for the calculation of non-compliance with refuge requirements.

(b): FQ: farmer questionnaires; Antama: Study sponsored by Spanish foundation supporting the use of new technologies in agriculture. In the surveys conducted by Antama, all farmers were from north-eastern Spain.

Appendix B – Growing area and adoption rate of maize MON 810 in north-eastern, Central and South-western Spain between 2014 and 2018

Season	Growing area of MON 810 (ha) ^(a)	Avances ^(b)	
		Total maize (ha)	Adoption rate (%)
North-eastern Spain (Aragón, Navarra and Cataluña)			
2014	97,686	154,134	63.4
2015	80,022	149,953	53.5
2016	96,180	149,843	64.2
2017	96,748	148,962 ^(c)	64.9 ^(c)
2018	91,784	145,287 ^(c)	63.2 ^(c)
Mean 2014–2018	–	–	61.8
Central Spain (Albacete)			
2014	5,696	14,700	38.8
2015	4,027	11,800	34.1
2016	4,388	9,600	45.7
2017	3,903	8,700 ^(c)	44.9 ^(c)
2018	2,406	7,092 ^(c)	33.9 ^(c)
Mean 2014–2018	–	–	39.5
South-western Spain (Extremadura and Andalucía)			
2014	24,507	96,999	25.3
2015	21,298	87,094	24.5
2016	25,958	72,257	35.9
2017	21,989	62,584 ^(c)	35.1 ^(c)
2018	19,109	61,207 ^(c)	31.2 ^(c)
Mean 2014–2018	–	–	30.4

(a): Source: Available online: <https://www.miteco.gob.es/es/calidad-y-evaluacion-ambiental/temas/biotecnologia/organismos-modificados-geneticamente-omg-/consejo-interministerial-de-ogms/superficie.aspx> (Accessed: 18 September 2020).

(b): Avances de superficies y producciones de cultivos: Available online: <http://www.mapa.gob.es/es/estadistica/temas/estadisticas-agrarias/agricultura/avances-superficies-producciones-agricolas/> (Accessed: 18 September 2020).

(c): Provisional data.

Appendix C – Field sampling of *Ostrinia nubilalis* (ECB) and *Sesamia nonagrioides* (MCB) larvae during the 2018 maize growing season in north-eastern Spain

[Table based on data provided in the 2018 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)	20	15
		Lanaja – 3 (Huesca)	130	40
		Cantalobos (Huesca)	121	55
		San Juan de Flumen (Huesca)	209	96
		Total	480	206 (43)
	2	Candasnos – 1 (Huesca)	214	126
		Candasnos – 3 (Huesca)	10	6
		Peñalba (Huesca)	143	53
		Total	367	185 (50)
	3	Aibar – 1 (Navarra)	11	14
		Aibar – 2 (Navarra)	12	
		Sangüesa – 1 (Navarra)	159	75
		Sangüesa – 2 (Navarra)	115	54
		Total	297	143 (48)
	Total		1,144	534 (47)
MCB	1	Lanaja – 1 (Huesca)	172	NR
		Lanaja – 3 (Huesca)	169	NR
		Cantalobos (Huesca)	175	NR
		Total	516	171 (33)
	2	Candasnos – 1 (Huesca)	206	NR
		Candasnos – 2 (Huesca)	181	NR
		Candasnos – 3 (Huesca)	166	NR
		Total	553	167 (30)
	3	Mendigorría – 2 (Navarra)	76	NR
		Mendigorría – 3 (Navarra)	44	NR
		Mendigorría – 4 (Navarra)	104	NR
		Mendigorría – 5 (Navarra)	197	NR
	Total	421	246 (58)	
	Total		1,490	584 (39)

