STATEMENT

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Assessment of the 2020 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 European Food Safety Authority (EFSA) assessed the 2020 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 2020 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 2020 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 2020, 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 2020 post-market environmental monitoring (PMEM) report on the cultivation of the Cry1Ab-expressing maize event MON 810. This report presents the results of the 2020 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 2020; and (ii) diagnostic bioassays conducted with European and Mediterranean corn borers collected from north-eastern Spain to monitor changes in susceptibility to the Cry1Ab protein.

Like in previous years, 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 achieve full compliance in areas of high adoption of MON 810 in Spain to delay resistance development. 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.

In the analysis of resistance monitoring data gathered through diagnostic bioassays with field-collected corn borers sampled during the 2020 maize growing season, moulting inhibition was lower than the expected > 99% in two out of the three Mediterranean corn borer (MCB) populations tested and in the two European corn borer (ECB) populations tested. Additional studies using plant material indicated that none of the MCB and ECB larvae tested from any of the populations were able to complete development on maize MON 810 leaves. However, EFSA considers that the low values of moult inhibition reported for field-collected ECB populations should have triggered further investigation.

As in previous years, EFSA identifies methodological and reporting shortcomings on 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) include a reference strain in the ECB diagnostic concentration assays; (3) recalculate (and validate) the diagnostic concentration for MCB; (4) apply the step-wise approach recommended by the US Environmental Protection Agency for confirming resistance of lepidopteran pests of *Bt* plants updating the harmonised IRM plan accordingly; and (5) consider EFSA's previous reporting recommendations for future resistance monitoring studies.

EFSA considers that it is timely for the consent holder to perform a F_2 screen on MCB populations from the same area where the Cry1Ab resistance allele was detected in 2016 by Camargo et al. (2018) as well as on ECB populations from north-eastern Spain, where the frequency of resistance alleles has never been estimated, and where low moult inhibition values have been detected for the first time in the diagnostic concentration assays.

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 2020 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 on corn borer damage that may be indicative of resistance emergence.

The general surveillance data set consists of a farmer survey based on 252 farmer questionnaires and relevant scientific publications published between May 2020 and May 2021 that were identified

through a systematic literature search complemented with an internet search in webpages 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.

Some areas of improvement of future literature searches were identified, e.g. providing a discussion/justification for the exclusion of other databases, details on the outcome of the pilot study, explaining better the reliability of publications identified in the literature search, providing a more detailed description of the reasons of discarding papers from further assessment. Furthermore, relevant information on teosinte should also be retrieved in future literature searches.

EFSA 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 2020 PMEM report does not invalidate previous EFSA and 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 (corn) fields in Europe which can substantially damage the crop. 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 days. 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 total cultivated area in the EU exceeded 100,000 ha.

Insect pests can develop resistance (i.e. become immune) 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. Assuming that resistance is recessive, 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 (https://www.efsa.europa.eu/en/glossary/adverse-effect) 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 2020, 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 2020 maize growing season.

General surveillance

The data from the 2020 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 May 2020 to May 2021, identified five scientific publications relevant to the food, feed, and environmental safety of maize MON 810 and Cry1Ab. EFSA evaluated all five 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 2020 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 2020 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 famers comply with refuge requirements.

It is recommended that all scientific information on teosinte (a type of grass considered to be a parent plant of maize) relevant for the environmental risk assessment and risk management (https://www.epa.gov/risk/risk-management) of maize MON 810 should be included in the future annual PMEM reports. The literature review could be improved by providing a discussion/justification for the exclusion of other databases, details on the outcome of the pilot study, explaining better the reliability of publications identified in the literature search and providing a more detailed description of the reasons of discarding papers from further assessment. Furthermore, relevant information on teosinte should also be retrieved in future literature searches.

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.

Table of contents

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 2020, maize MON 810 was cultivated in Spain (98,152 ha) and Portugal (4,216 ha) over a total area of 102,367 ha (DGAV, 2021; MAPA, 2020).

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.[2] (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 for single lepidopteran-active *Bt* maize events (Alcalde et al., 2007), which was updated in 2019 (EuropaBio, 2019)[3]. 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.[4]

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) a generic farmer complaint system where farmers can report product-related problems, including loss of efficacy in protection against corn borers.

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[5] contributing to the reported 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, pp. 32–33.

[2] Note that Monsanto has become a subsidiary of Bayer AG as of 21 August 2018.

[3] The responsibilities of EuropaBio in coordinating activities of technology providers on the post-market environmental monitoring of GM crops was taken over by Crop Life Europe as of 1st January 2021.

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

[5] 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 holder 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 2009–2019 growing seasons (EFSA, 2018, 2019a, 2020, 2021; EFSA GMO Panel, 2011a, 2012a, 2013, 2014a, 2015a,b 2016, 2017). 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). Some of the recommendations on insect resistance monitoring were included in the updated IRM plan.

1.1. Terms of Reference as provided by the requestor

On 15 October 2021, the European Commission received from the consent holder the annual PMEM report for the 2020 growing season of maize MON 810 (hereafter referred to as the '2020 PMEM report'). The reporting period of the 2020 PMEM report is from July 2020 until June 2021.

On 14 December 2021, 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*.

2. Data and methodologies

2.1. Data

In delivering this statement, EFSA considered the information provided in the 2020 PMEM report,[6] and comments submitted by the EU Member States. Additional information on literature searching was provided by the consent holder upon EFSA's request.

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 2020 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).

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.

[6] The 2020 PMEM report is publicly available at https://ec.europa.eu/food/plant/gmo/post_authorisation/plans_reports_opinions_en (Accessed 1 May 2022).

