efsa JOURNAL

STATEMENT

Assessment of the 2021 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 2021 post-market environmental monitoring (PMEM) report on the cultivation of Cry1Ab-expressing maize event MON 810. Evidence provided in the PMEM report shows that farmers growing maize MON 810 in Spain complied partially with refuge requirements, while full compliance was achieved in Portugal. Cry1Ab susceptibility tests performed on European and Mediterranean corn borer populations collected from north-eastern Spain in 2021 indicated no symptoms of resistance evolution to maize MON 810. However, unexpected damage to maize MON 810 plants was observed in a field trial in the province of Girona (north-eastern Spain), which may point to the presence of resistance alleles in this region. Information retrieved through farmer questionnaires and the scientific literature reveals no 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 2021 PMEM report does not invalidate its previous conclusions on the safety of maize MON 810. The possible presence of Cry1Ab resistance alleles at frequencies leading to damage to maize MON 810 plants in Girona requires twofold actions: (1) increase monitoring efforts in this area; and (2) implement remedial measures to limit the suspected evolution and spread of resistance. As in previous years, EFSA identified shortcomings on resistance monitoring that need revision. In particular, full refuge compliance must be achieved in Spain. Moreover, the sensitivity of the monitoring plan must be increased, which can be achieved by replacing the current susceptibility assays by periodic F_2 screens. EFSA also recommends the consent holder to revise the farmer questionnaires to account for the emergence of teosinte as a noxious agricultural weed in maize MON 810-growing areas in Spain.

KEYWORDS

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

CONTENTS

SUMMARY

Following a request from the European Commission, the European Food Safety Authority (EFSA) assessed the 2021 post-market environmental monitoring (PMEM) report on the cultivation of the Cry1Ab-expressing maize event MON 810. This report presents the results of the 2021 insect resistance management and monitoring activities on maize MON 810 (hereafter referred to as 'case-specific monitoring'), as well as the results of general surveillance.

The case-specific monitoring data set comprises of: (1) 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 2021; and (2) 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, while partial compliance with refuge obligations is observed in Spain. To delay resistance evolution, EFSA considers that the consent holder must ensure full compliance with refuge requirements, especially in areas where the uptake of maize MON 810 is high. In addition, EFSA recommends the consent holder and concerned EU Member States to develop proper information systems on genetically modified (GM) crop cultivation to ensure that structured refuges are planted in clustered areas greater than 5 ha.

In the analysis of resistance monitoring data gathered through diagnostic bioassays with field-collected corn borers sampled during the 2021 maize growing season, moulting inhibition was lower than the expected > 99% in two out of the three MCB populations tested and in the two 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.

As in previous years, EFSA spotted methodological and reporting shortcomings on resistance monitoring that need revision in future PMEM reports. Based on the estimated numbers of field-collected ECB and MCB larvae used in the diagnostic concentration bioassays, EFSA considers that the monitoring plan, as implemented in 2018, is not sufficiently sensitive to detect the recommended 3% resistance allele frequency for a timely and consistent detection of a surge of field resistance. Consequently, EFSA strongly recommends the consent holder to increase the sensitivity and precision of the monitoring strategy, which could be achieved by replacing the current strategy for assessing Cry1Ab susceptibility by a more sensitive testing method, such as F_2 screens. Periodic estimations of resistance alleles through F_2 screens, together with a robust farmer complaint system should replace annual diagnostic concentration assays. In addition, the consent holder should: (1) include a reference strain in the ECB leaf tissue assays; (2) recalculate (and validate) the diagnostic concentration for MCB; (3) apply the stepwise approach recommended by the US Environmental Protection Agency for confirming resistance of lepidopteran pests of *Bt* plants and thus update the harmonised insect resistance management (IRM) plan accordingly; and (4) address EFSA's previous reporting recommendations for future resistance monitoring studies.

The unexpected damage to maize MON 810 plants caused by MCB in a field trial performed in the province of Girona (north-eastern Spain) may point to the presence of resistance alleles in this region at frequencies capable of causing damage to maize MON 810 plants. To monitor any changes in Cry1Ab susceptibility that could point to resistance evolution, the consent holder included the affected area in the annual resistance monitoring programme, so that MCB populations would be collected in the area from the 2022 growing season onwards. EFSA urges the consent holder to revise the IRM plan, addressing the previous recommendations made by EFSA. In this respect, the events triggering the implementation of remedial measures and actions included in the remedial action plan should be defined more clearly.

In EFSA's view, it is timely for the consent holder to perform a F_2 screen on MCB populations, which must include populations from the same area where the Cry1Ab resistance allele was detected in 2016 by Camargo et al. ([2018](#)), as well as from the Girona area where unexpected damage to maize MON 810 plants was observed in the 2021 growing season. Additionally, a F_2 screen should be performed on ECB populations from north-eastern Spain, where the frequency of resistance alleles has not been estimated so far.

The consent holder and other companies marketing maize MON 810 seeds have put a farmer complaint system in place that allows farmers to report complaints about product performance. During the 2021 growing season, no farmer complaints about unexpected damage caused by corn borers were reported through this system. Since the farmer complaint system is not tailored to the detection of resistance evolution corn borers, the consent holder should substantiate the usefulness of this 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. Additionally, EFSA urges the consent holder and the Competent Authorities of the concerned Member States, mostly Spain and Portugal, to collaborate more closely together, so that the data recorded by the pest monitoring systems existing at national and/or regional level can be used to inform the PMEM of maize MON 810.

The general surveillance data set provided by the consent holder consisted of a farmer survey (based on 251 farmer questionnaires) and seven relevant scientific publications published between June 2021 and May 2022. The publications were identified through a systematic literature search, which was 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 publications does not indicate any unanticipated adverse effects on human and animal health or the environment arising from the cultivation of maize MON 810. Several areas of improvement of future literature searches were identified. These include seeking further information (e.g. by contacting the authors) to enable the inclusion/exclusion of publications of unclear relevance; better explaining the reliability assessment of those relevant publications identified by the literature

search; and providing a more detailed description of the reasons of discarding publications from further assessment. Furthermore, future literature searches should be tailored to retrieve relevant information on (EU) teosinte.

In the future annual PMEM reports, the consent holder should include and address all scientific evidence relevant for the environmental risk assessment and risk management of maize MON 810 in relation to teosinte.

The 2021 PMEM report does not report any analysis of information retrieved from existing environment networks (EENs) as recommended by EFSA guidance. Although EFSA acknowledges that integrating information from EENs entails several methodological challenges, competent authorities in concerned EU Member States (mostly Spain and Portugal), 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 maize MON 810 varieties.

EFSA reiterates its recommendation to risk managers to consider the implementation of risk mitigation measures to reduce the exposure of non-target lepidoptera to maize MON 810 pollen.

Overall, EFSA concludes that the evidence reported in the 2021 PMEM report does not invalidate previous EFSA and GMO Panel evaluations on the safety of maize MON 810. The insect resistance monitoring approach put in place lacks sensitivity which could be increased by replacing the current strategy for assessing Cry1Ab susceptibility by a more sensitive testing method, such as F_2 screens. Additionally, for the region of Girona where unexpected damage to maize MON 810 plants by MCB larvae was reported for the first time since the implementation of PMEM for the cultivation of maize MON 810, EFSA stresses the need to (1) increase monitoring efforts and (2) implement remedial measures to limit the suspected evolution and spread of resistance.

1 | INTRODUCTION

Genetically modified (GM) maize MON 810 expresses the insecticidal protein Cry1Ab, encoded by a gene from the soil bacterium *Bacillus thuringiensis* (*Bt*). Maize MON 810 confers protection 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). Currently, ECB and MCB are two of the most damaging maize pests in Europe.

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.[1] Since 2003, the transformation event MON 810 has been introduced into a wide range of maize varieties grown in the EU. In 2021, maize MON 810 was cultivated in Spain (96,606 ha) and Portugal (4321 ha) over a total area of 100,927 ha (DGAV, 2021b; MAPA, 2021).

According to the Commission Decision 98/294/EC, Monsanto Europe S.A.[2] (hereafter referred to as 'the consent holder') defined a management strategy to delay the evolution of resistance in corn borer populations and offered to report resistance monitoring results to the Commission and Competent Authorities of the Member States.

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 2023 (CropLife Europe, 2023, spontaneously provided on 12 October 2023).[3] The implemented resistance management measures are based on the high-dose/refuge strategy (e.g. Gould, 1998; Tabashnik et al., 2013). This strategy requires the planting of *Bt* crops that produce an extremely high dose of the insecticidal *Bt* protein, so that nearly all individuals of the target insect pest that are heterozygous for resistance do not survive on it. In addition, the strategy necessitates the cultivation of a structured refuge (i.e. blocks or strips of non-*Bt* maize that are located near, within or adjacent to the *Bt* maize field) where the target insect pest does not encounter the *Bt* protein, and thus which 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 indicating potential increases in Cry1Ab tolerance in field populations of the target pest. Timely detection of such signs enables implementing actions to limit the survival of resistant insects, thereby slowing or preventing the spread of resistance. In the case of maize MON 810, the consent holder follows a two-pronged approach for resistance monitoring. This approach relies on: (1) the monitoring for changes in susceptibility to the Cry1Ab protein in ECB/MCB field populations in laboratory bioassays; and (2) the reporting of product-related issues, including loss of efficacy in the protection against corn borers, by farmers (i.e. through a farmer complaint system).

