STATEMENT

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Assessment of the 2019 post-market environmental monitoring report on the cultivation of genetically modified maize MON 810 in the EU

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Abstract

Following a request from the European Commission, the EFSA assessed the 2019 post-market environmental monitoring (PMEM) report on the cultivation of Cry1Ab-expressing maize event MON 810. Like previous years, there was full compliance with refuge requirement in Portugal and 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 2019 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. Overall, EFSA concludes that the evidence reported in the 2019 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 2019, is not sufficiently sensitive to detect the recommended 3% resistance allele frequency. Consequently, EFSA strongly recommends the consent holder to achieve full compliance with refuge obligations in areas where adoption of maize MON 810 is high and increase the sensitivity of the monitoring plan by performing periodic F_2 screens on corn borer populations from north-eastern Spain. EFSA recommends revising the farmer questionnaires when new characteristics of the receiving environment emerge which are relevant for the environmental risk assessment of MON 810 such as the emergence of teosinte. EFSA encourages the Competent authorities of concerned EU Member States, the consent holder and environmental networks to engage in a dialogue to develop a framework on how to best identify and report unexpected adverse effects from the cultivation of *Bt* maize varieties.

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

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Technical summary

Following a request from the European Commission, the European Food Safety Authority (EFSA) assessed the 2019 post-market environmental monitoring (PMEM) report on the cultivation of the Cry1Ab-expressing maize event MON 810. This report presents the results of the 2019 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 2019; 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.

Full compliance with refuge obligations is observed in Portugal and partial compliance with refuge obligations is observed in Spain where maize MON 810 adoption is high. EFSA considers that the consent holder should strive to increase the level of compliance in areas of high adoption of MON 810 in Spain even further. Like in Portugal, the Spanish National Competent Authorities or other relevant stakeholders, including farmers' associations. 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 2019 maize growing season. For the Mediterranean corn borer (MCB), moulting inhibition was lower than the expected > 99% in 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 F_2 screening. Periodic estimations of resistance alleles through F_2 screening, together with a robust farmer complaint system should replace annual diagnostic concentration assays. In addition, the consent holder should: (2) recalculate (and validate) the diagnostic concentration for MCB; (3) apply the stepwise approach recommended by the US Environmental Protection Agency for confirming resistance of lepidopteran pests of *Bt* plants updating the harmonised IRM plan accordingly; and (4) consider EFSA's previous reporting recommendations for future resistance monitoring studies.

EFSA considers that it is timely for the consent holder to perform an F_2 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 2019 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 260 farmer questionnaires and relevant scientific publications published between May 2019 and May 2020 that were identified through a systematic literature search complemented with an internet search in web pages of relevant key organisations involved in the risk assessment of GM plants.

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.

EFSA evaluated information on the occurrence of teosinte and recommends that the consent holder includes and explicitly considers in the future annual PMEM reports all scientific evidence relevant for the environmental risk assessment and risk management of maize MON810 in relation to teosinte.

Competent Authorities in concerned EU Member States, the consent holder and representatives of environmental networks are encouraged to have a dialogue to develop a methodological framework to identify and report unexpected adverse effects from the cultivation of *Bt* maize varieties.

Overall, EFSA concludes that the evidence reported in the 2019 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 2019, 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 2019 maize growing season.

General surveillance

The data from the 2019 farmer surveys showed that all farmers in Portugal and 94% 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.

The literature search, covering the period from May 2019 to May 2020, identified 14 scientific publications relevant to the food, feed and environmental safety of maize MON 810 and Cry1Ab. EFSA evaluated all 14 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 2019 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.

Information on the occurrence of teosinte was evaluated by EFSA. It is recommended that all scientific information on teosinte relevant for the environmental risk assessment and risk management of maize MON810 should be included in the future annual PMEM reports.

EFSA encourages Competent Authorities in concerned EU Member States, the consent holder and representatives of environmental networks to have a dialogue to develop a methodological framework to identify and report unexpected adverse effects from the cultivation of Bt maize varieties.

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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 EuropaBio[4] 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 factor[6] 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)

[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, p. 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] 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 metres 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).

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 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, 2018, 2019a). 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 16 October 2020, the European Commission received from the consent holder the annual PMEM report for the 2019 growing season of maize MON 810 (hereafter referred to as the '2019 PMEM report'). The reporting period of the 2019 PMEM report is from July 2019 till June 2020.

On 23 December 2020, the European Commission mandated EFSA *'to evaluate the findings of these monitoring activities, taking into consideration the comments received from the Member States. In case, the monitoring methodology used is different compared to the previous season, EFSA is also requested to assess the appropriateness of this methodology.*

Following a recent publication on teosinte,[7] *the COMPERA WG of EFSA's GMO Panel indicated that further discussion is required on the potential need to update the post-market environmental monitoring for maize MON 8102.*[8] *Therefore, EFSA is requested to assess the relevance of this scientific publication for the cultivation of MON 810 in the EU and, if appropriate, to provide monitoring recommendations in this respect'.*

2. Data and methodologies

2.1. Data

In delivering this statement, EFSA considered the information provided in the 2019 PMEM report,[9] comments submitted by the EU Member States and additional information on insect resistance monitoring which was submitted spontaneously by the applicant. Additional information on literature searching was provided by the consent holder upon EFSA's request. Information on the occurrence of teosinte in maize growing areas provided by the competent authorities in France and Spain was received from the Commission.

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 2019 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 (2019b).

[7] Le Corre V, Siol M, Vigouroux Y, Tenaillon MI and Délye C, 2020. Adaptive introgression from maize has facilitated the establishment of teosinte as a noxious weed in Europe. Proceedings of the National Academy of Sciences of the United States of America, 117, 25618–25627. https://doi.org/10.1073/pnas.2006633117

[8] https://www.efsa.europa.eu/sites/default/files/wgs/gmo/gmocompera2019.pdf

[9] The 2019 PMEM report is publicly available at https://ec.europa.eu/food/plant/gmo/post_authorisation/plans_reports_opinions_en (Accessed: 10 May 2021).

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.

Relevant papers on teosinte and the information on the occurrence if teosinte were evaluated and discussed by EFSA's CompERA working group.[10] The evaluation regarding the need for updating the PMEM plan for maize MON810 is provided in Section 3.2.3 of the current statement.

3. Assessment

3.1. Case-specific monitoring

3.1.1. Implementation of non-*Bt* maize refuges[11]

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 2019, 239 farmers from Spain and 11 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, 226 out of the 239 maize MON 810-growing farmers surveyed stated that they complied with refuge obligations, either because they did implement a refuge (199 farmers) or because they planted less than 5 ha of maize MON 810 and were thus not required to plant a refuge (27 farmers) (Appendix A).

The 13 farmers who 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 feared yield losses in conventional maize, had small plots which complicates the sowing, did not know the technical regulations/did not read the label recommendations.

The locations of the *Bt* maize fields and total number of farmers where no refuges were planted were Huesca (2 farmers), Lleida (8 farmers) and Zaragoza (1 farmer) – north-eastern Spain; Cáceres (1 farmer) and Sevilla (1 farmer) – south-eastern Spain.

b) Portugal

In Portugal, the 11 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 49 farms (out of the 140 *Bt* maize cultivation notifications received in 2019) where maize MON 810 was grown to check compliance with refuge and coexistence obligations outlined in Portuguese law (DGAV, 2020). 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 2019 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. 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

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 the case 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

[10] Minutes of the 225th meeting of the working group on comparative analysis and environmental risk assessment: https://www.efsa.europa.eu/sites/default/files/wgs/gmo/gmocompera2019.pdf

[11] 2019 PMEM report: Section 3.2.1.1; Appendix 1.

have led to several cases of practical resistance to *Bt* crops by different lepidopteran pests (reviewed by Tabashnik et al., 2013 and 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).