3. Assessment

3.1. Case-specific monitoring

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

3.1.1.1. Consent holder's assessment

Compliance with non-*Bt* maize refuge requirements was available through the farmer questionnaires supplied as part of the general surveillance (Section 3.2.1). In 2020, 240 farmers from Spain and 12 farmers from Portugal completed a questionnaire which included the following question on compliance with the refuge strategy: *Did you plant a refuge in accordance to the technical guidelines?*

a) Spain

In Spain, 211 of the 240 maize MON 810-growing farmers surveyed stated that they complied with refuge obligations, either because they did implement a refuge (23 farmers) or because they planted less than 5 ha of maize MON 810 and were thus not required to plant a refuge (23 farmers) (Appendix A).

The 6 farmers that did not plant a refuge despite cultivating an area of maize MON 810 of more than 5 ha provided the following reasons for their non-compliance (as indicated in the survey): they feared yield losses in conventional maize (3 farmers), they had conventional maize as neighbouring plots (2 farmers), the planting would be too complicated (1 farmer).

The locations of the *Bt* maize fields and total number of farmers where no refuges were planted were: Huesca (3 farmers), Sevilla (2 farmers) and Lérida (1 farmer).

b) Portugal

In Portugal, the 12 maize MON 810-growing farmers surveyed followed the refuge requisites. None of them were exempted since they cultivated more than 5 ha with maize MON 810 varieties. In addition to the farmer questionnaires, the Portuguese authorities performed inspections on 42 farms (out of the 123 *Bt* maize cultivation notifications registered in 2020) where maize MON 810 was grown to check compliance with refuge and coexistence obligations outlined in Portuguese law (DGAV, 2021). 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 2020 season are consistent and do show a high level of refuge compliance (…)'. Besides, the consent holder proposed to integrate refuge planting '… as a 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 specially 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 have led to several cases of practical resistance to *Bt* crops by different lepidopteran pests (reviewed by Tabashnik et al., 2013 and Tabashnik and Carriere, 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 97.5% compliance with refuge planting obligation (see Appendix A), 2.5% of farmers did not implement a refuge although it was mandatory. An additional 9.6% 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

[7] 2020 PMEM report: Section 3.2.1.2; Appendix 1.

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[8]

3.1.2.1. Consent holder's assessment

Following the IRM plan, the 2020 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 2020 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 2020 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 • 1,118 neonates exposed to 28.22 ng Cry1Ab/cm^2 for 7 days • Separate bioassays performed for each sampling area • Endpoint: Mortality and moult inhibition	• Diet-overlay assay with purified Cry1Ab at a diagnostic concentration • Progeny of field-collected larvae • 3,658 neonates exposed to 1,091 ng Cry1Ab/cm^2 for 7 days • Separate bioassays performed for each sampling zone • Susceptible reference population tested for comparison • Endpoint: Moult inhibition
Susceptibility assay – Plant tissue	NE Spain (F_1 larvae)	• Assay using maize leaves • Larvae not used in the diagnostic assays (N = 7,250) • 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 = 16,350) • Neonates fed maize MON 810 leaves for 10 days • Susceptible reference population tested for comparison • Endpoint: Moult to L_2

[8] 2020 PMEM report: Sections 3.2.1.3 and 3.2.1.4 and Appendixes 7 and 8.

Assay	Population (generation)	ECB	MCB
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 = 141) • 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 = 59) • 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[(a)]	• Diet-overlay assay with purified Cry1Ab • Progeny of siblings of larvae that reached L_3 in confirmatory plant assay Step I and of larvae that reached L_2 in susceptibility plant assay • 105 neonates exposed to diagnostic concentration for 7 days • Endpoint: Moult inhibition
Confirmatory assay Step II – Plant tissue	NE Spain (F_2 larvae)	• Not conducted[(a)]	• 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 • 600 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): 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 2020, 651 ECB late-instars from the last generation were collected at the end of the maize growing season from five sampling sites (refuge areas or non-*Bt* maize fields) located in two zones across north-eastern Spain. Twenty-two 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 319 larvae reached the adult stage (49% of the field-sampled larvae) and were placed in 51 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. A first population was established from egg masses collected from Niedernberg (Germany) in 2005. In 2015, a second population was established from 145 diapausing larvae collected from three sampling sites in Galicia (Spain), of which 75 survived the diapause, reached the adult stage and were placed in oviposition cages for mating. Since their establishment, both populations 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). Bioassays listed as (1)–(3) only included ECB larvae from the two populations collected in the field.

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.[9]

In the 2020 bioassays, 1,118 neonates were tested against the diagnostic concentration. Two hundred and eighty-seven larvae treated with the same buffer solution used to dissolve the Cry1Ab protein were used as a negative control. Larval mortality and moulting inhibition, corresponding to dead larvae and larvae not reaching the second instar, was determined after 7 days. None of the reference populations were included in the diagnostic bioassay.

In the two zones, moulting inhibition was lower than the expected 99%, whereas in the control treatments it was 1.10% and 5.35% (Table 2). These results were lower than those reported in the previous seasons (Appendix C). The study authors indicated that *the reasons for the diagnostic concentration results are unclear* and that, *in the future, the reference strain will be used as a negative control (…) to aid in interpretation*.