Ensuring compliance with refuge requirements is a critical factor contributing to the success of IRM plans in delaying the rate at which resistance evolves. Instances of field-evolved resistance to certain *Bt* crops are attributed to lack or partial compliance with refuge requirements and the inability to carry out the operational details of IRM plans[5] (reviewed by Tabashnik & Carrière, 2017; Tabashnik et al., 2023). 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 thus, they are critical to the success of the high-dose/refuge strategy (Andow, 2008; Bates et al., 2005; Glaser & Matten, 2003; Head & Greenplate, 2012).

In 2005, the consent holder initiated, voluntarily, a general surveillance monitoring programme in anticipation of the mandatory obligation for post-market environmental monitoring (PMEM) for all market applications for deliberate release submitted under Directive 2001/18/EC and Regulation (EC) No 1829/2003 (including the pending application for the renewed market authorisation for the cultivation of maize MON 810). This general surveillance aims at detecting unanticipated adverse effects associated with the commercial use of GM plants. General surveillance activities include surveys based on questionnaires from EU farmers growing maize MON 810 and systematic literature searches to find relevant scientific publications.

Since 2005, the consent holder reports 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) to the European Commission and the EU Member States, as well as the results of general surveillance. EFSA has evaluated the annual PMEM reports on maize MON 810 corresponding to the 2009–2020 growing seasons (EFSA et al., 2018, 2019, 2020, 2021, 2022; EFSA GMO Panel, 2011a, 2012a, 2013, 2014a, 2015a, 2015b, 2016; EFSA GMO Panel et al., 2017). So far, the data provided in the annual PMEM reports suggest that the cultivation of maize MON 810 is not more harmful to human and animal health and the environment than conventional maize. However, EFSA noted several shortcomings in the methodology for both case-specific monitoring and general surveillance, and made several

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

[2]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 were taken over by CropLife 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).

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 (spontaneously provided on 12 October 2023).

1.1 | Terms of reference as provided by the requestor

On 14 October 2022, the European Commission received from the consent holder the annual PMEM report for the 2021 growing season of maize MON 810 (hereafter referred to as the '2021 PMEM report'). The reporting period of the 2021 PMEM report covers July 2021 until June 2022.

On 6 February 2023, 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 2021 PMEM report,[6] and comments submitted by the EU Member States. Additional information on the farmer's questionnaires, alerts on environmental issues, case-specific monitoring, literature searches and format of the report was provided by the consent holder upon EFSA's request. The consent holder was invited to provide additional information in a clarification teleconference that took place on 19 July 2023.

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 2021 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 et al. (2019).

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

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

3 | ASSESSMENT

3.1 | Case-specific monitoring

3.1.1 | Compliance with refuge requirements[7]

3.1.1.1 | *Consent holder's assessment*

Part of the information gathered through the farmer questionnaires provided as part of the general surveillance is designed to assess the level of compliance with non-*Bt* maize refuge requirements (Section 3.5.3 of the 2021 PMEM report). In 2021, 239 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, 233 of the 239 maize MON 810-growing farmers surveyed stated that they complied with refuge obligations, either because they did implement a refuge (212 farmers) or because they planted less than 5 ha of maize MON 810 and thus were not required to plant a refuge (21 farmers) (Appendix A).

The six 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 (four

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[6]The 2021 PMEM report is publicly available at https://food.ec.europa.eu/plants/genetically-modified-organisms/post-authorisation/monitoring-plans-and-reports/report-2021_en (Accessed 27 September 2023).

[7]2021 PMEM report: Section 3.1.3.1; Appendix 1.

farmers); they had conventional maize as neighbouring plots (one farmer); or they did not know the technical rules about refuges (one farmer).

The locations of the *Bt* maize fields where no refuges were planted and the total number of farmers who did not plant refuges were Lleida (three farmers); Huesca (two farmers); and Albacete (one farmer).

b. Portugal

In Portugal, the 12 maize MON 810-growing farmers surveyed followed the refuge requirements. None of them were exempted since they all cultivated more than 5 ha with maize MON 810. In addition to the farmer questionnaires, the Portuguese authorities performed inspections on 30 farms (out of the 122 *Bt* maize cultivation notifications registered in 2021) where maize MON 810 was grown to check compliance with refuge and coexistence obligations outlined in Portuguese law (DGAV, 2021a, 2021b). 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 2021 season are consistent and show a high level of refuge compliance (…)'*. Additionally, 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 delay resistance evolution to maize MON 810. This is specially the case in areas where maize MON 810 uptake is high and the selection pressure the highest, like north-eastern Spain (Castañera et al., 2016). Low levels of refuge compliance have contributed to several cases of practical resistance to *Bt* crops by different lepidopteran pests (reviewed by Tabashnik et al., 2023). Insufficient refuge areas might also have contributed to the first case of practical resistance to a *Bt* protein by ECB, detected in Canada in 2018 (Smith et al., 2019).

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

Farmer surveys in Spain resulted in 97.6% compliance with refuge requirements (see Appendix A), while 2.4% of farmers did not implement a refuge even though it is mandatory. It is important to note that 8.4% out of the 97.6% farmers surveyed in Spain that were compliant with refuge requirements did not plant a refuge because they planted less than 5 ha of *Bt* maize. However, the 2021 PMEM report does not report if these fields were in areas where the aggregated area planted with *Bt* maize is greater than 5 ha, for which EFSA considers that refuge requirements also apply, irrespective of individual field and farm size (EFSA, 2009).

Overall, a high level of compliance was achieved, which has been stable over the last years (Appendix A). 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, considering the findings on the frequency of Cry1Ab resistance alleles in MCB populations in the Ebro basin (Camargo et al., 2018), it is paramount to ensure full compliance in areas where the uptake of maize MON 810 is high, such as north-eastern Spain, regardless of the size of individual fields. EFSA therefore considers that the consent holder must increase the level of refuge compliance. To this end, EFSA recommends that:

– The message provided to farmers in all documents (including posters, postcards, technical user guides, etc.) must explain explicitly that non-compliance with refuge requirements may speed up resistance evolution in areas where the uptake of maize MON 810 is high and that, therefore, farmers would no longer 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 explore how to reinforce 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 2021 resistance monitoring activities focused on north-eastern Spain, around the Ebro basin, where the uptake of maize MON 810 was around 60% in the last years (Appendix B). The susceptibility of sampled ECB and MCB populations to the Cry1Ab protein was tested in diagnostic concentration and plant bioassays. An overview of the bioassays conducted for the 2021 PMEM report is presented in Table 1.

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[8]2021 PMEM report: Sections 3.2.1.3 and 3.2.1.4 and Appendixes 7 and 8; additional information provided on 22 June 2023 and 12 October 2023.

TABLE 1 Overview of bioassays conducted with European corn borer (*Ostrinia nubilalis*, ECB) and Mediterranean corn borer (*Sesamia nonagrioides*, MCB) larvae, as documented in the 2021 PMEM report on the cultivation of maize MON 810.

Assay	Population (generation)	ECB	MCB
Susceptibility assay – Diagnostic concentration (DC)	NE Spain (F_1 larvae)	• Diet-overlay assay with purified Cry1Ab at a diagnostic concentration • Progeny of field-collected larvae • 1652 neonates exposed to 28.22 ng Cry1Ab/cm^2 for 7 days • Separate bioassays performed for each sampling zone • Two susceptible reference populations tested for comparison • Endpoint: Mortality and moult inhibition (%)	• Diet-overlay assay with purified Cry1Ab at a diagnostic concentration • Progeny of field-collected larvae • 3467 neonates exposed to 1091 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 DC assays (N = 6900) • Neonates fed maize MON 810 leaves for 7 days • Endpoint: Mortality and moult inhibition (%)	• Assay using maize leaves • Larvae not used in the DC assays (N = 18,950) • Neonates fed maize MON 810 leaves for 10 days • Susceptible reference population tested for comparison • Endpoint: Moult inhibition (%)
Confirmatory assay Step I – Plant tissue	NE Spain (F_1 larvae)	• Assay using maize leaves • Larvae that survived and moulted to L_2 in the DC assays (N = 21) • L_2 survivors fed maize MON 810 leaves for 7 days • Endpoint: Not specified	• Assay using maize leaves • Larvae that survived and moulted to L_2 in the DC assays (N = 54) • L_2 survivors fed maize MON 810 leaves for 10 days • L_2 survivors of susceptible reference population after DC assays tested for comparison • Endpoint: Moult to L_3 (%)
Confirmatory assay Step II – Diagnostic concentration (DC)	NE Spain (F_2 larvae)	• Not conducted[a]	• Diet-overlay assay with purified Cry1Ab • Progeny of siblings of larvae that reached L_3 in Step I confirmatory assays • 168 neonates exposed to the DC for 7 days • Endpoint: Moult inhibition (%)
Confirmatory assay Step II – Plant tissue	NE Spain (F_2 larvae)	• Not conducted[a]	• Assay using maize leaves • Progeny of siblings of larvae that reached L_3 in Step I confirmatory assay • 1200 neonates fed maize MON 810 leaves for 10 days • Endpoint: Moult inhibition (%)
Confirmatory assay Step III – Plant tissue	NE Spain (F_2 larvae)	• Not conducted[a]	• Assay using maize leaves • Larvae that survived the DC and moulted to L_2 in the Step II confirmatory assays using a DC (N = 2) • L_2 survivors fed maize MON 810 leaves for 10 days • Endpoint: Moult to L_3 (%)
Concentration-response	Laboratory	• Diet-overlay assay with purified Cry1Ab • Susceptible reference populations (Galicia, Spain, 2015 & Niedernberg, Germany, 2005) • 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 (Galicia, Spain, 2020) • Seven concentrations (2–128 ng Cry1Ab/cm^2) • Duration: 7 days • Endpoint: $MIC_{50,95}$

Abbreviations: L_2, second instar; L_3, third instar; $MIC_{50,95}$, Cry1Ab 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 2021, 811 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-four 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 308 larvae reached the adult stage (38% of the field-sampled larvae) and were placed in 51 oviposition cages for mating. Thus, in the 2021 growing season, the detection limit for the recessive resistance alleles in ECB field populations was of 5.70%. Emerging adults from the different sampling zones were kept separately.