The data from farmer surveys and inspections from Portuguese authorities suggest full compliance with refuge planting requirements in Portugal as observed in previous years.

The farmer surveys in Spain resulted in 94% compliance with refuge planting obligation (see Appendix A), 5.4% of farmers did not implement a refuge although it was mandatory. An additional 11% of the farmers surveyed in Spain did not plant a refuge because the area where they planted Bt maize was less than 5 ha. However, it is not reported if these fields were in areas where the aggregated area planted with Bt maize is greater than 5 ha, for which EFSA considers that refuge requirements also apply, irrespective of individual field and farm size (EFSA, 2009).

Overall, the compliance has been stable over the last years at a rather high level. 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, given 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. EFSA therefore considers that the consent holder should strive to increase the level of compliance. To this end, EFSA recommends that

- The message provided to farmers in all documents (including poster, postcard, etc.) should always explain explicitly that non-compliance with refuge requirements may speed up resistance development in areas with high adoption rate and that, as a consequence, farmers would not benefit from the technology anymore in the future.
- The consent holder, EU Member States where maize MON 810 is cultivated and other relevant stakeholders should liaise to discuss how to contribute to reinforcing farmers' awareness of refuge compliance and develop 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 monitoring[12]

3.1.2.1. Consent holder's assessment

Following the IRM plan, the 2019 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 2019 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 2019 PMEM report

Assay	Population (Generation)	ECB	MCB
Susceptibility assay – Diagnostic	NE Spain (F_1 larvae)	• Diet-overlay assay with purified Cry1Ab at a diagnostic concentration • Progeny of field-collected larvae • 1,488 neonates exposed to 28.22 ng Cry1Ab/cm^2 for 7 days • Separate bioassays performed for each sampling area[(a)] • Endpoint: Mortality and moult inhibition	• Diet-overlay assay with purified Cry1Ab at a diagnostic concentration • Progeny of field-collected larvae • 3,551 neonates exposed to 1,091 ng Cry1Ab/cm^2 for 7 days • Separate bioassays performed for each sampling zone[(a)] • Susceptible reference population tested for comparison • Endpoint: Moult inhibition

[12] 2019 PMEM report: Sections 3.2.1.2 and 3.2.1.3 and Appendices 7 and 8.

Assay	Population (Generation)	ECB	MCB
Susceptibility assay – Plant tissue	NE Spain (F_1 larvae)	• Assay using maize leaves • Larvae not used in the diagnostic assays (N = 12,415) • Neonates fed maize MON 810 leaves for 5 days • Endpoint: Moult to L_2 and L_3	• Assay using maize leaves • Larvae not used in the diagnostic assays (N = 17,300) • Neonates fed maize MON 810 leaves for 10 days • Susceptible reference population tested for comparison • Endpoint: Moult to L_2
Confirmatory assay Step I – Plant tissue	NE Spain (F_1 larvae)	• Assay using maize leaves • Larvae that survived the diagnostic concentration and moulted to L_2 (N = 5) • L_2 fed maize MON 810 leaves for 5 days • Endpoint: Mortality	• Assay using maize leaves • Larvae that survived the diagnostic concentration and moulted to L_2 in the diagnostic assays (N = 67) and the susceptibility plant assay (N = 1) • L_2 fed maize MON 810 leaves for 10 days • Susceptible reference population tested for comparison • Endpoint: % Moult to L_3
Confirmatory assay Step II – Diagnostic	NE Spain (F_2 larvae)	• Not conducted[(b)]	• Diet-overlay assay with purified Cry1Ab • Siblings of larvae that reached L_3 in confirmatory plant assay Step I and of larvae that reached L_2 in susceptibility plant assay • 287 neonates exposed to diagnostic concentration for 7 days • Endpoint: Moult inhibition
Confirmatory assay Step II – Plant tissue	NE Spain (F_2 larvae)	• Not conducted[(b)]	• Assay using maize leaves • Siblings of larvae that reached L_3 in confirmatory plant assay Step I and of larvae that reached L_2 in susceptibility plant assays • 3,680 neonates fed maize MON 810 leaves for 10 days • Endpoint: Moult to L_2
Concentration-response	Laboratory	• Diet-overlay assay with purified Cry1Ab • Susceptible reference populations (Spain & Germany) • Nine concentrations (0.2–28.22 ng Cry1Ab/cm^2) • Duration: 7 days • Endpoint: $MIC_{50,95}$	• Diet-overlay assay with purified Cry1Ab • Susceptible reference population (Spain) • Seven concentrations (2–128 ng Cry1Ab/cm^2) • Duration: 7 days • Endpoint: $MIC_{50,95}$

L_2: second instar; L_3: third instar; $MIC_{50,95}$: concentration causing 50 or 95% moult 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 2019, 1,110 ECB late-instars from the last generation were collected at the end of the maize growing season from 14 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). Fourteen 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 'F_1 larvae') was tested for susceptibility to Cry1Ab. Larvae were

reared following a standardised protocol (Thieme et al., 2018). A total of 526 larvae reached the adult stage (47% of the field-sampled larvae) and were placed in 79 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. Since both populations were established, both have been reared in the laboratory 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 F_1 larvae to detect potential increases in resistance allele frequency; (2) an additional bioassay with F_1 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 F_1 neonates to purified Cry1Ab protein at a diagnostic concentration of 28.22 ng Cry1Ab/cm^2 of diet surface area in an artificial diet overlay assay.[13] A new batch of microbially produced Cry1Ab protein was used in the diagnostic concentration assay (see below for further details on the functional equivalence between the previous batch and the new one).

In the 2019 bioassays, 1,488 neonates were tested against the diagnostic concentration. Four hundred and thirty-two 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, were determined after 7 days. None of the reference populations were included in the diagnostic bioassay.

In the three zones, moulting inhibition was higher than the expected 99%, whereas in the control treatments, it ranged between 0.00% and 0.45% (Table 2). These results are similar to those reported in the previous seasons (Appendix E). The study authors indicated that *no decrease in Cry1Ab susceptibility of ECB has been observed during the monitoring duration*.

Table 2: Moult inhibition of European corn borer *(Ostrinia nubilalis)* larvae at a diagnostic concentration of Cry1Ab protein: 2019 field populations [Table based on data provided in the 2019 PMEM report]

Population	Sampling area[(a)]	Treatment % Moulting inhibition (No. of larvae tested)	
		Control	Cry1Ab[(b)]
North-eastern Spain	Huesca – 1	0.00 (144)	99.40 (496)
	Huesca – 2	0.45 (224)	99.86 (704)
	Navarra	0.00 (64)	99.65 (288)
	Total	0.15[(c)] (432)	99.64 ± 0.13[(d)] (1,488)

(a): Details on sampling sites are provided in Appendix C.
(b): A diagnostic concentration of 28.22 ng Cry1Ab/cm^2 of diet surface area was used.
(c): Of the 432 larvae tested, one larva died and 52, 365 and 14 larvae moulted to the second, third and fourth instar, respectively.
(d): Of the 1,488 larvae tested, 98 larvae died, 1,385 larvae survived but did not moult to the second instar, and five larvae moulted to the third instar.

Bioassay with maize MON 810 leaves: To complement the diagnostic bioassay, an additional assay was conducted with F_1 larvae from the field-collected populations using maize MON 810 leaves. To this end, 12,415 of the first instars not used in the diagnostic bioassays (between 1,350 and 7,300 larvae per sampling area) 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 544 larvae fed non-*Bt* maize leaves (between 64 and 256 larvae per sampling area), 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.