Table 2: Moult inhibition of European corn borer *(Ostrinia nubilalis)* larvae at a diagnostic concentration of Cry1Ab protein: 2020 field populations [Table based on data provided in the 2020 PMEM report]

Population	Sampling area	Treatment % Moulting inhibition (No. of larvae tested)	
		Control	Cry1Ab[(a)]
North-eastern Spain	Huesca – 1	1.10 (100)	95.09 (400)
	Huesca – 2	5.35 (187)	84.12 (718)
	Total	3.22[(b)] (287)	89.61 ± 5.49[(c)] (1,118)

(a): A diagnostic concentration of 28.22 ng Cry1Ab/cm^2 of diet surface area was used.
(b): Of the 287 larvae tested, 12 larvae died and 86 and 189 larvae moulted to the third and fourth instar, respectively.
(c): Of the 1,118 larvae tested, 65 larvae died, 912 larvae survived but did not moult to the second instar, and 141 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, 7,250 of the first instars not used in the diagnostic bioassays were fed maize MON 810 leaves. Larvae were placed in plastic boxes containing detached leaves of maize (~ 300 larvae per cage). 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 327 larvae fed non-*Bt* maize leaves, was included in the study. Larvae from the control group were placed individually onto leaf discs. Expression of Cry1Ab in maize MON 810 leaves used in the bioassay was verified using immunostrips.

All ECB larvae fed maize MON 810 leaves died. In the control group, 4.0% of the larvae died or did not reach the second or third instar, whereas 96.0% of the larvae moulted to the fourth instar.

Confirmatory bioassay with maize MON 810 leaves: A follow-up study using maize MON 810 leaves was conducted with the 141 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 larvae died within 7 days.

[9] 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). Batch 2c was used for the bioassays: 1.64 mg Cry1Ab/mL in 50 mM bicarbonate buffer; pH 10.25; 91% purity.

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).

MIC_{50} and MIC_{90} values estimated in 2020 for both reference populations were within the range of those obtained in previous years (Appendix C).

Mediterranean corn borer monitoring

a) Field sampling and laboratory rearing

In 2020, 1,569 MCB late-instars from the last generation were sampled at the end of the maize growing season from 11 sampling sites (refuge areas or non-*Bt* maize fields) in three zones across north-eastern Spain. Attempts were made to collect larvae from 16 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 852 larvae reached the adult stage (54% of the field-collected larvae) and were placed in 85 oviposition cages for mating. Emerging adults from the different sampling zones were kept separately. Eighty-one cages, containing 815 adults, were used to obtain F_1 progeny for the diagnostic bioassay (i.e. 52% 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) follow-up studies to the diagnostic bioassay (confirmatory studies); and (4) concentrationnresponse 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.[10] The reference population was also tested against the diagnostic concentration.

In the 2020 assays, between 1,171 and 1,315 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 a negative control. Moult inhibition was recorded after 7 days.

In two of the three zones, (corrected) moulting inhibition was lower than the expected 99%. In the control treatments it ranged between 4.49% and 9.20%. Moult inhibition observed in the reference population was 98.67% (see Table 3).

Average moulting inhibition of the progeny of field-collected larvae (98.31 $\pm$ 1.45%) 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.

[10] 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 mM sodium bicarbonate buffer; pH 10.25; purity 91%.

Table 3: Moult inhibition of Mediterranean corn borer (*Sesamia nonagrioides*) larvae at a diagnostic concentration of Cry1Ab protein: 2020 field populations [Table based on data provided in the 2020 PMEM report]

Population	Sampling area	Treatment % Moulting inhibition (No. of larvae tested)	
		Control	Cry1Ab[(a)]
North-eastern Spain	Huesca 1	5.33 (150)	97.75 (1,315)
	Huesca 2	9.20 (163)	99.06 (1,171)
	Navarra	4.49 (178)	98.12 (1,172)
	Total	6.34 ± 1.45[(b)] (491)	98.31 ± 0.39[(b)] (3,658)
Laboratory reference population		9.23 (130)	98.67 (1,074)

No statistically significant differences were observed between the north-eastern population and the expected value of 99% ($t = 1.7306$; $df = 2$; $p = 0.113$).
No statistically significant differences were observed between the north-eastern population and the reference population ($t = 0.6503$; $df = 2$; $p = 0.291$).
(a): A diagnostic concentration of 1,091 ng Cry1Ab/cm^2 of diet surface area was used. Values have been corrected using Abbott's formula (Abbott, 1925).
(b): Mean ± standard error.

Bioassay with maize MON 810 leaves: An additional bioassay was conducted with F_1 larvae from the collected field populations using maize MON 810 leaves. To this end, 16,350 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 830 larvae fed non-*Bt* maize leaves (approximately 10 larvae per cage), was included in the study. Neonates from the laboratory reference population were also fed on maize MON 810 leaves (6,500 larvae) and conventional maize leaves (340 larvae). All larvae were placed in plastic boxes containing leaves of maize MON 810. Larvae were fed fresh leaves *ad libitum* for 10 days and numbers of larvae moulting to the second instar were recorded. Expression of Cry1Ab in maize MON 810 leaves used in the bioassay was verified using immunostrips.

None of the larvae from either the field-collected populations or of the larvae from the reference population feeding on maize MON 810 leaves reached the second instar. Moulting in the control groups of the field-collected populations ranged between 94.07% and 99.62% and resulted in 96.18% 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: 2020 field populations [Table based on data provided in the 2020 PMEM report]

Population	Sampling area	Treatment % Moulting (No. of larvae tested)	
		Non-*Bt*	*Bt*
North-eastern Spain	Huesca – 1	99.62 (260)	0.0 (6,390)
	Huesca – 2	94.07 (270)	0.0 (5,130)
	Navarra	98.15 (270)	0.0 (4,830)
Laboratory reference population		96.18 (340)	0.0 (6,500)

Confirmatory bioassays: Experiments using maize MON 810 leaves were conducted with the 59 larvae that reached the second instar in the diagnostic bioassays to confirm that they were not potentially resistant to Cry1Ab. Larvae were individually placed on experimental arenas and fed maize

MON 810 leaves. Two larvae, from a single oviposition cage from Huesca – 1 reached the third instar and survived 10 days feeding on *Bt* maize leaves.