In addition, two laboratory populations were used as negative controls in the diagnostic concentration bioassays to evaluate potential changes in the biological activity of the test substance in dose–response bioassays. 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 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 protein Cry1Ab.

b. Monitoring assays

The following bioassays were performed: (1) a diagnostic bioassay with F_1 larvae to detect potential decrease in susceptibility to Cry1Ab; (2) an additional bioassay with F_1 larvae using maize MON 810 leaves ('positive control') and non-GM maize leaves ('negative control'); (3) a follow-up study to the diagnostic bioassay with exposure to maize MON 810 leaves, to further investigate cases of suspected reduction in Cry1Ab susceptibility; and (4) concentration-response assays with both susceptible reference populations (Table 1). Bioassays (2) and (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 2021 bioassays, 1652 neonates were tested against the diagnostic concentration. Two hundred and eighty larvae were treated with the same buffer solution used to dissolve the Cry1Ab protein and they were used as a negative control. Larval mortality and moult inhibition, corresponding to dead larvae and larvae not reaching the second instar, were recorded after 7 days. Neonates of the two reference strains were also tested against the diagnostic concentration and the negative control.

In the progeny of field-collected larvae from the two sampling zones, moult inhibition was below the expected 99%, although not significantly different from this value, whereas in the control treatments, it was 0.78% and 0.49% (Table 2). These results were higher than those reported in the previous growing season, but lower than those reported in the growing seasons 2016–2019 (Appendix C). For the two reference populations, moult inhibition at the diagnostic concentration was 100%, whereas all the larvae exposed to the control solution survived and moulted to second or third instar.

TABLE 2 Moult inhibition of European corn borer *(Ostrinia nubilalis)* larvae at a diagnostic concentration of Cry1Ab protein: 2021 growing season (Table based on data provided in the 2021 PMEM report).

Population	Sampling zone	Treatment % Moult inhibition (N larvae tested)	
		Control	**Cry1Ab[a]**
North-eastern Spain	Huesca 1	0.78 (76)	98.34 (576)
	Huesca 2	0.49 (204)	98.33 (1076)
	Total	0.64[b] (280)	98.33 ± 0.01[c] (1652)
Laboratory reference strain	ES Ref	0.00 (64)	100 (128)
	G04	0.00 (64)	100 (128)

[a]A diagnostic concentration of 28.22 ng Cry1Ab/cm^2 was used.

[b]Of the 280 larvae tested, 2 larvae died and 4, 243 and 31 larvae moulted to the second, third and fourth instar, respectively.

[c]Of the 1652 larvae tested, 57 larvae died, 1574 larvae survived but did not moult to the second instar and 21 larvae moulted to the second 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, 6900 of the first instars not used in the diagnostic bioassays were fed maize MON 810 leaves. Expression of Cry1Ab in maize MON 810 leaves used in the bioassay was verified using immunostrips. Larvae were placed in plastic boxes containing detached leaves of maize (a maximum of 300 larvae per box) where they were fed ad libitum for 7 days, after which mortality and the number of larvae moulting to the second instar were recorded. A negative control group, consisting of 279 larvae fed non-*Bt* maize leaves, was included in the study. Larvae from this control group were exposed individually to leaf discs.

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[9]The selected diagnostic concentration corresponds to the mean 99% moult 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 et al., 2018). Batch 2d was used for the bioassays: 1.64 mg Cry1Ab/ml in 50 mM bicarbonate buffer; pH 10.25; 91% purity.

All ECB larvae fed maize MON 810 leaves died within the exposure period. In the control group, 0.3% of the larvae died or did not reach the second instar, whereas 99.7% of the larvae moulted to the third or fourth instar.

Confirmatory bioassay with maize MON 810 leaves: A follow-up study using maize MON 810 leaves was conducted with the 21 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.

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). Moult 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 2021 for both reference populations were within the range of those obtained in previous years (Appendix D).

Mediterranean corn borer monitoring

a. Field sampling and laboratory rearing

In 2021, 1699 MCB late instars from the last generation were collected at the end of the maize growing season from 12 sampling sites (refuge areas or non-*Bt* maize fields) in three zones across north-eastern Spain. Attempts were made to collect larvae from 12 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 Cry1Ab susceptibility of MCB larvae was assessed. Larvae were reared following a standardised protocol (Farinós et al., 2004; González-Núñez et al., 2000). A total of 1117 larvae reached the adult stage (66% of the field-collected larvae) and were placed in 103 oviposition cages for mating. Emerging adults from the different sampling zones were kept separately. Ninety-seven cages, containing 1076 adults, were used to obtain F_1 progeny for the diagnostic bioassay (i.e. 63% of the field-collected larvae).

In addition, a population initiated from 800 larvae collected in 2020 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 progeny of field-collected larvae to detect potential decrease in susceptibility to the toxin Cry1Ab; (2) an additional bioassay with F_1 larvae using maize MON 810 leaves; (3) follow-up studies to the diagnostic bioassay (confirmatory studies, to further investigate cases of suspected reduction in susceptibility to Cry1Ab); and (4) concentration-response assays with the reference population (Table 1).

Diagnostic bioassay: Independent diagnostic bioassays were performed with F_1 larvae from each of the three sampling zones. Neonates were exposed to purified Cry1Ab protein at a diagnostic concentration of 1091 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 2021 assays, between 1141 and 1178 larvae per sampling zone were tested against the diagnostic concentration. Larvae treated with the same buffer solution used to dissolve the purified Cry1Ab protein served as negative control. Moult inhibition was recorded after 7 days.

In two of the three zones, corrected moult inhibition was lower than the expected 99%. In the control treatments, it ranged between 5.05% and 13.60%. Corrected moult inhibition observed in the reference population was 99.20% (see Table 3).

Average moult inhibition of the progeny of field-collected larvae (98.27 ± 1.02%) was not significantly lower than the expected 99%. No statistically significant differences were observed between larvae from the reference population and field-collected larvae.

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[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 B2-9 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: 2021 growing season (Table based on data provided in the 2021 PMEM report).

Population	Sampling zone	Treatment % Moulting inhibition (N larvae tested)	
		Control	Cry1Ab[a]
North-eastern Spain	Huesca 1	5.05 (198)	99.64 (1178)
	Huesca 2	13.60 (125)	98.88 (1141)
	Navarra	8.67 (150)	96.28 (1148)
	Total	9.11 ± 2.48[b] (476)	98.27 ± 1.02[b] (3467)
Laboratory reference population		13.39 (112)	99.20 (1152)

Notes: No statistically significant differences were observed between the north-eastern population and the expected value of 99% ($t = 0.2028$; $df = 2$; $p = 0.429$). No statistically significant differences were observed between the north-eastern population and the reference population ($t = 0.5276$; $df = 2$; $p = 0.355$).

[a]A diagnostic concentration of 1091 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 using maize MON 810 leaves was conducted with F_1 larvae from the collected field populations. To this end, 18,950 first instars not used in the diagnostic bioassays (approximately 200 larvae per oviposition cage) were fed maize MON 810 leaves. Expression of Cry1Ab in maize MON 810 leaves used in the bioassay was verified using immunostrips, and by exposing neonates of a susceptible population of ECB to this tissue for a week. A negative control group, consisting of 970 larvae fed non-*Bt* maize leaves (~ 10 larvae per cage), was included in the study. Neonates from the laboratory reference population were also fed leaves of maize MON 810 (5200 larvae) and conventional maize (260 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.

None of the larvae derived from either field-collected populations or the reference population reached the second instar or was alive on day 10 after the start of the experiment when fed maize MON 810 leaves. In the control groups of the field-collected populations, moulting ranged between 97.67% and 99.10%, whereas in the reference population, it was 97.31% (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: 2021 growing season (Table based on data provided in the 2021 PMEM report).