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

All ECB larvae-fed maize MON 810 leaves died. In the control group, 2.2% of the larvae died or did not reach the second instar, whereas 97.8% of the larvae moulted to the second or third instar.

Confirmatory bioassay with maize MON 810 leaves: A follow-up study using maize MON 810 leaves was conducted with the five 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/cm^2 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. MIC_{50} and MIC_{90} values, with a 95% confidence interval (CI), were estimated by probit analysis (Robertson et al., 2007).

To ascertain the functional equivalence between the new protein batch (2c) and the previous one (2b), a bridging assay was conducted with the Spanish reference population. The results of the bridging experiment revealed that both protein batches are biologically equivalent, as the 95% confidence limits of resistance ratios included the value of 1 (Appendix F).

MIC_{50} and MIC_{90} values estimated in 2019 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 2019, 1,644 MCB late-instars from the last generation were sampled at the end of the maize growing season from 13 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 15 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 982 larvae reached the adult stage (60% of the field-collected larvae) and were placed in 99 oviposition cages for mating. Emerging adults from the different sampling zones were kept separately. Ninety-two cages, containing 868 adults (393 males and 475 females), were used to obtain F_1-progeny for the diagnostic bioassay (i.e. 53% 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.

b) Monitoring assays

The following bioassays were performed: (1) a diagnostic bioassay with F_1 larvae to detect potential increases in resistance allele frequency; (2) an additional bioassay with F_1 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 F_1 larvae from each of the three sampling zones. Neonates were exposed to purified Cry1Ab protein at a diagnostic concentration of 1,091 ng Cry1Ab/cm^2 of diet surface area in an artificial diet-overlay assay.[14] The reference population was tested against the diagnostic concentration.

In the 2019 assays, between 1,162 and 1,194 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. Moult inhibition was recorded after seven days.

In the three zones, (corrected) moulting inhibition was lower than the expected 99%, whereas in the control treatments, it ranged between 3.59% and 11.52%. Moult inhibition observed in the reference population was 97.02% (see Table 3).

[14] The selected diagnostic concentration corresponds to the upper limit of the 95% confidence interval of the MIC_{99} estimated with data pooled from MCB populations collected in non-*Bt* maize fields from North-eastern Spain over 2009, 2011, 2013 and 2015. Batch B2-4 was used for the bioassays: 1.8 mg Cry1Ab/mL in 50 mmol/L sodium bicarbonate buffer; pH 10.25; purity 91%.

Average moulting inhibition of the progeny of field-collected larvae (97.97 ± 0.36%) 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.

Table 3: Moult inhibition of Mediterranean corn borer *(Sesamia nonagrioides)* larvae at a diagnostic concentration of Cry1Ab protein: 2019 field populations [Table based on data provided in the 2019 PMEM report]

Population	Sampling area[(a)]	Treatment % Moulting inhibition (No. of larvae tested)	
		Control	Cry1Ab[(b)]
North-eastern Spain	Huesca 1	4.15 (217)	98.20 (1,162)
	Huesca 2	11.53 (191)	97.26 (1,195)
	Navarra	3.59 (167)	98.44 (1,194)
	Total	6.42 ± 2.55[(c)] (575)	97.97 ± 0.36[(c)] (3,449)
Laboratory reference population		11.11 (108)	97.02 (679)

Statistically significant differences were observed between the north-eastern population and the expected value of 99% ($t = 4.000$; $df = 2$; $p = 0.029$).
No statistically significant differences were observed between the north-eastern population and the reference population. ($t = 2.440$; $df = 2$; $p = 0.067$).
(a): Details on sampling sites are provided in Appendix C.
(b): A diagnostic concentration of 1,091 ng Cry1Ab/cm^2 of diet surface area was used. Values have been corrected using Abbott's formula (Abbott, 1925).
(c): Mean ± standard error.

Bioassay with maize MON 810 leaves: An additional bioassay was conducted with F_1 larvae from the collected field populations using maize MON 810 leaves. To this end, 17,300 first instars not used in the diagnostic bioassays (~ 200 larvae per oviposition cage) were fed maize MON 810 leaves. A negative control group, consisting of 881 larvae fed non-*Bt* maize leaves (~ 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 (140 larvae). 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.

One larva from the field-collected populations feeding on maize MON 810 leaves reached the second instar whereas none of the larvae from the reference population moulted. Moulting in the control groups of the field-collected populations ranged between 97.57% and 98.83% and resulted in 95.71% in the reference population (see Table 4).

Table 4: Moult to second instar of Mediterranean corn borer *(Sesamia nonagrioides)* neonates feeding on *Bt* (MON 810) or non-*Bt* maize leaves: 2019 field populations [Table based on data provided in the 2019 PMEM report]

Population	Sampling area[(a)]	Treatment % Moulting (No. of larvae tested)	
		Non-*Bt*	*Bt*
North-eastern Spain	Huesca – 1	98.83 (342)	0.00 (6,850)
	Huesca – 2	97.90 (333)	0.02 (6,400)
	Navarra	97.57 (206)	0.00 (4,050)
Laboratory reference population		95.71 (140)	0.00 (2,675)

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

Confirmatory bioassays:

Experiments using maize MON 810 leaves were conducted with the 67 larvae that reached the second instar in the diagnostic bioassays and with the larvae that reached the second instar after feeding on *Bt* maize for 10 days to confirm that they were not potentially resistant to Cry1Ab. Larvae were individually placed on experimental arenas and fed maize MON 810 leaves. Eight of the 67 larvae reached the third instar whereas the larvae from the plant bioassay were not able to survive.

Siblings of the larvae that reached the third instar when fed on *Bt* maize leaves after the diagnostic bioassay or the second instar when directly fed on Bt maize leaves were reared on artificial diet. Additional diagnostic concentration and maize leaf bioassays were conducted with their progeny (F_2 larvae):

- in the diagnostic concentration bioassay, 287 F_2 larvae were tested and two larvae reached the second instar (99.30% 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 3,680 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/cm^2 of diet surface area, and a negative control (i.e. the same buffer solution in which the purified Cry1Ab protein was dissolved) were tested.

In all bioassays, three replicates were used per concentration including the control. Each replicate consisted of 32 larvae (64 for the controls), giving a total of 96 larvae tested for each concentration (192 for the controls). Moulting inhibition was assessed after 7 days of exposure. MIC_{50} and MIC_{90} values, with a 95% CI, were estimated by probit analysis.

Both MIC_{50} and MIC_{90} values estimated in 2019 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 farmer complaint 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 2019 growing season, no complaints related to corn borer infestation of maize MON 810 were received via the farmer complaint system. The consent holder also reports the outcome of a survey conducted by member companies of the National Breeder Association in Spain[15] selling maize MON 810 varieties to have an overview of the farmer complaint schemes. None of the 901 complaints received by these companies in 2019 were attributed to loss of efficacy of the *Bt* maize by corn borers.[16]

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,[17] north-eastern Spain; @RAIF_noticias in Andalucia,[18] southern Spain). These networks monitor and alert on incidence/outbreaks of agricultural pests and plant health issues and inform about IPM practices. However, the consent holder does not elaborate further on how this network could be used for resistance monitoring.