Siblings of the larvae that reached the third instar were reared on artificial diet and additional diagnostic concentration and maize leaf bioassays were conducted with their progeny (F_2 larvae):

- in the diagnostic concentration bioassay, 105 F_2 larvae were tested and two larvae reached the second instar (98.09% moulting inhibition). These two 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 600 F_2 first-instars moulted after feeding on maize MON 810 leaves for 10 days, while 97% of the larvae from the control group (non-*Bt* maize leaves) moulted to the 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 2020 are lower than those estimated in previous years. The historical results of the concentration assays with the reference population are given in Appendix D.

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 2020 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[11] selling maize MON 810 varieties to have an overview of the farmer complaint schemes. None of the 748 complaints received by these companies in 2020 were attributed to loss of efficacy of the *Bt* maize by corn borers.[12]

The consent holder also refers to regional monitoring networks that Spanish regional authorities have implemented for integrated pest management (IPM) (e.g. the Twitter accounts @redfaragon in Aragón,[13] north-eastern Spain; @RAIF_noticias in Andalucía,[14] southern Spain). These networks monitor and alert on incidence/outbreaks of agricultural pests and plant health issues and inform about IPM practices. 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 geographical areas where adoption rate of *Bt* maize hybrids is over 60% of the total maize acreage, and where these populations are multivoltine. Following this scheme, in 2020, the consent holder collected ECB and MCB larvae exclusively from two and three sampling zones in north-eastern Spain, respectively. 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).

[11] Asociación Nacional de Obtentores Vegetales (ANOVE): https://anove.es/ (Accessed 16 May 2022).
[12] Of the 748 complaints received in 2020, two were related to maize MON 810 efficacy. One complaint was a misunderstanding as it was confirmed that the variety planted was a conventional one. The other complaint was an attack caused by a larva of *Helicoverpa* sp.
[13] Red de avisos Fitosanitarios de Aragón: https://web.redfara.es/ (Accessed 16 May 2022).
[14] Noticias de la Red de Alerta e Información Fitosanitaria de Andalucía: https://www.juntadeandalucia.es/agriculturapescaydesarrollorural/raif (Accessed 16 May 2022).

ECB and MCB populations were collected from refuges and non-*Bt* maize fields. In 23 out of 27 and 16 out of 27 the sampling sites inspected in 2020, 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 for MCB. For ECB, however, the target could not be reached.

Overall pre-imaginal mortality values during the laboratory rearing of field-collected individuals were high for both target pests; 51% and 48% 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.

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 F_1 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 recognises 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, EFSA reiterates that 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, 2021). 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

The diagnostic bioassays with MCB included a reference population serving as a 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 E).

Selection of diagnostic concentrations: Moult inhibition values observed in the susceptible reference MCB populations are repeatedly below the expected 99% (Appendix C). Besides, the consent holder has not provided sufficient evidence to underpin the appropriateness of the diagnostic concentration selected for this target pest species (EFSA, 2021). 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 zones 1, 2 and 3 of north-eastern Spain, corrected moult inhibition values were 97.75%, 99.06% and 98.12%, and the mean (98.31%) was lower than the expected > 99%, although not significantly different from the values recorded in a reference strain. Moult inhibition values for both

ECB populations were as well below > 99% (95.09% and 84.12%). In line with previous assessments (EFSA, 2020, 2021), EFSA strongly recommends the consent holder to include a reference population as a negative control in the diagnostic concentration assays with ECB. This would allow to evaluate whether moult inhibition values below > 99% in field-collected populations could indicate a decrease of susceptibility to Cry1Ab or could be associated to other factors, such as a low efficacy of the toxin batch used in the assays or the use of an inadequate diagnostic Cry1Ab concentration. This is the first time that moult inhibition values below the expected 99% are recorded in field-collected ECB populations, and weak reasoning against the potential relevance of these results as a sign of a decrease in susceptibility to the protein Cry1Ab is provided by the consent holder. EFSA considers that the cause(s) for the low moult inhibition values reported for the field-collected ECB populations should have been further investigated.

EFSA considers that moulting inhibition values lower than the expected > 99% in the diagnostic bioassays should always trigger further investigations to determine if the population has field-relevant resistance to the trait. EFSA encourages the consent holder to apply the step-wise approach recommended by the US Environmental Protection Agency for confirming resistance of lepidopteran pests of *Bt* plants (US EPA, 2010, 2018) to the corn borer monitoring programme (Appendix E).

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/or 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 siblings of larvae surviving the diagnostic bioassays, following the step-wise approach presented in Appendix E.

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 (Siegfried et al. 2007; Andreadis 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 (see Section 3.2.1.3 for further insights), 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 6 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 have been no previous estimations in this area and considering the outcome of the diagnostic concentration assay with the populations sampled in 2020 (see Section 3.1.2.1).

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 F 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

Diagnostic concentration bioassays with the progeny of the field-collected corn borer populations resulted in moulting inhibition values lower than the expected > 99% in the two ECB and in two out of the three MCB populations tested. Additional studies with plant material indicated that none of the ECB and MCB larvae tested from those populations could complete development on maize MON 810 leaves. EFSA encourages the consent holder to follow the step-wise 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 2020 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. EFSA recommends performing periodic F_2 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[15]

3.2.1.1. Consent holder's assessment

2020 Questionnaires

In the annual 2020 PMEM report, the consent holder submitted a survey based on 252 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.

[15] 2020 PMEM report: Sections 3.1.2.1 and 3.1.4.1 and Appendixes 1 and 2.

The 2020 PMEM report represented the 15th reporting year, with the completion of a total of 3,879 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 2021. The response rate was 56.2% in Spain,[16] and 100% in Portugal. Forty-nine of the 240 farmers in Spain (20%) and 4 out of 11 farmers in Portugal (33%) were interviewed for the first time.