Population	Sampling zone	Treatment % Moulting (N larvae tested)	
		Non-*Bt*	*Bt*
North-eastern Spain	Huesca 1	98.48 (330)	0.0 (6450)
	Huesca 2	97.67 (300)	0.0 (5850)
	Navarra	99.12 (340)	0.0 (6650)
Laboratory reference population		97.31 (260)	0.0 (5200)

Confirmatory bioassays: Experiments using maize MON 810 leaves were conducted with the 54 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. One larva, from Huesca 2, reached the third instar and survived 10 days feeding on *Bt* maize leaves.

Siblings of the larva that reached the third instar were reared on artificial diet, and their progeny (F_2 larvae) was subject to additional diagnostic concentration and maize leaf bioassays:

- In the diagnostic concentration bioassay, 168 F_2 larvae were tested and one larva reached the second instar (99.4% moulting inhibition). This larva did not survive after subsequently being fed maize MON 810 leaves for 10 days;
- In the maize leaf bioassays, none of the 1200 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.

The MIC_{50} value estimated in 2021 falls within the range of values estimated in previous years. However, the MIC_{90} value and its CI 95% (292 [139–1336] ng Cry1Ab/cm^2) were higher than the values previously recorded for the laboratory reference strain. Furthermore, in all three replicates, moult inhibition at the highest concentration tested was below 90%, which compromised the fit of the regression line at the top range of the tested doses. 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 product-related issues such as complaints about product performance to seed suppliers via the local sales representatives or customer service routes. This system enables farmers to report unexpected crop damage caused by or failure in protection against target pests in maize MON 810 varieties. The consent holder states that, during the 2021 growing season, no complaints about loss of efficacy of maize MON 810 against target pests were received via the farmer complaint system.

The consent holder reports the outcome of a survey conducted by member companies of the National Breeder Association in Spain[11] selling maize MON 810 varieties to have an overview of the farmer complaint schemes. None of the 788 complaints received by these companies in 2021 was attributed to the 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 and resistance management.

Investigation of unexpected damage on MON 810 caused by MCB in a field trial

Unexpected damage to maize MON 810 plants caused by MCB was observed in October 2021 in a field trial, comprising maize MON 810 and conventional maize, performed in a research station in the province of Girona (north-eastern Spain). The damage was subsequently notified to the consent holder. In the additional information provided by the consent holder upon request from EFSA (22 June 2023 and 12 October 2023), the consent holder described damage in around 25% of the maize MON 810 plants present in the field trial, which corresponded to approximately 992 maize MON 810 plants. The damage induced by MCB larvae to maize MON 810 plants was generally less than that observed in conventional maize plants of an adjacent field that was heavily infested with MCB. Both dead and alive larvae were recovered from maize MON 810 plants. Alive larvae were generally in earlier developmental stages (second to fourth instar, mainly) compared to the predominantly sixth-instar larvae observed in the conventional maize plants of the adjacent field. The consent holder indicates that the observation was investigated following the steps outlined in CropLife Europe's IRM plan (spontaneously provided on 12 October 2023). These investigations included: (1) collection of larvae from both the damaged maize MON 810 plants and the adjacent conventional maize field, and their transport to the laboratory; (2) rearing those larvae on semiartificial diet; and (3) testing the Cry1Ab susceptibility of the F_1 progeny of the maize MON 810-collected population in both diagnostic concentration and leaf tissue assays. No bioassays were performed to assess Cry1Ab susceptibility of the population collected from conventional maize plants. Cry1Ab expression in maize MON 810 plants was confirmed with immunostrips for plants with more than two alive larvae, and for plants used for the leaf tissue bioassays.

1. *Field collection*: 212 larvae were collected from the damaged maize MON 810 plants and 42 from conventional maize plants of the adjacent field;
2. *Transport and rearing*: The population collected from maize MON 810 plants experienced high mortality during both transport to the laboratory and rearing (72.6%): It was more than double the mortality observed in the population collected from the conventional maize plants of the adjacent field (33.3%). However, no statistically significant differences in fecundity and fertility were observed among the two populations in the F_0 generation.
3. *Testing for Cry1Ab susceptibility*: Corrected moult inhibition of the population collected from the damaged maize MON 810 plants at the diagnostic concentration was 97.91% ($N = 564$ F_1 larvae tested). This concentration fell within the range of values observed in field populations collected in north-east Spain in the period 2018–2021 (94.10%–98.66%) and laboratory reference strains tested in the same period (97.02%–99.20%). The 11 larvae that had moulted to second instar and which were alive after exposure to the diagnostic concentration were subjected to confirmatory tests. These confirmatory tests involved feeding them with maize MON 810 leaf tissue for 10 days. At the end of this period, three larvae, which came from the same

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[11]Asociación Nacional de Obtentores Vegetales (ANOVE): https://anove.es/ (Accessed 15 October 2023).

[12]Of the 788 complaints received in 2021, one was related to maize MON 810 efficacy. This complaint was a misunderstanding, as it was confirmed that the variety planted was a conventional one, and not a MON 810 one. The remaining complaints were not product-related to maize MON 810.

[13]Red de avisos Fitosanitarios de Aragón: http://web.redfara.es/ (Accessed 15 October 2023).

[14]https://twitter.com/raif_noticias?lang=en (Accessed 15 October 2023).

oviposition cage, had moulted to at least third instar. No further tests with these potentially resistant larvae were conducted, as all larvae died before moulting to fourth instar.

As for the leaf tissue assay, five larvae coming from the same oviposition cage as those moulting in the confirmatory tests previously described were alive and moulted to the second instar after 10 days of exposure. This is the first time that moulting to the second instar was recorded in larvae feeding on maize MON 810 leaves since this type of assay has been carried out. No confirmatory tests were performed with these larvae.

Based on the available evidence, the consent holder concluded there was no evidence of resistance evolution in the MCB population in the region where the field damage was reported (see PMEM report). However, in the additional information provided (22 June 2023 and 12 October 2023), the consent holder concluded that, while population-level resistance has not evolved, there are signs of the presence of resistance alleles in the MCB population collected from maize MON 810 plants. Moreover, the consent holder indicated that further studies with a larger population would be needed to confirm the preliminary results. As a follow-up action, the consent holder pro-actively included the region of Girona in the annual resistance monitoring programme. This will ensure that resistance evolution is monitored in the region from the 2022 growing season onwards.

Since the unexpected damage reported is an instance of suspected resistance, it triggered the implementation of remedial measures in the area. These measures consisted of: (1) the confirmation of the unexpected damage on plants expressing Cry1Ab and the investigation of potential practical resistance (i.e. field-evolved resistance that reduces pesticide efficacy with practical consequences for pest control (Tabashnik et al., 2014)); and (2) the inclusion of the area of Girona in the annual resistance monitoring plan.

3.1.2.2 | EFSA's assessment

European and Mediterranean corn borer resistance monitoring
a. Laboratory reference strains

Since 2018, the MCB laboratory strain originates from Galicia, which is an area in north-western Spain where the target pests have not been exposed to a high selective pressure from maize MON 810, as it has not been commercially cultivated in this area to the date. The MCB laboratory strain has been replaced by new field populations collected in Galicia three times in the last four growing seasons (new stocks obtained in 2018, 2019 and 2020). The replacement of the 2018 population by one collected in 2019 from Galicia was justified by an infection by *Nosema* spp., whereas a new stock was collected in 2020 due to the observed 'discrepancies in susceptibility to Cry1Ab' during the laboratory assays, with some larvae surviving longer than those from previous reference populations when exposed to MON 810 leaf tissue or diet treated with high Cry1Ab doses. The consent holder indicated that the three populations were collected from the same three municipalities in Galicia, and the two strains collected in 2019 and 2020 had similar susceptibility to Cry1Ab, as indicated by the results of separate susceptibility assays performed in 2021 with the same toxin batch.

As indicated by the consent holder, the MIC_{90} and its CI 95% obtained in the dose–response susceptibility bioassay of the MCB laboratory strain were above the historical range (Section 3.2 in Appendix 7), but the CI 95% overlaps with those of the MIC_{90} values reported in the growing seasons in which MCB reference strains from Galicia have been used (2018–2020). This points to lack of significant differences with historical susceptibility values. Due to the high variability in the MIC_{90} of the MCB reference strain, it is not unexpected that no significant statistical trend is observed over time. However, this could be attributed to the low statistical power of the test of difference. Consequently, during the assessment of the PMEM report, EFSA requested the consent holder to analyse the data using a test of equivalence instead of the test of difference. The consent holder indicated they will consider this recommendation in future monitoring reports (Additional Information provided on 12 October 2023).

The higher MIC_{90} values recorded in the MCB reference strain, together with the longer survival than previously observed of some larvae of the strain collected in Galicia in 2019 when exposed to high toxin doses or MON 810 leaves, indicate a high variability in Cry1Ab susceptibility in populations from Galicia, with some individuals possibly exhibiting higher Cry1Ab tolerance. High variability in Cry1Ab susceptibility had been previously reported in MCB populations from Galicia, which had broader CI 95% ranges for both the LC_{50} and LC_{90} than those recorded in populations from Madrid, Andalucía and the Ebro Valley (González-Núñez et al., 2000).