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

[15] Asociación Nacional de Obtentores Vegetales (ANOVE): http://anove.es/ (Accessed: 24 May 2021).
[16] Of the 901 complaints received in 2019, eight were related to maize MON 810 efficacy. Of these, seven complaints were about damage caused by lepidopteran pests other than ECB and MCB and one complaint was about the quality of the grains.
[17] Red de avisos Fitosanitarios de Aragón: http://web.redfara.es/ (Accessed: 24 May 2021).
[18] Noticias de la Red de Alerta e Información Fitosanitaria de Andalucía: http://www.juntadeandalucia.es/agriculturapescaydesarrollorural/raif (Accessed: 24 May 2021).

maize acreage, and where these populations are multivoltine. Following this scheme, in 2019, 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 six of 17 and eight of 18 sampling sites inspected in 2019, none or very few numbers of ECB and MCB larvae were found, respectively. The consent holder underlines the increasing difficulties to find fields infested with ECB and MCB larvae for sampling. Yet still, the consent holder reached the target sampling size of 1,000 larvae (corresponding to 2,000 genomes) established in the current IRM plan. Overall pre-imaginal mortality values during the laboratory rearing of field-collected individuals were high for both target pests; 53% and 40% of the ECB and MCB larvae collected in the fields did not reach adulthood.

Together with the limited number of larvae collected in fields, this fact prevented from reaching the recommended detection level of 3% (recessive) resistance allele frequency to detect a possible insurgence of field resistance timely.

As for sampling, EFSA acknowledges the efforts made by the consent holder and recognises that it might not always be possible in practice to collate large amounts of larvae due to several factors such as natural fluctuation in pest density, environmental conditions and regional pest suppression (Dively et al., 2018).

Also, the consent holder indicated that the laboratories performing the bioassays have extensive experience working with ECB and MCB populations, and that both have optimised the rearing process applying good experimental practices. EFSA acknowledges that rearing and maintenance of insect populations entails some practical challenges and that many factors contribute to mortality before susceptibility testing, and that it is not possible to control some of those (e.g. parasitism of corn borer larvae by hymenopteran species, insect pathogens).

Overall, EFSA acknowledges that under current conditions in north-eastern Spain, it is not feasible to reach the targeted threshold. Therefore, an alternative monitoring strategy is needed (more details are provided below, under 'alternative testing methods').

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, 2018, 2019a, 2020). 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.

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. 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: Moult inhibition values observed in the susceptible reference MCB populations are repeatedly below the expected 99% (Appendix F). Besides, the consent holder has not provided sufficient evidence to underpin the appropriateness of the diagnostic concentration selected for this target pest species (EFSA, 2020). Thus, uncertainty remains on whether the diagnostic concentration for MCB is able to reliably discriminate between homozygous resistant and susceptible individuals. To overcome this issue, the consent holder could recalculate the diagnostic concentration for MCB by, for instance, using data from bioassays in which only > 80% moult inhibition

values were observed. The new diagnostic concentration should then be validated with susceptible to prove that > 99% moult inhibition values are obtained.

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 98.20%, 97.26% and 98.44%, and the mean (97.97%) 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 encourages the consent holder to apply the stepwise 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 program (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. Since 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, the only alternative to increase the sensitivity of the monitoring strategy is using a more sensitive method.

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 stepwise approach presented in Appendix G.

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, 2018, 2019a), 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 advocates modifying the current monitoring strategy, primarily based on diagnostic concentration assays, and using a more precise and sensitive testing method, like F_2 screen (Andow and Alstad, 1998). EFSA is aware that the F_2 screen is costly and resource intensive (Andow and Alstad, 1998; Huang et al., 2012) and entails practical challenges (Andreadis et al., 2007; Siegfried et al., 2007; Engels et al., 2010; Siegfried and Spencer, 2012). To overcome such limitations, F_2 screens could be performed periodically with ECB and MCB populations. Periodic estimations of resistance alleles through F_2 screening, together with a robust farmer complaint system, should 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 model parameters (e.g. the proportion of maize MON 810, refuge compliance). The new estimated allele frequency and the outcome of these simulations would help to decide when to conduct the next F_2 screen. Although performing an F_2 screen is, overall, more costly than conducting diagnostic assays, the proposed strategy, based on periodic estimations of resistance allele frequencies, would reduce the expenses as insect collection and rearing and travel for field sampling would not be required every year.

Considering that 5 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.

The consent holder might also try to develop *Bt*-resistant populations of ECB and MCB would be available, e.g. by laboratory selection) and perform F_1 screens. 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.

Farmer complaint system

EFSA considers that a farmer complaint system could complement the other strategies used for managing insect resistance as, in principle, it may allow those managing crops to comment on pest infestation levels and product performance as well as to 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 current invitation to 'report damages higher than expected' lacks clarity and is likely to trigger only report of those damages that would actually affect the yield while the mere presence of borers might remain unreported, despite their potential relevance to detect early signs of resistance. Adequate communication mechanisms and educational programmes (e.g. field scouting techniques and characterisation of the damage caused by corn borers) should therefore be in place to ensure the prompt and effective reporting of farmer complaints relevant for resistance monitoring. 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 2019 maize growing season. For MCB, moulting inhibition observed in the diagnostic concentration bioassays was lower than the expected > 99% in the three populations tested. Additional studies with plant material indicate that none of the MCB larvae tested from those populations could complete development on maize MON 810 leaves. EFSA encourages the consent holder to apply the stepwise approach recommended by the US Environmental Protection Agency for confirming resistance of lepidopteran pests of *Bt* plants updating the harmonised IRM plan accordingly.

Based on the estimated numbers of ECB and MCB field-collected larvae represented in the diagnostic concentration bioassays, the monitoring strategy implemented in the 2019 growing season was not sensitive enough to detect the recommended 3% resistance allele frequency (EFSA, 2015a). EFSA notes the efforts made by the consent holder to increase the sampling size as well as to reduce laboratory mortality prior to susceptibility testing. Likewise, EFSA acknowledges that there are strong limitations that prevent reaching the 3% resistance allele frequency threshold. Consequently, EFSA considers that a more sensitive alternative testing method should be used so that alternative management measures can be implemented timely to delay resistance evolution. Given that no resistant ECB and MCB populations are available for F_1 screens EFSA recommends performing periodic F2 screens. EFSA considers that it is timely to perform an F_2 screen on MCB populations from the same area where the Cry1Ab resistance allele was detected by Camargo et al. (2018) as well as on ECB populations from north-eastern Spain, where the frequency of resistance alleles has never been estimated.

EFSA also notes that 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 questionnaires[19]

3.2.1.1. Consent holder's assessment

2019 Questionnaires

In the annual 2019 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 2019 PMEM report represented the fourteenth reporting year, with the completion of a total of 3,627 questionnaires since 2006.

The surveys were performed in each country by external companies with experience in agricultural surveys and were completed between February and March 2020. The response rate was 55.3% in Spain,[20] and 100% in Portugal. Seventy-four of the 250 farmers (29.6%) were interviewed for the first time.

Table 5: Farmers surveyed and maize MON 810 areas monitored in 2019 through questionnaires [Table based on data provided in the 2019 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	239(a)	21.0	5,012	107,127	4.7
Portugal	11(b)	83.9	923	4,718	19.6
Total	250	23.8	5,935	111,845	5.3

(a): One-hundred and seventy-seven farmers were from Aragón/Cataluña, 18 from Navarra, 28 from Extremadura, 9 from Andalucía and 8 from Castilla la Mancha. Sixty-nine of the 239 farmers were interviewed for the first time.
(b): Six farmers were from Alentejo, two from Lisbon and Vale do Tejo and three from centre. Five out of the 11 farmers were interviewed for the first time.

The questionnaire collected information on four specific areas: (1) maize growing area; (2) typical agronomic practices to grow maize on the farm; (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 2019 farmer questionnaires on maize MON 810 did not identify potential adverse effects that might be related to MON 810 plants and their cultivation*.

A pooled analysis of farmer questionnaires covering the years 2006–2015 was provided in the PMEM report of 2018 and evaluated by EFSA (EFSA, 2020).

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 2019 growing season are similar to those from previous annual PMEM reports.

The following summarises the evaluation of the methodology of the 2019 farmer questionnaire. The same observations were made in last years statement, and for further details, we would like to refer to EFSA, 2020.