Table 5: Farmers surveyed and maize MON 810 areas monitored in 2020 through questionnaires [Table based on data provided in the 2020 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	240[(a)]	27.0	6,469	98,152	6.6
Portugal	12[(b)]	46.4	557	4,216	13.2
Total	252	27.9	7,026	102,368	6.9

(a): One hundred seventy-nine farmers were from Aragón/Cataluña, 21 from Navarra, 26 from Extremadura, 7 from Andalucía and 7 from Castilla la Mancha.
(b): Six farmers were from Alentejo, two from Lisbon and Tagus Valley and four from Centre.

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 2020 farmer questionnaires on maize MON 810 did not identify potential adverse effects that might be related to MON 810 plants and their cultivation'*.

No information on the occurrence of teosinte was collected and the following justification was provided: *'…the emergence/occurrence of teosinte in Spain cannot be classified as information linked to the safety of MON 810 to human and animal health or the environment, nor can it be regarded as information that influences the evaluation of the MON 810 safety in all uses as conventional maize, including cultivation. Bayer is of the opinion that reporting the activities of teosinte monitoring in Spain limited to MON 810 alone or scientific literature on teosinte would not bring any additional value to the environmental risk assessment of MON 810 maize. The appearance of teosinte in Spain is a generic agronomic problem that concerns all commercial, MON 810, conventional and organic, maize fields. The monitoring of its occurrence and the management of teosinte by related good agronomic practices are relevant for conventional commercial maize, organic maize (specific measures might be needed) as well as MON 810 commercial cultivated fields'*.

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 2020 growing season are similar to those from previous annual PMEM reports.

The following summarises the evaluation of the methodology of the 2020 farmer questionnaire. The same observations were made in the previous EFSA statements. For further details, we would like to refer to EFSA (2020, 2021).

- 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 2020, 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

[16] The questionnaire was completed by 240 out of the 427 farmers that were contacted in Spain. The 187 farmers that did not respond gave the following reasons: (1) because they did not grow maize MON 810 in 2020 (71 farmers); (2) they did not grow maize in 2020 (58 farmers); (3) they grew MON 810 in 2020 but refused to answer the interview (37 farmers); (4) they were absent or could not be localised (11 farmers); (5) they were retired (10 farmers).

beginning of the growing season, so that they know upfront which questions are included and which observations they have to pay attention to all 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, possible presence of borers), and an effort should be made to use objective measurable outcomes, whenever this is possible.

EFSA (2021) recommended that information of teosinte monitoring activities in Spain and France should be included in the PMEM report and that all scientific evidence relevant for the environmental risk assessment and risk management of maize MON810 in relation to teosinte should be considered explicitly. In addition, it was recommended that the farmer questionnaires are revised to include the reporting of both the occurrence of teosinte and teosinte hybrid plants as well as the corresponding level of infestation.

It is acknowledged that last year's recommendations from EFSA (2021) could not be implemented in the 2020 PMEM report, but these recommendations should be considered in the 2021 PMEM report.

3.2.1.3. Conclusions on farmer questionnaires

From the data provided by the 2020 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).

The consent holder suggested 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[17]), and ensure that farmers growing maize MON 810 varieties are 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.

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.

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.

3.2.2. Existing monitoring networks[18]

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

3.2.2.1. Consent holder's assessment

The consent holder identified four groups of different networks, (1) governmental networks; (2) academic networks; (3) nature conservation networks and (4) professional networks.

However, no information was gathered through the existing monitoring networks. The consent holder recognised the monitoring expertise of these networks but concluded that it would not be possible for these networks to establish a cause-and-effect relationship since none of the identified

[17] Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides.
[18] 2020 PMEM report: Sections 3.1.2.3 and 3.1.4.3.

EENs measured GM crop cultivation as an influencing factor, making it difficult to establish accurate correlations based on the collected data. In addition, the consent holder lists some limitations for using EENs as an early warning system in the context of the general surveillance: '*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, 2014a)…*'.

3.2.2.2. EFSA's assessment

EFSA acknowledges the challenges of using EENs to identify impacts of GM crops. Nevertheless, several networks were identified in an external report commissioned by EFSA (Centre for Ecology and Hydrology et al., 2014) and associated publications (e.g. Smets et al., 2014) 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 recognises that the use of such networks raises a 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). Also, the purpose of EENs is not to identify cause–effect relationships but they can help detect whether key environmental endpoints/proxys are significantly affected in a receiving environment where MON 810 is grown and may point to potential adverse effects. In such a case, additional investigations would be triggered to assess to what extent MON 810 cultivation might contribute to the observed effects. EFSA acknowledges that such a strategy should go beyond the monitoring of MON 810. These systems would equally inform the potential effect of other agricultural practices (e.g. pesticides).

Therefore, 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.

As a starting point, 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

Teosinte was not considered in the original risk assessment of maize MON 810. Potential pathways to harm for non-target organisms were identified in EFSA (2016) and more recently in EFSA (2022). The completion of the pathway to harm cannot be excluded but it requires a succession of rare events, of which the combined probabilities are very low. One of the conditions for low probability of environmental harm is that the presence of teosinte is minimised in areas where MON 810 is grown.

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. The 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.

EFSA re-iterates its recommendations that:

- the consent holder explicitly considers all new scientific evidence on teosinte relevant for the ERA and RM of maize MON 810;
- the consent holder includes the reporting of both the occurrence of ES teosinte and corresponding levels of infestation in the farmer questionnaires;

- the consent holder and the Competent Authorities share relevant information on teosinte for regions where maize MON 810 cultivation may co-occur with teosinte.

3.2.4. Literature searching[19]

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 28 May 2020 and 31 May 2021.