EFSA is of the opinion that reference strains must exhibit consistent and high levels of Cry1Ab susceptibility over time. The regular replacement of the MCB laboratory strain with new stocks collected in Galicia in the last years and the high natural variability in Cry1Ab susceptibility of MCB populations from this area suggest that the MCB reference strains used in the last seasons might not be an adequate reference population.

b. Field sampling and laboratory rearing

The sampling scheme of the IRM plan implemented in the EU establishes that target pest populations should be monitored annually in those geographic areas where *Bt* maize hybrids represent more than 60% of the total maize acreage, and where the target pests are multivoltine. In line with this scheme, in 2021, the consent holder collected ECB and MCB larvae exclusively from two and three sampling zones in north-eastern Spain, respectively. Since around 60% of the total maize acreage was cropped to maize MON 810 hybrids in north-eastern Spain in the last years (Appendix B), and corn borer

populations complete two generations annually in this area (Alfaro, 1972; Cordero et al., 1998), it currently represents the only hotspot for resistance evolution in the EU.

In 2021, ECB and MCB populations were collected from non-*Bt* maize fields located within 500 m from the nearest maize MON 810 field. In 24 out of the 29 (83%) and 12 out of the 24 (50%) sampling sites inspected in 2021, none or very few numbers of ECB and MCB larvae were found, respectively, highlighting that finding fields infested with ECB and MCB larvae for sampling can be a challenge. Nevertheless, for MCB, the consent holder managed to reach the target sampling size of 1000 larvae (corresponding to 2000 genomes) as established in the current IRM plan, with a total of 1699 late-instar larvae collected in the 2021 growing season. For ECB, the target sample size could not be reached, as 811 larvae were collected.

Overall pre-imaginal mortality values during the laboratory rearing of field-collected individuals were high for both target pests: 62% and 44% of the ECB and MCB larvae collected in field failed to reach adulthood, or to produce viable offspring expected to undergo testing in the susceptibility assays. The consent holder indicated that the laboratories performing the bioassays have extensive experience working with ECB and MCB populations, and have optimised the rearing process. EFSA recognises that rearing and maintenance of insect populations entail some practical challenges, with many factors (some of which are impossible to control (e.g. parasitism of corn borer larvae by hymenopteran species, insect pathogens)) contributing to mortality before susceptibility testing.

High levels of pre-imaginal mortality together with the limited number of larvae collected in the field prevented from reaching the recommended detection level of 3% (recessive) resistance allele frequency in ECB, which is needed to detect a possible insurgence of field resistance timely. In 2021, the upper bound of resistance allele frequency for ECB was the highest since this parameter was first estimated in 2016 (5.70%). For MCB, the higher number of larvae collected, together with the higher percentage of field-collected individuals contributing to the F_1 generation tested in the susceptibility bioassays, may have allowed the upper bound of the estimate of resistance allele frequency to meet the detection threshold of 3% for the first time (in case no resistant individual is detected). It must be noted that this statement is only valid in case all the individuals included in the mating cages (generally 3–6 couples per cage) had succeeded in mating and producing viable offspring, and offspring descending from all adults had been tested in the susceptibility assays, which cannot be demonstrated. To accurately determine the detection threshold for resistance allele frequency, offspring descending from single pair crosses must be used in the susceptibility bioassays.

Additionally, the missing details about the rearing of the ECB field population must be provided (i.e. number of adults that emerged from the field-collected larvae, number of adults used in oviposition cages, number of cages that produced viable offspring used in the susceptibility assays). EFSA recommends the consent holder to report the data using the same format as applied for MCB (Table 8 in Appendix 7).

In spite of the larger number of ECB individuals collected from the field in comparison with the previous growing season, the target sample size of 1000 individuals was not reached once more for this target pest. Additionally, ECB larvae were sampled from only two out of the three zones indicated in the CropLife Europe IRM plan (spontaneously provided on 12 October 2023). EFSA acknowledges the efforts made by the consent holder and recognises that it might not always be possible in practice to collate large amounts of larvae due to several factors such as natural fluctuation in pest density, environmental conditions and regional pest suppression (Dively et al., 2018). Nevertheless, EFSA reiterates the need to optimise ECB field sampling, so that enough field-collected individuals are tested to reach the target detection threshold of 3%, which, in turn, would allow the early detection of signs of resistance evolution. To achieve this, and considering the potential population suppression in north-eastern Spain, contact with field technicians from seed companies must be intensified to identify fields infested with ECB. Additionally, the consent holder is strongly recommended to liaise with the Competent Authorities of the concerned regions in Spain so as to facilitate the use of the data reported in the phytosanitary alerts and/or reports on the phytosanitary situation, which are regularly published by Pest Monitoring Systems like RedFAragon[15] and Gencat,[16] to locate fields with ECB and/or MCB damage.

Additionally, in the growing seasons 2017–2021, the ECB and MCB populations have been commonly sampled from the same zones, which include fields located in the municipalities of Candasnos, Lanaja (both in Huesca) and Mendigorría (Navarra). This sampling strategy followed EFSA's opinion which recommends repeated sampling over the years in areas where target pest pressure and/or maize MON 810 uptake are consistently high over time (EFSA, 2015a). However, EFSA also recommends focusing sampling in areas where farmers have indications of potential resistance evolution (EFSA, 2015a). No populations have been collected from Cataluña, which is the area with the highest uptake of maize MON 810 in Spain (García et al., 2023), and where unexpected damage on maize MON 810 plants by MCB was observed in 2021. In some regions of Cataluña, such as Baix Empordà (Girona), maize MON 810 uptake has consistently surpassed 60% nearly every year since 2007, with adoption values around 80% some years.[17] Given the consistently high uptake of maize MON 810 in the region, together with the signs of the presence of resistance alleles at frequencies high enough to cause damage to maize MON 810 plants in the MCB population collected from damaged maize MON 810 plants, EFSA welcomes the consent holder's initiative to sample MCB populations in Girona, where unexpected damage was reported in 2021. Moreover, MCB populations should be collected from the zone where a resistance allele was detected in a F_2 screen performed in 2016.

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[15]https://web.redfara.es/?page_id=5808 (Accessed 16 October 2023).

[16]http://agricultura.gencat.cat/ca/ambits/agricultura/dar_sanitat_vegetal_nou/avisos-fitosanitaris/ (Accessed 16 October 2023).

[17]Superfícies de conreu OMG. Distribució comarcal OMG 2006–2021 (OGM cultivation surfaces. Commarcal distribution 2006–2021) (https://agricultura.gencat.cat/ca/departament/estadistiques/agricultura/estadistiques-omg/ (Accessed 16 October 2023).

EFSA acknowledges the increasing difficulties to locate maize fields infested with the target pests in north-eastern Spain, which might make it challenging to consistently reach the targeted threshold for both target pests in this region. Therefore, EFSA reiterates the need for an alternative more sensitive monitoring strategy (see Section *'Additional testing methods'* for more details).

Currently, the Technical User Guide (TUG) provided in Spain instructs farmers cultivating maize MON 810 to immediately report corn borer damage that is higher than expected (Appendix 3.2). However, they are not instructed to regularly survey the fields for signs of damage. The TUG received by farmers that cultivate maize MON 810 in Portugal does not ask farmers to report unexpected corn borer damage. Therefore, EFSA recommends the consent holder to encourage farmers to actively and regularly inspect maize MON 810 fields to detect any unexpected damage to maize MON 810 plants caused by ECB and/or MCB as part of their communication and grower education activities.

c. 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 Cry1Ab susceptibility of target pests, instead of concentration-response assays. EFSA previously agreed with the principles driving the revision of the testing approach, but it expressed reservations on the actual implementation of this approach and made considerations on the design of the diagnostic bioassays, the selection of the diagnostic concentrations and the confirmatory studies performed with suspected-resistant individuals (EFSA et al., 2018, 2019, 2020, 2021, 2022). While the consent holder has been repeatedly invited to improve the IRM plan accordingly, and consider alternative testing methods, the consent holder has implemented only part of EFSA's recommendations.

Design of diagnostic assays: The diagnostic bioassays with both target pests included reference populations that served both as negative control and as an additional comparator. EFSA reiterates the need to include a susceptible reference population in leaf tissue bioassays with ECB. As explained in previous statements (EFSA, 2022 and earlier), reference populations must be used solely as a quality control and thus not as an additional comparator for field populations. In this regard, moult inhibition observed in diagnostic bioassays in field-collected ECB and MCB populations should not be compared statistically with the reference population; they must only be compared with the expected 99% (see proposed testing approach in Appendix E). This is further supported by the aforementioned frequent replacement of reference strains that took place in the last years for both MCB (three different populations used since 2018, high variability in Cry1Ab susceptibility reported) and ECB (two different populations used since 2017), which renders these laboratory strains inconsistent comparators across years. Additionally, to detect potential inter-population variation in the susceptibility of target pest populations and guarantee early detection of resistance, which would emerge at smaller geographic scales, EFSA recommends analysing the data from populations sampled in different zones independently (EFSA GMO Panel, 2012a). Thus, the consent holder is recommended to compare the moult inhibition recorded in field populations with the expected 99% for each sampling zone independently.