- 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. The claim that *The whole sampling procedure ensured*

[19] 2019 PMEM report: Sections 3.1.2.1 and 3.1.4.1 and Appendices 1 and 2.
[20] The questionnaire was completed by 239 out of the 432 farmers that were contacted in Spain. The 193 farmers that did not respond gave the following reasons: (1) because they did not grow maize MON 810 in 2019 (69 farmers); (2) they did not grow maize in 2019 (64 farmers); (3) they grew maize MON 810 in 2019 but refused to sign the consent form (21 farmers); (4) they grew MON 810 in 2019 but refused to answer the interview (20 farmers); (5) they were absent or could not be localized (10 farmers); (6) they were retired (9 farmers).

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 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 specific comparators selected by the farmers for the survey should also be summarised in the monitoring report.
- Farmers completed the questionnaires after the harvest of maize cultivated in 2019, and growers might not recall 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 need to take along the growing season.
- 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 an effort should be made to use objective measurable outcomes, whenever this is possible.

It is also recommended that the farmer questionnaire is updated when new characteristics of the receiving environment are relevant for the environmental risk assessment from MON 810 (e.g. emergence of teosinte).

3.2.1.3. Conclusions on farmer questionnaires

From the data provided by the 2019 farmer survey, EFSA could not identify any unintended effects associated with the cultivation of maize MON 810 varieties.

The current 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).

With respect to the suggestion made by the consent holder to discontinue farmer questionnaires and to use the farmer complaint system instead, EFSA believes that a robust and fit-for-purpose farmer alert system could support both the IRM and address general surveillance purposes. However, the current farmer complaint system is insufficient to address these challenges. In addition to the considerations made about its implementation for IRM, it 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 2009/128[21]), and ensure that farmers growing maize MON 810 varieties could be encouraged to report any unusual observations. To facilitate this, it may be envisaged to use instruments of the Common Agricultural Policy, cross-compliance requirements 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).

EFSA reiterates its recommendation that the Competent Authorities in concerned EU Member States have a dialogue with the companies to discuss 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 networks[22]

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.

[21] 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.

[22] 2019 PMEM report: Sections 3.1.2.3 and 3.1.4.3.

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 identified four groups of different networks, (1) governmental networks; (2) academic networks; (3) nature conservation networks and (4) professional networks. Their expertise in monitoring was recognised but the consent holder concluded that it would not be possible for these networks to establish a cause and effect relationship since none of the identified EENs measured GM crop cultivation as an influencing factor, making it difficult to establish accurate correlations based on the collected data. The consent holder also pointed out that: *'additional limitations in the use of EENs as an early warning system part of GS efforts are (1) technical constraints (e.g. delayed publication of monitoring data); (2) lack of public availability of (raw) data; (3) harmonisation between networks (e.g. data collection and processing)... In addition, the EFSA has published a scientific opinion on the use of EENs for PMEM reports based on internal expertise and a report issued by a contracted consortium (Henrys et al., 2014). EFSA's opinion concluded that "In compliance with these assessment criteria, several existing ESNs have been identified as potentially suitable for GS of GMPs subject to further examination. However, the EFSA GMO Panel also identified several limitations pertaining to ESNs such as limited data accessibility, data reporting format and data connectivity with GMO registers" (EFSA, 2014b)...'*

3.2.2.2. EFSA's assessment

An external report commissioned by EFSA (Centre for Ecology and Hydrology, 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 networks 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).

While EFSA acknowledges the challenges of using EENs to identify impacts of GM crops, EFSA encourages the European Commission, the consent holder, the National Competent Authorities and relevant stakeholders to discuss how to make the best use of EENs.

For transparency reasons, it is suggested that the consent holder provides a list of EENs identified as being active in the areas where GM maize is cultivated and an evaluation of the EENs according to the assessment criteria outlined under point 3 on p. 8–9 in EFSA 2014b.

Overall, as part of the general framework on general surveillance that could also include a robust farmer alert system as outlined above, EFSA encourages the concerned 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. Information on teosinte and the potential need to update the post-market environmental monitoring for maize MON 810

EFSA notes the existence of monitoring activities of national authorities directly linked to maize cultivation, such as the monitoring of teosinte populations in Spain and in France. As part of general surveillance and given their potential relevance for MON 810, EFSA is of the opinion that the consent holder should include the outcome of such monitoring activities in the PMEM report.

Details on the available information on teosinte monitoring and its implications on the PMEM of MON 810 were discussed by the EFSA WG on 4 May 2021.[10] It is recommended that the consent holder includes and explicitly considers in future annual PMEM reports all scientific evidence relevant for the environmental risk assessment and risk management of maize MON810 in relation to teosinte, including the outcome of existing monitoring activities as mentioned above. In addition, EFSA recommends that the farmer questionnaires are revised to include the reporting of both the occurrence of teosinte and teosinte hybrid plants and the corresponding level of infestation. The

consent holder and the Competent Authorities of the EU Member States where maize MON810 is grown should ensure that robust information systems are in place to promote the sharing of relevant information on teosinte.

3.2.4. Literature searching[23]

3.2.4.1. 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 1 May 2019 and 28 May 2020.

The consent holder searched in the electronic bibliographic databases SciSearch (Science Citation Index) and CABA (CAB Abstracts®) using the STN® database catalogue and complemented with an internet search in web pages of relevant key organisations involved in the risk assessment of GM plants.

Altogether, 460 publications were retrieved (excluding duplicates). After applying the predefined eligibility/inclusion criteria, the consent holder identified 14 publications as relevant for the assessment of food and feed (eight publications) or environmental safety (six publications).

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.

3.2.4.2. EFSA's assessment

Systematic literature search

The systematic literature search was evaluated using a modified version of the EFSA critical appraisal tool for assessing quality of extensive literature searches (EFSA, 2015b) which integrates the relevant principles and criteria outlined in EFSA (2010) and the recommendations provided in EFSA (2019a,b).

A description of the information sources searched is provided with limited discussion on the reasons for their selection. It is not clear on what basis the searched databases were selected. A description of the information sources searched is provided, but without the reasons for their selection or any discussion/justification why also other information sources were not included or considered (e.g. EMBASE) and what might be the impact of their non-inclusion.

Three reference publications are mentioned for validating the search strategy as part of the protocol development. However, the conduct of the validating pilot study is not documented i.e. no information on the pilot study and its outcomes is provided to confirm the validity of the selected search strategy/methodology or to allow its fine-tuning based on the outcome of the pilot study.

According to EFSA (2019a,b), details on the criteria to appraise the reliability of the studies identified in the review should be provided. Some further information was provided in the additional information but more details on the individual criteria applied should be provided.

3.2.4.3. Conclusions on literature searching

Overall the quality of the literature review performed by the consent holder is acceptable. EFSA acknowledges the efforts made by the consent holder to take into consideration EFSA's recommendations and to comply with the guidance given in EFSA (2019a,b). However, some areas of improvement of future literature searches were identified. It is recommended that the consent holder provides a discussion/justification for the selection of the searched databases and the exclusion of other databases (e.g. EMBASE) and what might be the impact of their non-inclusion. Furthermore, the consent holder should provide details on the outcome of the pilot study and explain and list the criteria which were used for assessing the reliability of publications identified in the literature search. Relevant information on teosinte should also be retrieved in future literature searches.

None of the publications point to new hazards, modified exposure or new scientific uncertainties that would change the 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 2019 PMEM report, additional information provided by the consent holder on insect resistance management and literature

[23] 2019 PMEM report: Section 3.1.6 and Appendix 5; additional information 20/5/2021.

searching, comments provided by EU Member States and relevant scientific publications, following a weight of evidence approach (EFSA Scientific Committee, 2017).