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

Altogether, 529 publications were retrieved (excluding duplicates). After applying the pre-defined eligibility/inclusion criteria, the consent holder identified five publications as relevant for the assessment of food and feed or environmental safety.

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. Additional information submitted by the consent holder upon EFSA's request, clarified that other information sources were not considered necessary, because the SciSearch and CABA databases allow for a wide-ranging coverage for literature searching, meeting the minimum requirement in accordance to EFSA (2019a,b). A discussion on what might be the impact of non-inclusion of other databases is lacking.

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. In the additional information, the consent holder clarified that a pilot study was not deemed necessary, due to the conservative approach taken when defining the eligibility/inclusion criteria and the long-term experience in literature searches for MON 810 maize conducted for over 16 years.

EFSA noticed that papers which can potentially contribute to the assessment of MON 810 were excluded from further assessment (e.g. Baudrot et al., 2021). The provided explanation for all excluded studies is given as: 'It is not a safety study on MON 810'. This is considered as not precise enough and a more detailed explanation of the rationale for excluding a publication should be provided. The applicant should support the explanation by referring to the eligibility criteria defined in Table 1 of the literature review.

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 reliability criteria applied should be provided in future searches.

Despite the recommendations provided in the last statement, EFSA noticed that scientific information on teosinte was not included in the current PMEM. It is important that all scientific information on teosinte relevant for the environmental risk assessment and risk management of maize MON810 be included in the annual PMEM report.

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

[19] 2020 PMEM report: Section 3.1.6 and Appendix 5; additional information 14/4/2022.

improvement of future literature searches were identified. It is recommended that the consent holder provides a discussion/justification for 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. EFSA recommends that a more detailed description is provided for the reasons of discarding papers from further assessment. Relevant information on teosinte should also be retrieved in future literature searches.

None of the publications which were identified by the applicant as relevant points to new hazards, modified exposure or new scientific uncertainties that would change the former conclusions on risk assessment and risk management recommendations for maize MON 810.

3.3. Weight of evidence assessment

EFSA assembled, weighed and integrated the evidence provided in the 2020 PMEM report, additional information provided by the consent holder on insect resistance management and literature searching, comments provided by EU Member States and relevant scientific publications, following a weight of evidence approach (EFSA Scientific Committee, 2017).

Table 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 2020 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 2020 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 2020 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 (LoEs)	A summary of the evidence provided is as follows: **Case-specific monitoring** – **LoE 1:** Farmer compliance with refuge requirements. Survey of 240 Spanish and 12 Portuguese farmers growing maize MON 810 *(Section 3.1.1)* – **LoE 2:** ECB and MCB resistance monitoring *(Section 3.1.2)*: • Sampling of 651 ECB and 1,569 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 2020 growing season *(Section 3.1.2)* **General surveillance** – **LoE 4:** Systematic literature search (28 May 2020–31 May 2021). Five 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 252 questionnaires received from farmers in Spain and (240) and Portugal (12) *(Section 3.2.1)*

Weigh the evidence	Methods	– **LoE 1:** Best professional judgement – **LoE 2:** The relevance and validity of the bioassays was assessed by best professional judgement considering EFSA's previous recommendations. In the DC bioassays, MI values of the field populations were compared with the expected > 99% MI and with the results reported for the susceptible reference populations (MCB only) – **LoE 3:** The methodology of the search was assessed by best professional judgement considering the principles for literature searching laid down in EFSA (2010) and the recommendations given in EFSA (2019). 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 (97.5%) with refuge requirements in Spain and full compliance in Portugal was reported in the farmer's questionnaires – **LoE 2:** 1) ECB: MI of larvae tested against the DC was lower than the expected 99% in the two populations sampled. No resistant larvae were found in the follow-up/confirmatory bioassays with maize MON 810 leaves. 2) MCB: MI was lower than the expected 99% in two of the three sampling zones. No resistant larvae were found in the follow-up/confirmatory bioassays with maize MON 810 leaves. – **LoE 3:** None of the 748 complaints received in 2020 were attributed to loss of efficacy of maize MON 810 to provide protection against ECB/MCB damage. **General surveillance** – **LoE 4:** The information reported in the food and feed- and the 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 2020 is not sensitive enough to detect the recommended 3% resistance allele frequency – The information reported in the 2020 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 2020 growing season – EFSA concludes that no new evidence has been reported in the context of the 2020 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 a F_2 screen on European and Mediterranean corn borer populations from north-eastern Spain 4) To report all the relevant information on teosinte, including those derived from national monitoring programmes and to revise farmer questionnaires to report occurrence of teosinte and teosinte hybrids. – 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 2020 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, 2019a,b; 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.

Despite the consent holder's efforts to put in place most of the EFSA's recommendations, the monitoring plan, as it has been implemented over the last years, is not capable of timely detection of a surge of field resistance. EFSA's recurrent recommendation of replacing the current testing strategy and using a more sensitive method, like F_2 screening, has not been implemented. EFSA considers that conducting F_2 screens is proportionate and timely for populations of both target pests from north-eastern Spain, given the results of the diagnostic concentration tests for ECB and the six years elapsed since a resistance allele was detected in a population of MCB from this area. A dialogue between all relevant stakeholders (including the consent holder and companies marketing maize MON 810 varieties, risk assessors, the European Commission and Member States) is urgently needed for revising and improving the current IRM plan.

In particular, EFSA notes that the monitoring strategy implemented in the 2020 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 EFSAs 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 2020 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.

Future literature searches should provide a discussion/justification for the exclusion of other databases, details on the outcome of the pilot study, explaining better the reliability of publications identified in the literature search and provide a more detailed description of the reasons of discarding papers from further assessment. Furthermore, relevant information on teosinte should also be retrieved in future literature searches.