Selection of diagnostic concentrations: Moult inhibition values observed in the susceptible reference MCB populations have been consistently below the expected 99% since the diagnostic concentration was first tested in the 2016 growing season (Appendix C). Moreover, the consent holder has not provided sufficient evidence to underpin the appropriateness of the diagnostic concentration selected for this target pest species (EFSA, 2021). Therefore, 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 a susceptible population 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 99.64%, 98.88% and 96.28%, respectively, and the mean (98.27%) was lower than the expected >99%. Moult inhibition values for the ECB populations collected in two zones were below >99% (98.33% and 98.34%). While it is a longstanding EFSA recommendation (EFSA et al., 2020, 2021, 2022), it is the first time that reference strains were tested in the diagnostic concentration assays for ECB. No moulting to second instar was observed in the two ECB susceptible reference strains, whereas moult inhibition in the MCB laboratory reference strain was 99.20%.

EFSA considers that moult inhibition values lower than the expected >99% in the diagnostic bioassays should always trigger further investigation to determine if the population has field-relevant resistance to the trait. EFSA encourages the consent holder to replace the current approach to confirm suspected resistance by the stepwise approach recommended by the US Environmental Protection Agency for confirming resistance of lepidopteran pests of *Bt* plants (US EPA, 2010, 2018) in the corn borer resistance monitoring programme (Appendix E). This approach allows to assess whether resistance is heritable and field relevant. On this aspect, EFSA considers that the current IRM plan of CropLife Europe should be updated in line with US EPA's approach, while each step taken to confirm resistance in a suspected population must be thoroughly described. Furthermore, EFSA recommends the consent holder to explore the feasibility of replacing the current plant assays, which use leaf tissue to confirm field relevance of resistance, with assays that rely on whole maize plants. This method would be more realistic to assess resistance evolution of ECB and MCB to maize MON 810, since it would account for the tunnelling feeding behaviour in stalks of both target pests, especially in the case of MCB, which enters the stalk after only 1–2 days feeding on whorl tissue (Kaçar et al., 2023), as well as the lower toxin concentrations expressed in stalks compared to leaves in maize MON 810 across phenological stages (Székács et al., 2010).

EFSA observes that the detection limit for resistance allele frequency achieved in the diagnostic bioassays was higher than the recommended 3% for ECB (5.7%). For MCB, the detection threshold of 3.0% may have been achieved for the first time. Increasing the sensitivity and precision of the monitoring strategy is key for a timely implementation of remedial measures to delay resistance evolution for both target pests. As indicated in EFSA et al. (2019), increased sensitivity 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; and/or (2) replacing diagnostic bioassays by more sensitive testing methods. The consent holder has repeatedly highlighted that it is challenging to find sampling sites with sufficient numbers of corn borer larvae and reduce the mortality of field-collected individuals before laboratory testing, in spite of the results achieved for MCB this year. Therefore, in EFSA's view, the only way forward to increase the sensitivity of the monitoring strategy is to use a more sensitive method (see below *Alternative testing methods*).

Bioassays with plant tissue: The consent holder conducted supplementary bioassays in which ECB and MCB larvae surviving the diagnostic concentration and moulting to second instar, and neonates that were not used in the bioassays were fed maize MON 810 leaves. These assays aim to verify whether resistant individuals are present in the field-collected populations. EFSA recognises the value of conducting such studies with plant material, but considers that they should be performed with the progeny of siblings of larvae surviving the diagnostic bioassays in the case of suspected resistance, following the stepwise approach presented in Appendix E. According to the suggested approach, the first step to confirm resistance is to test whether a detected reduction in susceptibility (e.g. susceptibility lower than expected) is reproducible and heritable. To this aim, siblings of the larvae that moulted to second instar when exposed to the diagnostic concentration should be reared in a medium devoid of Cry1Ab, and their offspring (F_2) should be tested in the same type of susceptibility assays. Moulting to second instar after exposure to a discriminating dose of Cry1Ab for two consecutive generations would confirm heritability of resistance. The current approach followed to confirm resistance (i.e. feeding *Bt* maize leaves to larvae that are already greatly weakened due to their previous exposure to a high dose of Cry1Ab) will not serve the purpose of confirming whether a suspected decrease in susceptibility is heritable, and may underestimate the presence of resistant individuals.

Additionally, the reference strains were not tested in the assays that exposed ECB neonates to leaves of either maize MON 810 (termed by the consent holder as 'positive control') or non-GM maize ('negative control'). As indicated in previous statements, EFSA considers that bioassays testing field-collected populations should include stable laboratory susceptible strains to be used to assess the suitability of the test system.

Alternative testing methods: EFSA advocates modifying the current monitoring strategy, primarily based on diagnostic concentration assays, and replacing it by a more precise and sensitive testing method, such as the F_2 screen (Andow & Alstad, 1998). 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 3.2.3.3 for further insights), should replace annual diagnostic concentration assays. While performing an F_2 screen is, overall, more resource intensive than conducting diagnostic assays (Andow & Alstad, 1998; Huang et al., 2012), insect collection and rearing and travelling for field sampling would no longer be required every year. Moreover, this approach would yield more accurate estimations on the Cry1Ab susceptibility of field populations. To obtain adequate sensitivity for detecting Cry1Ab resistance alleles before they become widespread in target pest populations leading to resistant individuals causing measurable field damage, the target population size to test in the F_2 generation larvae must be at least 100 isolines, each of which is started from a field-mated female or two field-collected individuals (Andow & Alstad, 1998).

There is an urgent need to perform an F_2 screen on MCB populations from north-eastern Spain. This is due to the fact that: (1) 7 years have passed since the last estimation of the frequency of resistance alleles, in which Camargo et al. (2018) identified a Cry1Ab resistance allele in a MCB population from north-eastern Spain; and (2) the results of an investigation of unexpected damage of maize MON 810 plants by MCB concluded that resistant alleles are potentially present in Girona at frequencies capable of causing damage to maize MON 810 plants. This F_2 screen should consider MCB field populations from Girona where unexpected damage was reported in 2021, as well as the area where a resistance allele was detected in 2016 by Camargo et al. (2018). 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 of this parameter in ECB populations from this area. After each F_2 screen, new simulations with resistance evolution models must be run using the latest resistance frequency estimations and accounting for the relevant changes in the model parameters (e.g. the uptake of maize MON 810, refuge compliance). The newly estimated allele frequency and simulation outcomes will indicate whether the frequency of resistance alleles is increasing in comparison with the last values, and thus help to decide when to conduct the next F_2 screen.

A modified F_2 screen has recently been proposed by Santiago-González et al. (2023). This adjusted method aims at increasing the percentage of successful F_0 crossings, which is typically very low under laboratory conditions for the tested species, *Helicoverpa zea*, and entails mating one female from a known susceptible laboratory strain with three field-collected males. Using this method, the authors achieved successful mating in the F_0 and F_1 crossings leading to the production of viable offspring in 24%–34% of the isolines started. Such modified F_2 screen method might not be needed for ECB and MCB, given that for both species, a relatively high proportion of the female isolines initially started from single-pair crossings of field-collected individuals was represented in the F_2. The success rate in the last F_2 screen performed with MCB using single pairs of field-collected individuals was of 36% (Camargo et al., 2018), whereas the previous time that this method was applied to field populations of this pest species the proportion of initial lines represented in the F_2 screen was of 16%–22% (Andreadis et al., 2007). In the case of ECB, 38% of the isolines started with pairs of field-collected

individuals could be tested in the F_2 generation (Engels et al., 2010). Additionally, it must be noted that the proposed modified F_2 screen uses only males from field populations. On the one hand, this overlooks any potential resistance alleles sexually linked to females. On the other hand, it will require to collect a higher number of individuals from the field, to make up for the fact that only males are used, and sex cannot be determined visually at larval stage in neither of the target pests. Finally, to implement this method, it is necessary to have a laboratory strain with confirmed susceptibility to Cry1Ab. This could be a problem with the current MCB laboratory population, which has been regularly replaced by new stocks in the last years, and for which a high variability in susceptibility to Cry1Ab has been reported. Taking these points into consideration, the consent holder could evaluate whether using the modified F_2 screen would allow to overcome some of the limitations repeatedly raised for the use of the classic F_2 screen in ECB and MCB field populations.

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 information to be reported by the consent holder (presented as a checklist in Appendix F of this statement). This list aims at facilitating open data reporting of monitoring assays. The checklist focuses on several elements relevant to the evaluation of study design and interpretation of results. The consent holder and study authors should follow these recommendations when preparing the reports of resistance monitoring assays, and justify whenever it is not possible to meet a recommendation.

Farmer complaint system. EFSA considers that a farmer complaint system could complement the existing insect resistance management strategies as, in principle, it may allow those managing crops to provide relevant information on pest infestation levels and product performance, as well as to report possible damages to maize MON 810 plants. Therefore, a farmer complaint system may provide an additional source of first-hand information to field sampling and laboratory monitoring assays. As it was the case for previous annual PMEM reports on maize MON 810 cultivation, EFSA is unable to evaluate whether the existing farmer complaint system can be used as a complementary resistance monitoring tool. This is mainly due to the broad nature of the current invitation to 'report damages higher than expected'. Based on this requirement, it is likely that farmers will only report the occurrence of borers on maize MON 810 plants whose presence may point to early signs of resistance. Additionally, the consent holder does not report the nature of the product-related complaints received, making it impossible to determine whether this system is adequate to collect information on potential resistance evolution in a timely manner.