Table 6 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 6: Weight of evidence approach followed to assess the evidence provided in the 2019 PMEM report on maize MON 810

Question:	*Do the findings of the insect resistance monitoring and general surveillance activities indicate any adverse effects on human and animal health or the environment arising from the cultivation of maize MON 810 during the 2019 growing season that would invalidate previous GMO Panel evaluations on the safety of this GM maize?*	
Assemble the evidence	Select the evidence	The evidence was obtained from: – The 2019 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: **Case-specific monitoring** – **LoE 1:** Farmer compliance with refuge requirements. Survey of 239 Spanish and 11 Portuguese farmers growing maize MON 810 *(Section 3.1.1)* – **LoE 2:** ECB and MCB resistance monitoring *(Section 3.1.2)*: • Sampling of 1,110 ECB and 1,644 MCB larvae from three zones in North-eastern Spain • 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 2019 growing season *(Section 3.1.2)* **General surveillance** – **LoE 4:** Systematic literature search (1 May 2019 to 28 May 2020). Fourteen food and feed- and environmental safety relevant publications were identified and assessed. *(Section 3.2.3)* – **LoE 5:** Existing monitoring networks – **LoE 6:** Farmer survey based on 250 questionnaires received from farmers in Spain and (239) and Portugal (11) *(Section 3.2.1)*
Weigh the evidence	Methods	– **LoE 1:** Best professional judgement – **LoE 2:** The relevance and validity of the bioassays were 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 (2019a,b). A critical appraisal tool was used (EFSA, 2015b). The implications of each of the publications identified in the search were assessed by best professional judgement – **LoE 4:** Best professional judgement – **LoE 5:** Best professional judgement – **LoE 6:** The methodology of the farmer questionnaire was assessed by best professional judgement based on an evaluation grid for surveys used for general surveillance on GM plants (see Appendix 1 of EFSA GMO Panel, 2011a,b)

	Results	**Case-specific monitoring** – **LoE 1:** Partial compliance (94%) with refuge requirements in Spain and full compliance in Portugal was reported in the farmer's questionnaires – **LoE 2:** 1) ECB: MI inhibition of larvae tested against the DC was 99.64%. The five larvae that moulted to the second instar in the DC assay died within 7 days of feeding on maize MON 810 leaves 2) MCB: MI inhibition was lower than the expected 99% in three sampling zones. No resistant larvae were found in the follow-up/confirmatory bioassays with maize MON 810 leaves. – **LoE 3:** None of the 901 complaints received in 2019 were attributed to loss of efficacy of maize MON 810 **General surveillance** – **LoE 4:** The information reported in the eight food and feed- and six environmental-safety relevant publications identified through the systematic literature search do not point to new hazards, modified exposure or new scientific uncertainties that would invalidate the risk assessment conclusions on and risk management recommendations for maize MON 810 – **LoE 5:** The consent holder did not report information gathered through existing networks involved in environmental monitoring in the EU – **LoE 6:** No adverse effects that might be caused by the cultivation of maize MON 810 were reported in the analysis of the farmer questionnaires.
Integrate the evidence	Methods	– The different LoE were integrated by best professional judgement (i.e. no formal method was used) 1) LoE 1–LoE 3 were integrated to conclude on resistance management strategies and insect resistance monitoring 2) LoE 4–LoE 6 were integrated to conclude on unexpected adverse effects due to the cultivation of maize MON 810 in the EU during the 2019 growing season
	Results	**Conclusions** *(Section 4)* – The monitoring strategy implemented in 2019 is not sensitive enough to detect the recommended 3% resistance allele frequency – The information reported in the 2019 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 2019 growing season – EFSA concludes that no new evidence has been reported in the context of the 2019 PMEM report that would invalidate previous GMO Panel evaluations on the safety of maize MON 810 **Recommendations** – EFSA strongly recommends the consent holder to 1) Achieve full compliance with refuge 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 F_2 screen on European and Mediterranean corn borer populations from North-eastern Spain – EFSA gives other practical recommendations on insect resistance monitoring, farmer questionnaires, existing environmental networks and literature searching that should be implemented by the consent holder in future reports *(Section 5)* – EFSA gives other practical recommendations on insect resistance monitoring, farmer questionnaires, existing environmental networks and literature searching that should be implemented by the consent holder in future reports *(Section 5)*

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

4. Conclusions

The evidence from the 2019 PMEM report does not indicate any adverse effects on human and animal health or the environment arising from the cultivation of maize MON 810 during the 2019 growing season. Consequently, previous evaluations on the safety of maize MON 810 (EFSA, 2009; EFSA GMO Panel, 2012b,c) remain valid.

However, 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. EFSA notes that the monitoring strategy implemented in the 2019 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 F_2 screening as soon as possible.

Full compliance with refuge requirements was observed in Portugal and EFSA considers that the consent holder should strive to achieve full compliance also in Spain in areas of high adoption of MON 810.

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. Together with the use of existing environmental monitoring networks, this farmer alert system would be part of a framework on general surveillance. 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 2019 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 by replacing annual diagnostic assays with periodic F_2 screening on European and Mediterranean corn borer populations from north-eastern Spain; and (3) address previously mentioned methodological, analytical and reporting limitations of resistance monitoring and farmer questionnaires.

All the relevant information on teosinte, including those derived from national monitoring programs, should be reported in future annual PMEM reports. Moreover, the relevance and implications of the teosinte-related information for the environmental risk assessment and risk management of maize MON 810 should be assessed. The farmer questionnaires should be revised to include the reporting of both the occurrence of teosinte and teosinte hybrid plants and the corresponding level of infestation. The consent holder and the Competent Authorities where maize MON 810 is grown should collaborate to ensure that robust information systems are in place to promote the sharing of relevant information such as occurrence of teosinte in maize MON 810 growing areas.

EFSA encourages the concerned 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. EFSA recommends that Competent Authorities in concerned EU Member States, the consent holder and representatives of environmental networks have a dialogue to discuss and agree on how to best identify and report unexpected adverse effects from the cultivation of *Bt* maize varieties. Based on this dialogue, a methodological framework for the general surveillance of GM plants could be developed.

For transparency reasons, it is suggested that the consent holder provides a list of EENs identified as being active in the areas where GM maize is cultivated and an evaluation of the EENs according to the assessment criteria outlined under point 3 on p. 8–9 in EFSA 2014b.

A full list of all recommendations is provided in Table 7 below.

Table 7: 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-*Bt* maize refuges (Section 3.1.1.2)	– To take relevant actions, in order to reinforce the adoption of sufficient refuge areas, especially in regions of high maize MON 810 adoption – Be more explicit in the information provided to farmers that that non-compliance with refuge requirements may speed up resistance development in areas with high adoption rate and that, as a consequence, farmers would not benefit from the technology anymore in the future	– 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)	Monitoring strategy – To increase the sensitivity of the monitoring strategy so that it achieves a detection level of 3% resistance allele frequency in target pest populations (see below on 'testing')	– Consent holder
		Testing – To recalculate (and validate) the diagnostic concentration for MCB – To include a reference laboratory population in the bioassays with ECB – To follow the stepwise approach recommended by the US Environmental Protection Agency for confirming resistance of suspected resistant populations (see Appendix G) – To replace annual diagnostic assays by more sensitive testing methods (periodic F_2 screening on European and Mediterranean corn borer populations in North-eastern Spain)	
		Reporting – 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
General surveillance	Farmer questionnaires (Section 3.2.1.2)	– To report the occurrence of teosinte and teosinte hybrid plants and the corresponding level of infestation – To update the farmer questionnaire when new characteristics of the receiving environment are relevant for the	– Consent holder