All the relevant information on teosinte, including those derived from national monitoring programmes, 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. It is acknowledged that last year's recommendations from EFSA (2021) could not be implemented in the 2020 PMEM report, but that applicant should strive to implement these recommendations in the 2021 PMEM report.

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.

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)	<u>Monitoring strategy</u> • To increase the sensitivity of the monitoring strategy so that it achieves a detection level of 3% resistance allele frequency in target pest populations (see blow on 'testing')	• Consent holder and other relevant stakeholders
		<u>Testing</u> • To recalculate (and validate) the diagnostic concentration for MCB • To include a reference laboratory population in the diagnostic concentration and leaf-tissue assays with ECB • To follow the step-wise approach recommended by the US Environmental Protection Agency for confirming resistance of suspected resistant populations (see Appendix E) • 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)	• Consent holder
		<u>Reporting</u> • To consider recommendations outlined in Appendix F of this statement when preparing the reports of bioassays	• Consent holder
	Farmer complaint system (Section 3.1.2.2)	• To provide more information on the farmer complaint system complementary resistance monitoring tool to determine whether proper communication mechanisms and fit-for-purpose educational programmes exist ensuring the prompt and effective reporting of farmer complaints	• Consent holder

Area (section)		Recommendation[(a)]	Responsible for implementation
General surveillance	Farmer questionnaires (Section 3.2.1.2)	• To 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 environmental risk assessment from MON 810 (e.g. emergence of teosinte)	• Consent holder
	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 3.2.4.3)	• Provide a discussion/justification for 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 • Provide a more detailed description for the reasons of discarding papers from further assessment • 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 14 December 2021, requesting EFSA to assess the annual PMEM report on the cultivation of maize MON 810 during the 2020 season provided by the consent holder.
2) Comments from the EU Member States on the 2020 PMEM report.
3) Additional information, dated 14 April 2022 provided by the consent holder.

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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
2020	240	211	23	6	97.5	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 2016 and 2020

Season	Growing area of MON 810 (ha)[a]	Avances[b]	
		Total maize (ha)	Adoption rate (%)
North-eastern Spain (Aragón, Navarra and Cataluña)			
2016	96,180	149,843	64.2
2017	96,748	148,962	64.9
2018	91,784	145,287	63.2
2019	87,329	159,261	54.8
2020	81,138	157,396[c]	51.5
Mean 2016–2020	90,636	152,150	**59.7**
Central Spain (Albacete)			
2016	4,388	9,600	45.7
2017	3,903	8,700	44.9
2018	2,406	7,092	33.9
2019	3,193	7,300	43.7
2020	2,084	7,475[c]	27.9
Mean 2016–2020	3,195	8,033	**39.2**
South-western Spain (Extremadura and Andalucía)			
2016	25,958	72,257	35.9
2017	21,989	62,584	35.1
2018	19,109	61,207	31.2
2019	16,050	64,690	25.5
2020	13,442	51,639[c]	26.0
Mean 2016–2020	19,310	62,475	**30.7**

(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 12 May 2022).
(b): Avances de superficies y producciones de cultivos: https://www.mapa.gob.es/es/estadistica/temas/estadisticas-agrarias/agricultura/avances-superficies-producciones-agricolas/ (Accessed 12 May 2022).
(c): Provisional data.

Appendix C – 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 2008–2020 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	1,110	NP	NP	NP	NP	NP	99.64 ± 0.13
	2020	651	NP	NP	NP	NP	NP	89.61 ± 5.49
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]
	2020	1,569	B2-8	NP	NP	NP	NP	98.31 ± 0.39[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.
(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.
(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 D – Cry1Ab susceptibility of reference susceptible populations of *Ostrinia nubilalis* (ECB) and *Sesamia nonagrioides* (MCB) [Table based on data provided in the 2006–2020 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
		2020	2c	2.84 (1.88–4.06)	6.97 (4.79–13.45)	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
		2020	2c	3.68 (2.78–4.40)	6.60 (5.46–9.33)	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
	Population 3[f]	2020	B2-8	14 (10–19)	93 (59–180)	98.67

Shaded rows correspond to values from the 2020 PMEM report. NP = not performed.