EFSA considers that adequate communication mechanisms and educational programmes (e.g. field scouting techniques and characterisation of the damage caused by corn borers) should be put in place to ensure the prompt and effective reporting of farmer complaints relevant for resistance monitoring.

While the regional monitoring networks mentioned (see Section 3.1.2.1) currently do not address resistance evolution in target pests, they might help to alert farmers about a possible outbreak, as some of the networks regularly monitor the incidence of pests, including corn borers. However, the consent holder did not clarify how such networks are actually used for resistance monitoring.

EFSA recommends strengthening the collaboration between the consent holder and the Competent Authorities of the Member States where maize MON 810 is grown. This would allow for the use of relevant data collected by those pest monitoring systems compliant with the Directive on Sustainable Use of Pesticides (2009/128)[18] to contribute to the IRM of MCB and ECB, as well as to monitor potential damage by secondary or emerging maize pests (e.g. *Mythimna unipuncta, Spodoptera frugiperda*) which have shown lower susceptibility to maize MON 810 or have developed resistance to it (Eizaguirre et al., 2010; Omoto et al., 2016).

Investigation of MCB unexpected damage on MON 810 in a field trial. The observation of unexpected damage by MCB to maize MON 810 plants reported in October 2021 in a research trial in the province of Girona (north-east Spain) is the first notification of damage by a target pest on maize MON 810 plants in Europe since insect resistance monitoring was initiated. The results of the follow-up investigation performed by the consent holder indicate that the population collected from the damaged maize MON 810 plants potentially harboured alleles conferring resistance to this *Bt* maize event. EFSA notes that the area where the unexpected damage was observed is located in Girona. Maize MON 810 uptake has been consistently high over time in some areas of the province of Girona, with adoption levels ranging between 55% and 82% in the period 2007–2021 (data obtained from Gencat).[19] This implies a substantial selective pressure on the target pest populations in the region. Since no MCB populations from Girona have been sampled as part of annual insect resistance monitoring previously (Farinós et al., 2018; PMEM reports of growing seasons 2015–2021), there are no data to assess whether the susceptibility of MCB populations from this area has changed over time.

Results of the laboratory assays and confirmatory tests suggest that some individuals of the population collected from maize MON 810 plants have a low susceptibility to Cry1Ab, which may point to the presence of resistance alleles in this population at frequencies capable of causing damage to maize MON810 plants. Further studies are needed to confirm this hypothesis and characterise the resistance if the presence of such resistance alleles is confirmed (e.g. dominance, potential sexual linkage, association to fitness costs). In EFSA's view, a first step in this direction would involve the selection of a resistant population from those individuals moulting and surviving the leaf tissue and confirmatory assays. The consent

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[18]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.

[19]https://agricultura.gencat.cat/ca/departament/estadistiques/agricultura/estadistiques-omg/ (Accessed 3 July 2023).

holder confirmed that a first attempt to select a resistant population from the MCB larvae collected from damaged maize MON 810 plants was unsuccessful.

In order to evaluate whether the resistance alleles are present beyond the area where damage to MON 810 plants was reported, there is a need to test Cry1Ab susceptibility of MCB populations collected in maize fields located near the area of Girona where unexpected damage was observed. Since Cry1Ab susceptibility of the population collected from the conventional maize adjacent to the damaged maize MON 810 plants was not evaluated, such information is not available so far.

EFSA strongly supports the inclusion of the region of Girona in the annual resistance monitoring programme, which should include the sampling of MCB populations from conventional maize fields and the scouting of MCB presence and damage in maize MON 810 fields. EFSA also strongly encourages the consent holder to alert the farmers in the province of Girona about the potential presence of resistance alleles in MCB populations in the area at levels that could lead to unexpected damage on *Bt* maize fields, and instruct them to inspect maize MON 810 fields for signs of MCB damage, which should be promptly notified.

Crop Life Europe's IRM plan (spontaneously provided on 12 October 2023) establishes that following *Bt* maize failure *'Appropriate integrated pest management (IPM) options will be identified and implemented to minimize spread of the problem. The remedial actions should be implemented as soon as resistance is suspected'*. However, the plan does not clearly define what suspected resistance entails, neither does it provide information on the range of IPM options that could be applied to limit the spread of resistance, nor on what would drive the choice among the different options. The consent holder confirmed that the observation of unexpected damage by MCB to maize MON 810 plants is to be considered as an event of suspected resistance, that triggers the implementation of remedial measures. However, currently, none of the remedial measures taken were tailored to reduce the spread of resistance in the field, due to the lack of population level resistance. EFSA considers that CropLife Europe's IRM plan should be amended; it should clearly define what is meant by 'suspected resistance' and propose specific remedial or mitigation actions to be implemented on a case-by-case basis. Such actions should be designed to limit the spread of resistance outside the affected area and could include stopping the sales/growing of maize MON 810 in the region where resistance has evolved/is evolving, intensifying grower education activities, implementation of alternative pest control measures, increasing refuge sizes or increasing insect resistance monitoring in the affected area, among others (US EPA, 2010).

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 populations and two out of the three MCB populations tested. Additional studies with plant material indicated that none of the ECB and MCB larvae tested from these populations could complete development on maize MON 810 leaves.

EFSA encourages the consent holder to: (1) follow the stepwise approach recommended by the US Environmental Protection Agency for confirming resistance of lepidopteran pests of *Bt* plants; and (2) update 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 2021 growing season was not sensitive enough to detect the recommended 3% resistance allele frequency in ECB (EFSA, 2015a). For MCB, the consent holder took several measures to increase the sampling size and reduce laboratory mortality prior to susceptibility testing. This may have enabled to reach the detection threshold in MCB diagnostic concentration assays for the first time. It is also noted that reaching the 3% resistance allele frequency threshold in a consistent manner is challenging. This emphasises the need to use an alternative, more sensitive testing method, so that the necessary remedial measures to delay resistance evolution can be implemented in a timely manner. In this respect, EFSA recommends the consent holder to replace the current strategy to assess Cry1Ab susceptibility by periodic F_2 screens. In EFSA's view, it is timely to perform a F_2 screen on MCB populations from north-eastern Spain, including individuals from the same area where the Cry1Ab resistance allele was detected by Camargo et al. (2018) and from the area where unexpected damage on maize MON 810 plants was detected in 2021, as well as on ECB populations from north-eastern Spain, where the frequency of resistance alleles has never been estimated.

The consent holder is strongly encouraged to implement several other of EFSA's recommendations that remain unfollowed at present. This will enable to resolve previously identified shortcomings and improve the monitoring plan (for a summary of these, see Section 5).

The investigation triggered by the observation of unexpected damage to MON 810 plants by MCB in the growing season of 2021 point to the potential evolution of field resistance ongoing in the area of Girona. To avoid resistance build up and spread, the consent holder must continue to closely monitor resistance in the Girona area, and implement remedial measures that limit further spread of resistance. Such measures could include reducing the uptake of maize MON 810 in the region and applying alternative pest management measures to control MCB populations, alerting farmers of the area to pay special attention to MCB damage on their MON 810 fields and enforcing strict refuge compliance.

3.2 | General surveillance

3.2.1 | Farmer's questionnaires[20]

3.2.1.1 | *Consent holder's assessment*

In the annual 2021 PMEM report, the consent holder provides a survey based on 251 farmer questionnaires completed by farmers in Spain and Portugal (Table 5). Both Member States accounted for all the maize MON 810 grown in the EU in that year.

The 2021 PMEM report represents the sixteenth reporting year, with the completion of a total of 4130 questionnaires since 2006.

The surveys, which were completed between February and March 2022, were performed in each country by external companies with experience in agricultural surveys. The response rate was 60.4% in Spain,[21] and 100% in Portugal. Thirty-eight of the 239 farmers in Spain (16%) and five out of 12 farmers in Portugal (42%) were interviewed for the first time.

TABLE 5 Farmers surveyed and maize MON 810 areas monitored in 2021 through questionnaires (Table based on data provided in the 2021 PMEM report)

Country	N farmers surveyed	Mean maize MON 810 area monitored per farmer (ha)	Monitored maize MON 810 area (ha)	Total planted MON 810 area (ha)	Monitored maize MON 810 (% of total area)
Spain	239[a]	23.1	5528	96,606	5.7
Portugal	12[b]	41.3	495	4313	11.5
Total	251	24.0	6023	100,919	6.0

[a]One hundred and eighty-three farmers were from Aragón/Cataluña, 22 from Navarra, 22 from Extremadura, seven from Castilla la Mancha and five from Andalucía.
[b]Seven farmers were from Alentejo, three from Centre and two from Lisbon and Tagus Valley.

The questionnaire enabled the consent holder to collect 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 specific measures to maize MON 810. Overall, the questionnaire aimed at identifying unintended effects caused by the cultivation of maize MON 810.

The consent holder concluded that '*The analysis of 251 questionnaires from a survey of farmers cultivating MON 810 in 2021 in the two MON 810 cultivating European countries, Spain and Port(u)gal, did not reveal unexpected adverse effects that could be associated with maize hybrids containing the genetic modification in MON 810*'.