Area (Section)		Recommendation[(a)]	Responsible for implementation
		environmental risk assessment from MON 810 (e.g. emergence of teosinte)	
	Existing environmental networks (Section 3.2.2.2)	– List EENs being active in the areas where GM maize is cultivated and evaluate the EENs according to the assessment criteria outlined under point 3 on p. 8–9 in EFSA 2014b – To implement a methodological framework enabling the use of environmental networks in the broader context of environmental monitoring – Competent Authorities in concerned EU Member States, the consent holder and representatives of environmental networks should have a dialogue to discuss and agree on the development of a framework which could best identify and report unexpected adverse effects from the cultivation of maize MON 810.	– Consent holder – Competent authorities of concerned EU Member States – Environmental networks active in the area of cultivation of MON 810.
	Literature searching (Section 3.2.4)	– provide a discussion/justification for the selection of the searched databases and the exclusion of other databases (e.g. EMBASE) and what might be the impact of their non-inclusion. – provide details on the outcome of the pilot study – explain and list the criteria which were used for assessing the reliability of publications identified in the literature search. – Include relevant information on teosinte in the literature search)	– Consent holder

ECB: European corn borer; MCB: Mediterranean corn borer.
(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 2019 PMEM report.
3) Additional information, dated 20 May 2021 provided by the consent holder.

Supporting information

Annex 1: Replies to EU Member States' comments.
Annex 2: Appraisal of systematic literature search.
Annex 3: Assessment of relevant scientific publications.

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Abbreviations

CI	confidence interval
ECB	European corn borer
GM	genetically modified
GMO	genetically modified organism
GS	general surveillance
IRM	insect resistance management
MCB	Mediterranean corn borer
MIC	moulting inhibition concentration
PMEM	post-market environmental monitoring
WoE	weight of evidence

Appendix A – Farmer compliance with refuge requirements in Spain between 2004 and 2019

[Table based on data provided in 2004–2019 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
2019	239	199	27	13	94	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)			
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]
2019	87,329	159,261[c]	54.8
Mean 2014–2019	–	–	**60**
Central Spain (Albacete)			
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]
2019	3,193	7,300[c]	43.7[c]
Mean 2014–2019	–	–	**41**[c]
South-western Spain (Extremadura and Andalucía)			
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]
2019	16,050	64,690[c]	24.8[c]
Mean 2014–2019	–	–	**31**

(a): Source: https://www.miteco.gob.es/es/calidad-y-evaluacion-ambiental/temas/biotecnologia/organismos-modificados-geneticamente-omg-/consejo-interministerial-de-ogms/superficie.aspx (Accessed: 28 May 2021).
(b): Avances de superficies y producciones de cultivos: http://www.mapa.gob.es/es/estadistica/temas/estadisticas-agrarias/agricultura/avances-superficies-producciones-agricolas/ (Accessed: 28 May 2021).
(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 2019 PMEM report on maize MON 810]

Species	Sampling zone	Sampling site location – code (province)	No. of larvae collected	No. of adults emerged (% over larvae collected)
ECB	1	Lanaja – 2 (Huesca)	79	31 (39)
		Lanaja – 4 (Huesca)	66	35 (53)
		Cantalobos (Huesca)	223	103 (46)
		Total	**5,368**	**169 (46)**
	2	Candasnos – 1 (Huesca)	247	129 (52)
		Candasnos – 6 (Huesca)	102	61 (60)
		Candasnos – 9 (Huesca)	72	51 (71)
		Candasnos – 10 (Huesca)	126	20 (16)
		Total	**547**	**261 (48)**
	3	Mendigorría – 2 (Navarra)	6	Larvae from all sites were pooled
		Mendigorría – 3 (Navarra)	1	
		Mendigorría – 5 (Navarra)	1	
		Mendigorría – 6 (Navarra)	2	
		Mendigorría – 8 (Navarra)	182	
		Mendigorría – 9 (Navarra)	2	
		Mendigorría – 10 (Navarra)	1	
		Total	**195**	**96 (49)**
	Total		**1,110**	**526 (48)**
MCB	1	Lanaja – 1 (Huesca)	261	NR
		Lanaja – 2 (Huesca)	37	NR
		Lanaja – 3 (Huesca)	161	NR
		Cantalobos (Huesca)	196	NR
		Total	**655**	**404 (62)**
	2	Candasnos – 1 (Huesca)	206	NR
		Candasnos – 5 (Huesca)	53	NR
		Candasnos – 6 (Huesca)	204	NR
		Candasnos – 9 (Huesca)	79	NR
		Candasnos – 10 (Huesca)	18	NR
		Total	**560**	**346 (62)**
	3	Mendigorría – 2 (Navarra)	165	NR
		Mendigorría – 5 (Navarra)	13	NR
		Mendigorría – 6 (Navarra)	63	NR
		Mendigorría – 8 (Navarra)	188	NR
		Total	**429**	**232 (54)**
	Total		**1,644**	**982 (60)**

Late-instars were collected from refuges and non-*Bt* maize fields between 16 September and 17 October 2019.
NR: not reported.

Appendix D – 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 2008–2019 PMEM reports on maize MON 810]

Target pest	Season	Larvae collected	Protein batch[(a)]	Concentration response				Diagnostic concentration
				MIC_{50} (95% CI)[(b)]	MIC_{90} (95% CI)[(b)]	RR MIC_{50} (95% CI)[(c)]	RR MIC_{90} (95% CI)[(c)]	% Moult inhibition
ECB	2008	401	1	7.03 (4.89–10.03)	23.91 (15.76–46.84)	3.11/3.18*,[(d)] (NR)	2.93/5.35*,[(d)] (NR)	NP
	2009	509	1	6.40 (5.32–7.75)	13.68 (10.77–20.02)	1.75* (NR)	1.43 (NR)	NP
	2011	382	2	1.79 (1.54–2.07)	4.19 (3.45–5.48)	0.61* (NR)	0.67 (NR)	NP
	2013	452	2a	2.48 (2.03–3.02)	5.41 (4.27–7.61)	1.26 (NR)	0.82 (NR)	NP
	2015	376	2a	2.12 (1.75–2.55)	5.43 (4.36–7.29)	0.53* (NR)	0.77 (NR)	NP
	2016	1,111	2b	NP	NP	NP	NP	99.23
	2017	1,111	2b	NP	NP	NP	NP	99.19
	2018	1,144	2b	NP	NP	NP	NP	99.83
	2019	NP	NP	NP	NP	NP	NP	
MCB	2004	424	B1	63 (34–99)	570 (333–1,318)	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)]
	2019	1,644	B2-7	NP	NP	NP	NP	97.97 ± 0.36[(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/cm^2 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 (MIC_{50} and MIC_{90}) and their 95% confidence intervals (CI 95%) are expressed in ng Cry1Ab/cm^2 of diet surface area.
(c): Resistance ratio (RR) between MIC values of the field-collected populations and of the susceptible laboratory population for each growing season.
(d): The reference population was tested two times in 2008 (see Appendix E).
(e): MIC_{50} and MIC_{90} values of the reference population used to calculate RR MIC_{50} and RR MIC_{90} correspond to those estimated in 2004.
(f): Mean ± standard error of three independent assays corresponding to the different sampling zones.