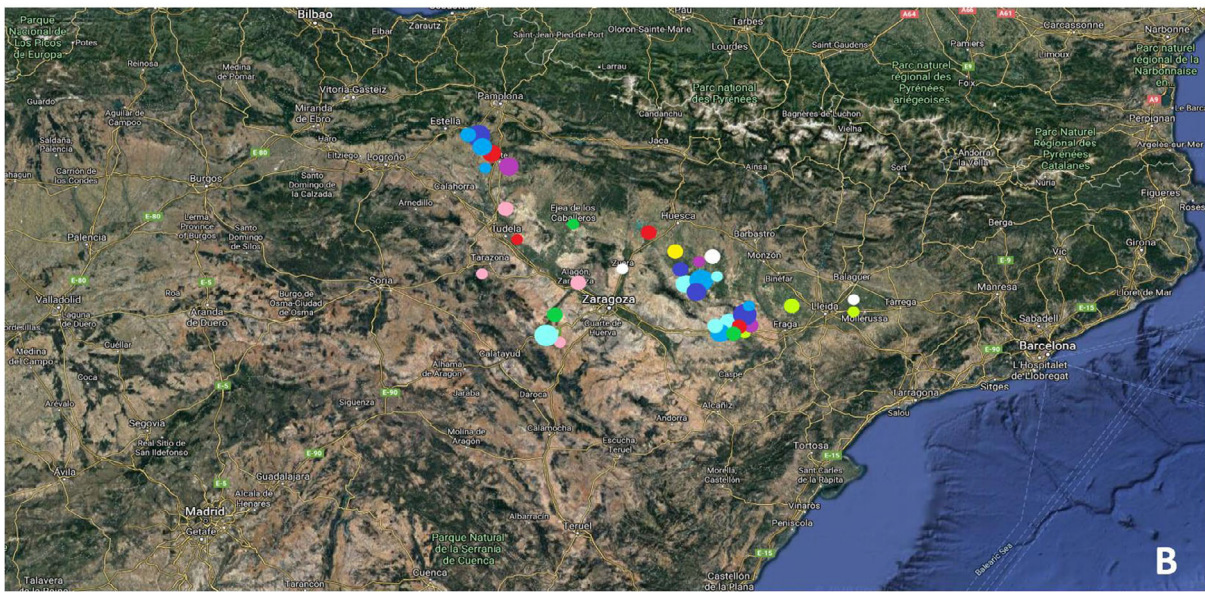
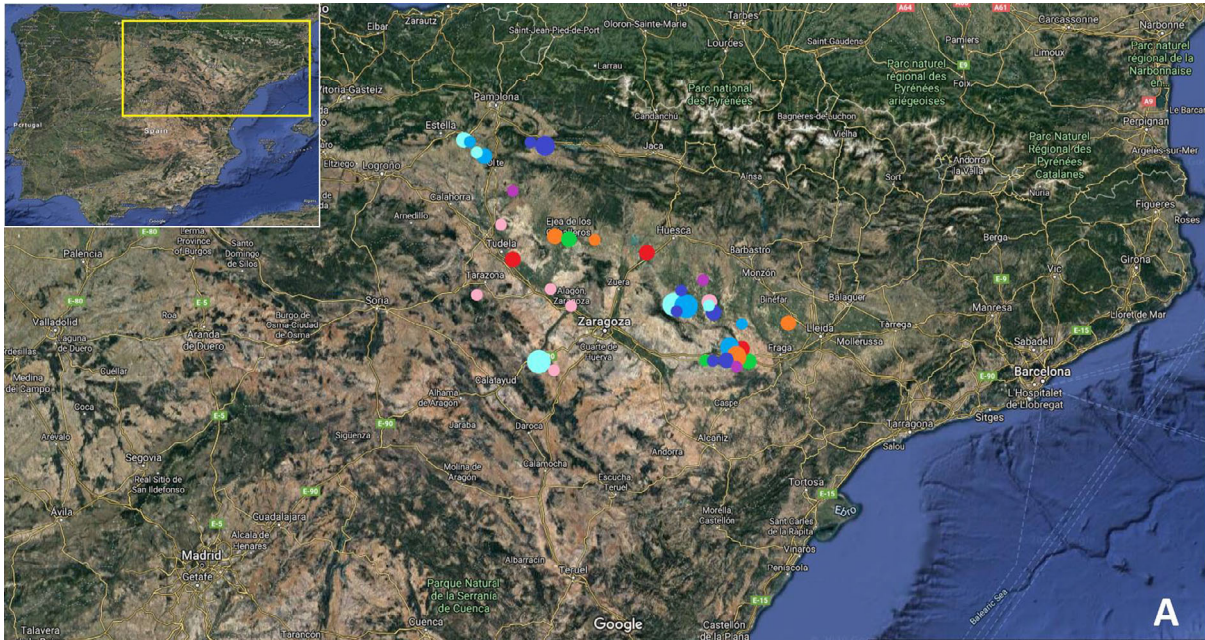
Late-instars were collected from refuges and non-Bt maize fields between 18 September and 17 October 2018. No geographical coordinates were provided for the sampling sites. All ECB and MCB larvae collected were in diapause.

NR: not reported.

Six and eight additional sites were inspected for ECB and MCB, respectively, but the minimum number of larvae established in the harmonised insect resistance management (EuropaBio, 2019) plan could not be reached for these sites.

Appendix D – Sites for *Ostrinia nubilalis* (A) and *Sesamia nonagrioides* (B) collection in north-eastern Spain between 2004 and 2018

[Based on data provided in the 2004–2018 PMEM reports on maize MON 810]



Appendix E – Historical data on Cry1Ab susceptibility of *Ostrinia nubilalis* (ECB) and *Sesamia nonagrioides* (MCB) populations from north-eastern Spain

[Table based on data provided in the 2008–2018 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)	% Moulting 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
	2018	1,144	2b	NP	NP	NP	NP	99.83
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)
	2018	1,490	B2-6	NP	NP	NP	NP	98.65 ± 0.40 ^(f)

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 population 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 E).