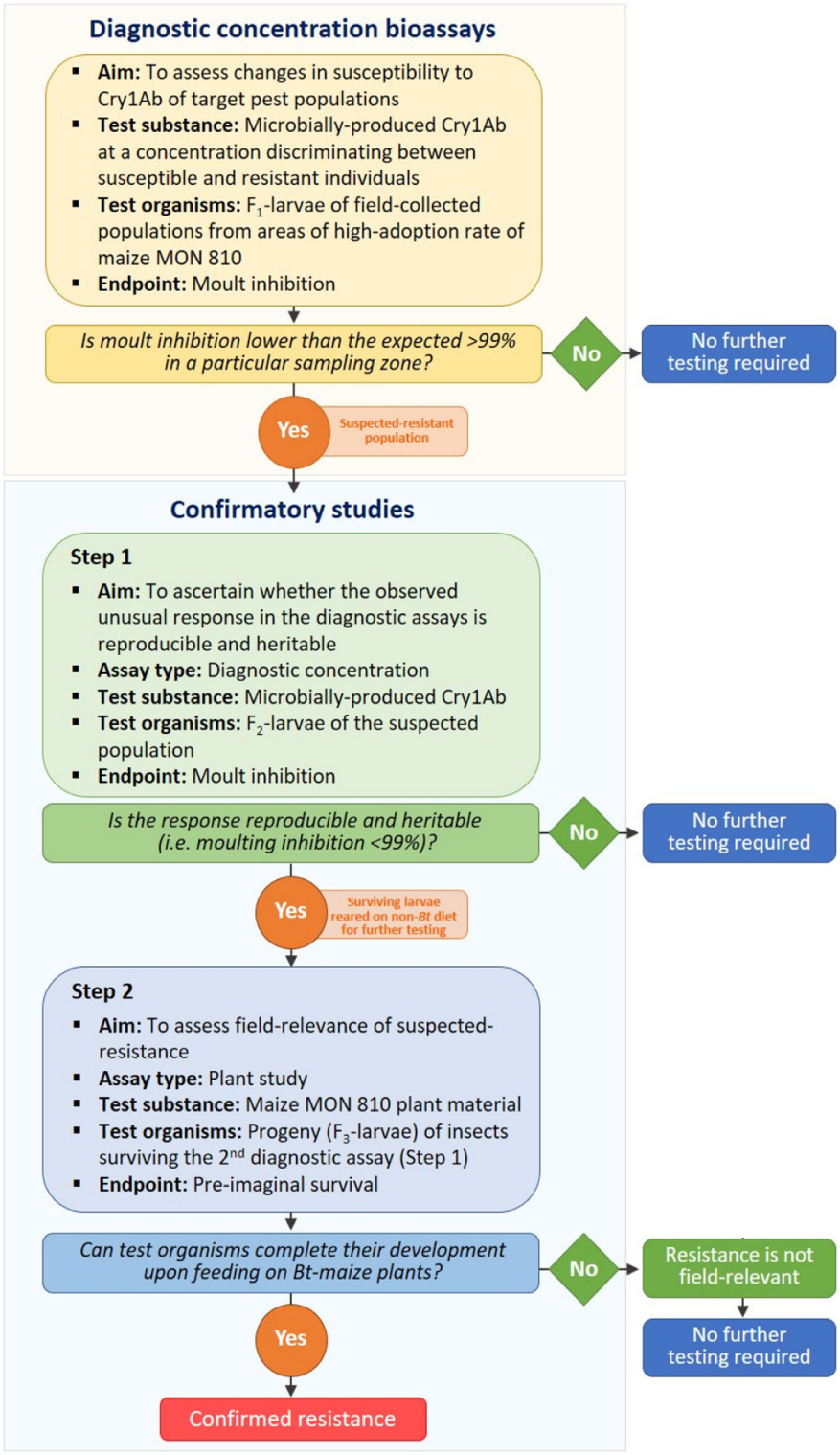
(a): 50% and 90% moulting inhibition concentration (MIC_{50} and MIC_{90}) and their 95% confidence intervals (CI 95%) are expressed in ng Cry1Ab/cm^2 of diet surface area.

(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.
(f): The population was established in 2020 from larvae collected from Galicia (Spain), where *Bt* maize has never been cultivated.

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

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



[20] US EPA (United States Environmental Protection Agency), 2010. Biopesticide Registration Action Document: Cry1Ab and Cry1F *Bacillus thuringiensis* (*Bt*) corn plant-incorporated protectants.
US EPA (United States Environmental Protection Agency), 2018. White paper on resistance in lepidopteran pests of *Bacillus thuringiensis* (*Bt*) plant incorporated protectants in the United States.

Appendix F – 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	1) Geographical area where the test organisms were collected[(a)] 2) 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) 3) Sampling source (e.g. non-*Bt* maize field, refuge) and distance to the nearest *Bt* maize field
Test organism	1) Number and life stage of collected individuals (per sampling zone/field) 2) Sampling date(s) 3) Measures taken to avoid the collection of siblings 4) Diapause and health status of field-collected populations 5) Description of the laboratory rearing protocol (including environmental conditions during laboratory rearing of field-collected individuals) 6) Number of field-collected individuals reaching adulthood after laboratory rearing of field-collected individuals (pre-imaginal mortality) 7) Number, sex and location of adults placed in oviposition cages for obtaining F_1 larvae 8) 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	1) Biochemical characterisation of the test substance (e.g. source, % purity, batch/lot used, nominal concentration, solvent/vehicle used) 2) Method used to quantify the concentration of the test substance (e.g. Bradford, ELISA, SDS–PAGE/densitometry) 3) Description of the storage conditions of the test substance 4) Biological activity (in case of new batch, comparison of biological activity to the former batch(es) 5) Equivalence to the plant-expressed protein[(b)]
Study design	1) Study performed according to standardised guideline/peer-reviewed protocol 2) Study performed according to GLP or other standards 3) Description of control(s) 4) Preparation of stock solutions, including solvent concentrations in control(s) 5) Nominal concentration(s) of test substance and rationale for their selection 6) Administration of test substance (e.g. diet-overlay, mixed with artificial diet) 7) Age and generation of individuals tested (e.g. < 24-h-old larvae from F_1 generation) 8) Duration of the assay(s) 9) Description of measurement endpoints (e.g. mortality, moult inhibition) 10) Environmental-controlled conditions (e.g. temperature, humidity and light regime) 11) Validity criteria of the study (e.g. mortality in the control group < 20%) 12) Blinding of personnel
Statistical design	1) Number of replicates for control(s) and test concentration(s); set-up of replicates (to avoid pseudo-replication) 2) Number of individuals tested per replicate 3) Treatment design (e.g. block, randomised) 4) Statistical method used 5) Statistical software used
Results and discussion	1) Deviations from the protocol 2) Description of the response effects for each of the measurement endpoints followed

Category	Specific reporting recommendations
	3) Control mortality and other observed endpoints, and comparison to validity criteria from protocol 4) Estimation of variability for measurement endpoints (if relevant, e.g. 95% confidence intervals for MIC_x values) 5) Comparison to laboratory reference population (i.e. use of resistance ratios in case of concentration/response assays) 6) Estimation of slope, chi-square (for Probit analysis) 7) Relevance of the results (in the context of baseline susceptibility and natural variability to the test substance) 8) 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 pest populations 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.