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

[Table based on data provided in the 2006–2019 PMEM reports on maize MON 810]

Target pest	Population	Year	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	2b	3.93 (2.97–4.98)	7.23 (5.64–10.85)	NP
		2019	2c	1.36 (1.16–1.57)	2.00 (1.72–2.61)	NP
	ES.ref[c]	2015	2a	1.82 (1.53–2.16)	2.95 (2.43–4.54)	NP
		2016	2b	5.02 (3.61–6.33)	14.25 (11.29–19.87)	NP
		2017	2b	5.15 (4.20–6.05)	9.68 (8.15–12.37)	NP
		2018	2b	2.91 (2.21–3.76)	6.13 (4.61–9.75)	NP
		2019	2b	2.49 (1.88–3.31)	6.26 (4.53–10.39)	NP
		2019	2c	1.93 (1.55–2.38)	4.87 (3.81–6.92)	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	B2-6	19 (13–26)	116 (76–224)	97.75
		2019	B2-7	27 (16–40)	233 (133–656)	97.02

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

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

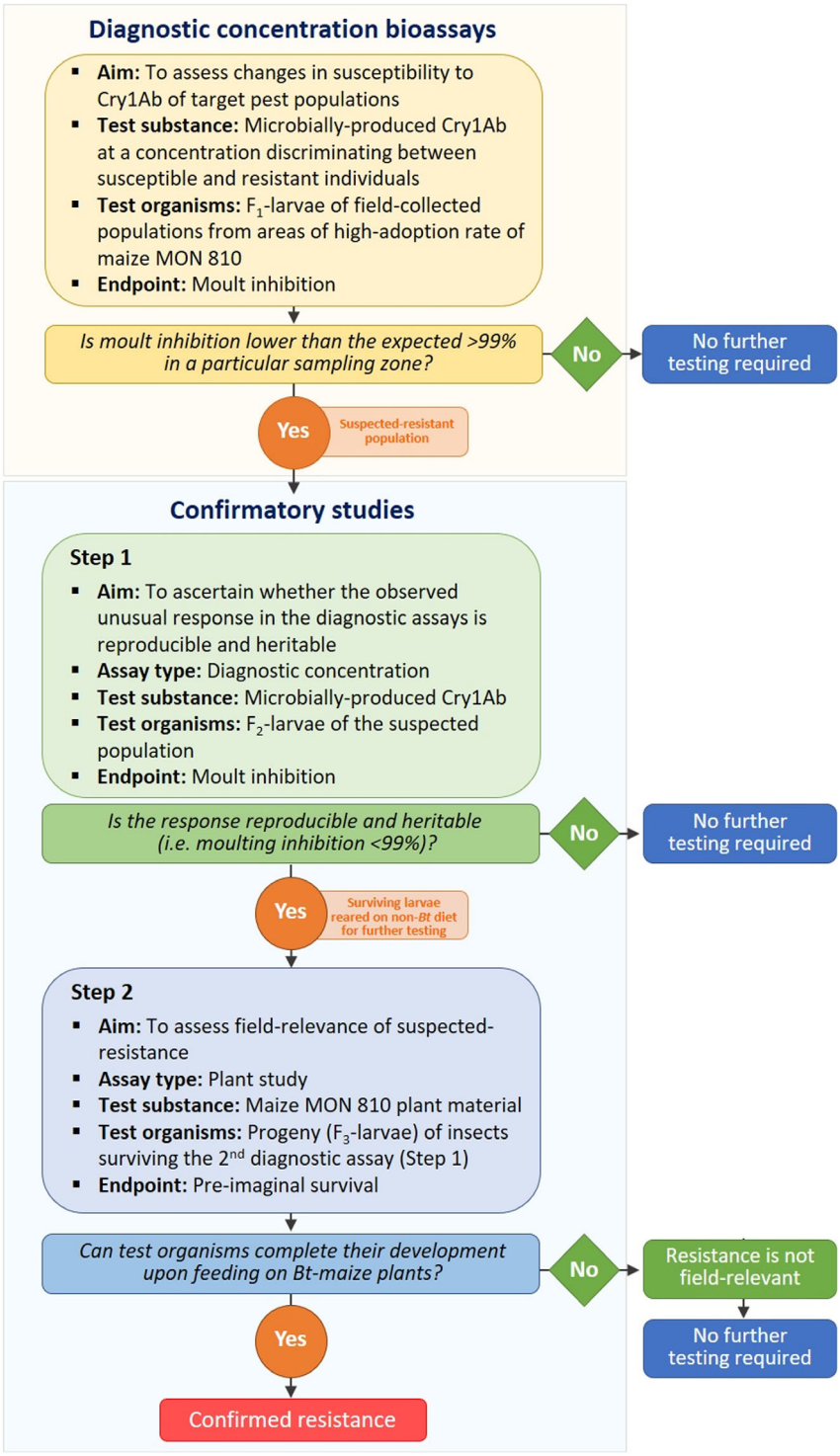
(b): The 'G.04' population was established from egg masses collected from Niedernberg (Germany) in 2005.

(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 F – Proposed stepwise approach for confirming resistance to *Bt* plants of suspected resistant populations

[Adapted from US EPA (2010, 2018).[24] Once resistance is confirmed, the EuropaBio insect resistance management plan foresees the implementation of remedial actions]



[24] 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 G – 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	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	4) Geographical area where the test organisms were collected[(a)] 5) Locations, number and type of fields (e.g. refuge areas, non-*Bt* maize field) per location where test organisms were collected (e.g. geographical coordinates, nearest municipality) 6) Sampling source (e.g. non-*Bt*–maize field, refuge) and distance to the nearest *Bt* maize field
Test organism	7) Number and life stage of collected individuals (per sampling zone/field) 8) Sampling date(s) 9) Measures taken to avoid the collection of siblings 10) Diapause and health status of field-collected populations 11) Description of the laboratory rearing protocol (including environmental conditions during laboratory rearing of field-collected individuals) 12) Number of field-collected individuals reaching adulthood after laboratory rearing of field-collected individuals (pre-imaginal mortality) 13) Number, sex and location of adults placed in oviposition cages for obtaining F_1 larvae[‡] 14) 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	15) Biochemical characterisation of the test substance (e.g. source, % purity, batch/lot used, nominal concentration, solvent/vehicle used) 16) Method used to quantify the concentration of the test substance (e.g. Bradford, ELISA, SDS–PAGE/densitometry)[†] 17) Description of the storage conditions of the test substance 18) Biological activity (in case of new batch, comparison of biological activity to the former batch(es) 19) Equivalence to the plant-expressed protein[(b),†]
Study design	20) Study performed according to standardised guideline/peer-reviewed protocol 21) Study performed according to GLP or other standards[§] 22) Description of control(s) 23) Preparation of stock solutions, including solvent concentrations in control(s) 24) Nominal concentration(s) of test substance and rationale for their selection 25) Administration of test substance (e.g. diet-overlay, mixed with artificial diet) 26) Age and generation of individuals tested (e.g. < 24-h-old larvae from F_1 generation) 27) Duration of the assay(s) 28) Description of measurement endpoints (e.g. mortality, moult inhibition) 29) Environmental-controlled conditions (e.g. temperature, humidity and light regime) 30) Validity criteria of the study (e.g. mortality in the control group < 20%) 31) Blinding of personnel[†]
Statistical design	32) Number of replicates for control(s) and test concentration(s); set up of replicates (to avoid pseudo-replication) 33) Number of individuals tested per replicate 34) Treatment design (e.g. block, randomised) 35) Statistical method used 36) Statistical software used

Category	Specific reporting recommendations
Results and discussion	37) Deviations from the protocol[†] 38) Description of the response effects for each of the measurement endpoints followed 39) Control mortality and other observed endpoints, and comparison to validity criteria from protocol 40) Estimation of variability for measurement endpoints (if relevant, e.g. 95% confidence intervals for MIC_x values) 41) Comparison to laboratory reference population (i.e. use of resistance ratios in case of concentration/response assays) 42) Estimation of slope, Chi-square (for Probit analysis) 43) Relevance of the results (in the context of baseline susceptibility and natural variability to the test substance) 44) Availability of raw data

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

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

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