(b): 50% and 90% moulting inhibition concentration (MIC₅₀ and MIC₉₀) and their 95% confidence intervals (CI 95%) are expressed in ng Cry1Ab/cm² of diet surface area.

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

(d): The reference population was tested two times in 2008 (see Appendix E).

(e): MIC₅₀ and MIC₉₀ values of the reference population used to calculate RR MIC₅₀ and RR MIC₉₀ correspond to those estimated in 2004.

(f): Mean ± standard error of three independent assays corresponding to the different sampling zones.

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

[Table based on data provided in the 2006–2018 PMEM reports on maize MON 810]

Target pest	Population	Year	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	
		2018		3.93 (2.97–4.98)	7.23 (5.64–10.85)	NP	
			ES.ref ^(c)	2015	2a	1.82 (1.53–2.16)	2.95 (2.43–4.54)
		2016		2b	5.02 (3.61–6.33)	14.25 (11.29–19.87)	NP
		2017		2b	5.15 (4.20–6.05)	9.68 (8.15–12.37)	NP
		2018		2b	2.91 (2.21–3.76)	6.13 (4.61–9.75)	NP
MCB	Population 1 ^(d)	2004 ^(d)	B1	18 (11–25)	99 (66–208)	NP	
		2007 ^(d)	B1	16 (11–22)	94 (69–147)	NP	
		2008 ^(d)	B1	19 (10–30)	120 (76–255)	NP	
		2010 ^(d)	B1	8 (5–11)	74 (51–117)	NP	
		2011 ^(d)	B2-1	9 (6–13)	68 (45–127)	NP	
		2012 ^(d)	B2-1	7 (5–10)	62 (41–107)	NP	
		2013 ^(d)	B2-1	7 (5–10)	48 (31–88)	NP	
		2013 ^(d)	B2-2	5 (3–9)	42 (26–87)	NP	
		2014 ^(d)	B2-2	17 (11–25)	91 (57–209)	NP	
		2015 ^(d)	B2-2	28 (21–36)	67 (50–110)	NP	
		2016 ^(d)	B2-3	30 (24–38)	83 (62–132)	99.23	
		2017 ^(d)	B2-4	24 (16–35)	162 (100–363)	97.69	
			Population 2 ^(e)	2018 ^(e)	B2-6	19 (13–26)	116 (76–224)

Shaded rows correspond to values from the 2018 PMEM report. NP: not performed.

(a): 50% and 90% moulting inhibition concentration (MIC₅₀ and MIC₉₀) and their 95% confidence intervals (CI 95%) are expressed in ng Cry1Ab/cm² of diet surface area.

(b): The 'G.04' population was established from egg masses collected from Niedernberg (Germany) in 2005.

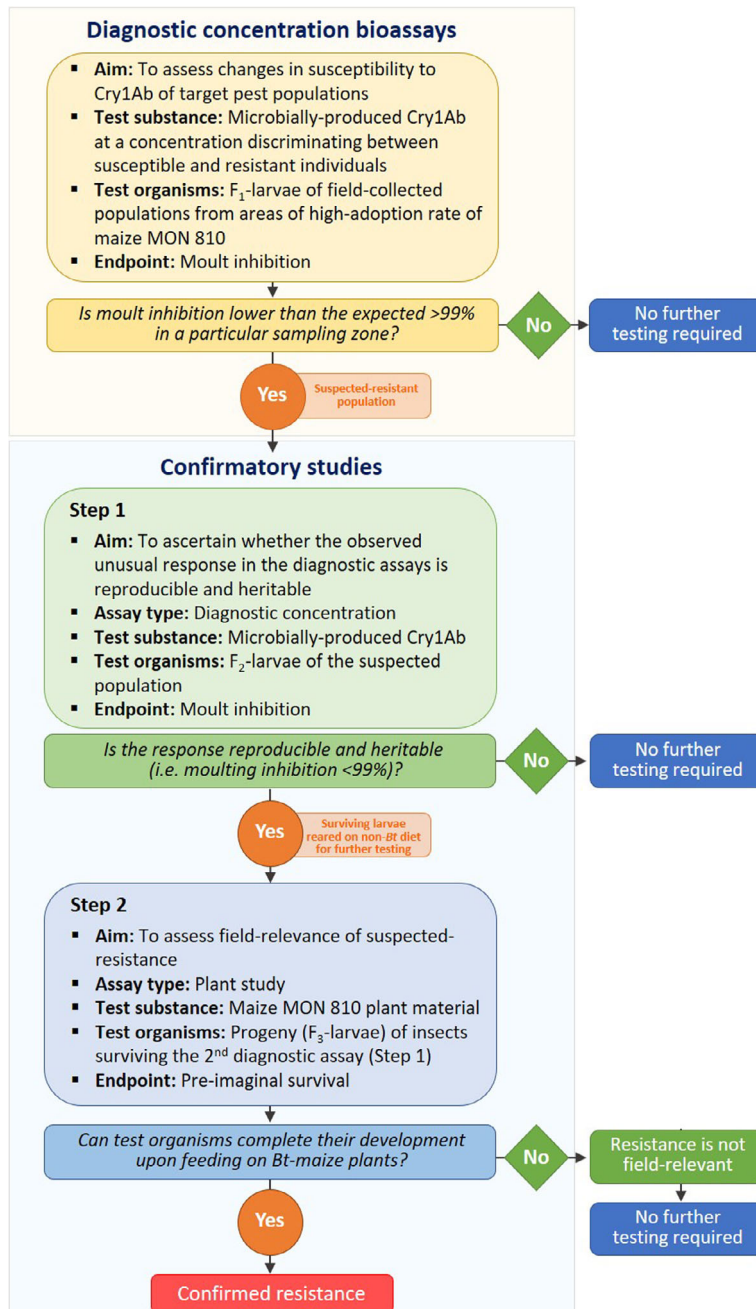
(c): The 'ES.ref' population was established from 145 diapausing larvae collected from three sampling sites in Galicia (Spain) in 2015, of which 75 survived the diapause, reached the adult stage and were placed in oviposition cages for mating.

(d): The population was established from larvae collected from Andalucía (661 larvae), Madrid (793 larvae), north-eastern Spain (857 larvae) and Galicia (665 larvae) (Spain) in 1998 (González-Núñez et al., 2000). To preserve its vigour, the population 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 population are introduced.

(e): The population was established in 2018 from larvae collected from Galicia (Spain) where *Bt* maize has never been cultivated.

Appendix G – Proposed step-wise approach for confirming resistance to *Bt* plants of suspected resistant populations

[Adapted from US EPA (2010, 2018).²⁰ Once resistance is confirmed, the EuropaBio insect resistance management plan foresees the implementation of remedial actions]



²⁰ US EPA (United States Environmental Protection Agency), 2010. Biopesticide Registration Action Document: Cry1Ab and Cry1F *Bacillus thuringiensis* (*Bt*) corn plant-incorporated protectants. 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.

Appendix H – Recommended minimum reporting information for insect resistance monitoring studies

To assist open data reporting, EFSA has compiled a list of recommended reporting information for insect resistance monitoring studies. The list is not inclusive and EFSA might revise it in the future.

Category	Specific reporting recommendations
General information	<ol style="list-style-type: none"> 1) Scientific name of the lepidopteran species tested 2) Assay type (e.g. concentration-response, diagnostic concentration, follow-up/confirmatory study with plant material/survival assays on plants) 3) Purpose of the study
Field collection	<ol style="list-style-type: none"> 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-<i>Bt</i> maize field, refuge) and distance to the nearest <i>Bt</i> maize field 7) Adoption rate of <i>Bt</i> maize compared to conventional maize (in the geographical area or in the sampling zone if data are available)^{(b),†}
Test organism	<ol style="list-style-type: none"> 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 and health 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 F₁ larvae[‡] 15) Description of the use of susceptible/resistant laboratory reference population, including information on how the population was initiated and how it is maintained and invigorated[‡]
Test substance	<ol style="list-style-type: none"> 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^{(c),†}
Study design	<ol style="list-style-type: none"> 21) Study performed according to standardised guideline/peer-reviewed protocol 22) Study performed according to GLP or other standards[§] 23) Description of control(s) 24) Preparation of stock solutions, including solvent concentrations in control(s) 25) Nominal concentration(s) of test substance and rationale for their selection 26) Administration of test substance (e.g. diet-overlay, mixed with artificial diet) 27) Age and generation of individuals tested (e.g. < 24-h-old larvae from F₁ generation) 28) Duration of the assay(s) 29) Description of measurement endpoints (e.g. mortality, moult inhibition) 30) 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	<ol style="list-style-type: none"> 33) Number of replicates for control(s) and test concentration(s); set-up of replicates (to avoid pseudo-replication) 34) Number of individuals tested per replicate 35) Treatment design (e.g., block, randomised) 36) Statistical method used 37) Statistical software used
Results and discussion	<ol style="list-style-type: none"> 38) Deviations from the protocol[†] 39) Description of the response effects for each of the measurement endpoints followed

Category	Specific reporting recommendations
	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 laboratories practices; MIC_x = x % moulting inhibition concentration.

†: Information not reported in the 2018 PMEM report for any of the two target pests.

§: Information not reported in the 2018 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): Mean adoption rates of the last 3–5 previous years could be provided included the sources used to estimate the rates.
- (c): 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.