In the annual PMEM monitoring report, the consent holder states that the farmers questionnaires will be revised to address EFSA's recommendations in the statements assessing the annual PMEM reports from 2018, 2019 and 2020 (EFSA et al., 2020, 2021, 2022). However, no additional details were provided in response to EFSA's request for further information on the nature of the revisions.

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 2021 growing season are identical to those from the previous annual PMEM reports.

The following points summarise the evaluation of the methodology of the 2021 farmer questionnaire. EFSA made similar observations in its previous statements (EFSA et al., 2022):

- The questionnaire provides a list of GM and non-GM varieties grown by each farmer, but it is unclear which conventional and GM varieties have been actually compared in the different fields. 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 2021, and growers might not recall everything that occurred in the field or is required in the questionnaire. It would be advisable to send the questionnaire to the selected farmers at the beginning of the growing season, so that they know 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 uptake of maize MON 810 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 possible;
- Farmer questionnaires should include an additional question to report the occurrence of any novel/emerging pest in maize, which could arrive, for instance, due to climate change or commodity trade (e.g. *S. frugiperda*), and which might affect maize MON 810.

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[20]2021 PMEM report: Sections 3.1.3.1 and 3.1.5.1 and Appendixes 1 and 2.

[21]The questionnaire was completed by 239 out of the 396 farmers that were contacted in Spain. The 157 farmers that did not respond gave the following reasons: (1) because they did not grow maize MON 810 in 2021 (69 farmers); (2) they did not grow maize in 2021 (51 farmers); (3) they grew MON 810 in 2021 but refused to answer the interview (25 farmers); (4) they were absent or could not be localised (seven farmers); (5) they were retired (five farmers).

3.2.1.3 | *Conclusions on farmer questionnaires*

From the data provided by the 2021 farmer survey, EFSA could not identify any unintended effects associated with the cultivation of maize MON 810.

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, instead, to use the farmer complaint system. However, since insufficient information is reported about the current farmer complaint system, it is unclear whether this system is fit-for-purpose to address these challenges. EFSA considers that a robust and fit-for-purpose farmer alert system could support both the IRM and address general surveillance purposes. The farmer alert system should also be linked or integrated into existing pest monitoring systems such as those established to support the implementation of IPM systems across Member States (See Directive on sustainable use of pesticides 2009/128), including the regular phytosanitary alerts issued by the Competent Authorities of Cataluña (Gencat) and Aragón (RedFAra), and ensure that farmers growing maize MON 810 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).

The Competent Authorities in concerned EU Member States must have a dialogue with the consent holder 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.

In the meantime, EFSA is of the opinion that farmer questionnaires must remain in place and their implementation should integrate the above-mentioned recommendations to improve their efficiency and support their potential to detect unexpected adverse effects.

3.2.2 | Existing monitoring networks[22]

Directive 2001/18/EC and Council Decision 2002/811/EC propose to make use of existing networks involved in environmental monitoring because they provide an additional tool for the general surveillance of GM plants, and could complement farmer questionnaires. 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.

The consent holder recognises the monitoring expertise of existing monitoring networks but concludes that these networks cannot establish a cause-and-effect relationship, as none of the identified EENs (Smets et al., 2014) 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 to use 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 GMO Panel, 2014b).*"

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 scientific publications (e.g. Smets et al., 2014). These networks may provide useful information on how agricultural practices at large impact the environment and, as such, may be useful for the general surveillance of GM plants. EFSA 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. An example of an environmental monitoring network that could be used for this purpose is the Catalan Butterfly monitoring scheme (Lee et al., 2020). Also, the purpose of EENs is not to identify cause–effect relationships. Instead, they could help to detect whether key environmental endpoints/proxies are significantly affected in a receiving environment where maize MON 810 is grown, which, in turn, could point to potential adverse effects

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[22]2021 PMEM report: Sections 3.1.3.3 and 3.1.5.3.

caused by the GM maize. In such a case, additional investigations would be triggered to assess to what extent maize MON 810 cultivation might contribute to the observed effects. EFSA acknowledges that such a strategy should go beyond the monitoring of maize 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 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 in EFSA GMO Panel (2014b).

Overall, 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 to make best use of existing ones involved in environmental monitoring of agricultural practices.

3.2.3 | Literature searches[23]

3.2.3.1 | *Consent holder's assessment*

The consent holder performed a systematic literature search to find scientific publications relevant to the food and feed and environmental safety assessment of maize MON 810 and the Cry1Ab protein published between 1 June 2021 and 31 May 2022.

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

Altogether, 540 scientific publications were retrieved (excluding duplicates). After applying the predefined eligibility/inclusion criteria, the consent holder identified seven 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 scientific publications and indicated that none of them would invalidate the initial conclusions of the maize MON 810 risk assessment.

3.2.3.2 | *EFSA's assessment*

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 et al. (2019).

Eight scientific publications identified in the search were excluded from further assessment. The provided explanation for all excluded publications was '*It is not a safety study on MON 810'*. This is not sufficiently precise, and a more detailed explanation of the rationale followed to exclude a publication should be provided, as already indicated in the last statement (EFSA et al., 2022).

The relevance of four scientific publications could not be ascertained as, based on the available information, it was no possible to determine whether the event used was MON 810. The consent holder did not make any attempt to contact the authors of these unclear publications for seeking clarification on the event used in the publications. The reason provided by the consent holder pointed out to the authors, indicating that '*they are responsible for transparent reporting of methods and results ensuring reproducibility and reliability of the data'*. EFSA considers that the consent holder should contact authors of unclear publications in order to obtain the necessary information to assess the relevance of the publication for risk assessment and risk management.

According to EFSA et al. (2019), details on the criteria to appraise the reliability of the scientific publications identified in the review should be provided. Although additional information was requested, the consent holder did not provide sufficient details on how the reliability (internal quality) of the relevant publications was evaluated.

Despite EFSA's recommendations to include relevant information on teosinte, no scientific information on teosinte was 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 MON 810 be included in the annual PMEM report (see Section 3.2.4).

3.2.3.3 | *Conclusions on literature searches*

Overall, the quality of the literature review performed by the consent holder is acceptable. EFSA acknowledges the efforts made by the consent holder to address EFSA's recommendations and comply with the guidance given in EFSA et al. (2019). However, some areas of improvement of future literature searches were identified. When the eligibility of a publication remains unclear after full-text screening, further information should be sought, if feasible (e.g. by contacting the authors) to enable the publication to be included or excluded. Regarding the reliability (internal quality) assessment of all relevant scientific publications identified in the literature searches, the consent holder should list all criteria that were used and clearly indicate how all these criteria were finally considered in the overall reliability categorisation of the publications. Also, a more detailed justification should be provided for the reasons of discarding publications from further assessment. Finally, relevant information on (EU) teosinte should be retrieved in future literature searches.

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[23]2021 PMEM report: Section 3.1.5.5 and Appendix 5; additional information 22/6/2023.

None of the relevant scientific publications identified by the consent holder 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.

3.2.4 | Teosinte

Teosinte, wild maize relatives originating from Mexico and Central America, emerged as a noxious agricultural weed in France and Spain, where they are subject to control and/or eradication measures and monitoring.

Risk concerns have been expressed that maize MON 810 may hybridise with teosinte in regions where they co-occur, leading to the development of more persistent and invasive weeds that may pose unconsidered risks to the environment, including target organisms and non-target organisms (e.g. Bauer-Panskus et al., 2020; Lohn et al., 2021; Trtikova et al., 2017). In its 2016 technical report and 2022 statement on teosinte, EFSA followed a pathway to harm approach to assess the plausibility of the above risk concerns and their relevance for the ERA and risk management of maize MON 810 cultivation (EFSA, 2016; EFSA et al., 2022). EFSA concluded that the completion of the pathway to harm requires a succession of rare events, of which the combined probabilities are very low. Consequently, it is unlikely that environmental harm will be realised through the postulated pathway to harm.

In its 2022 statement on teosinte, EFSA recommended that:

1. The consent holder explicitly considers all new scientific evidence on teosinte relevant for the ERA and risk management of maize MON 810;
2. The consent holder revises farmer questionnaires to include the reporting of both the occurrence of teosinte and corresponding levels of infestation (see also EFSA et al., 2020, 2021);
3. The consent holder and the Competent Authorities of Spain share relevant information on teosinte for regions where maize MON 810 cultivation may co-occur with teosinte.

3.2.4.1 | *Consent holder's assessment*

In the 2021 PMEM report on the cultivation of maize MON 810, the consent holder concludes that the general surveillance, including the farmer questionnaires and literature searches, did not identify any adverse effects attributable to the cultivation of maize MON 810 in the EU due to the potential presence of teosinte in maize fields in the 2021 growing season.

The consent holder reported to have taken note of EFSA's recommendations (EFSA et al., 2022) to revise farmer questionnaires in order to include the reporting of both the occurrence of teosinte and corresponding levels of infestation, and consider all new scientific evidence on teosinte relevant for the ERA and risk management of maize MON 810.

3.2.4.2 | *EFSA's assessment*

EFSA observes that no specific information on the occurrence of teosinte and corresponding levels of infestation was collected through the farmer questionnaires for the 2021 growing season of maize MON 810. This is partially due to the fact that the farmer questionnaire template has not been revised yet to address EFSA's recommendations on teosinte (see EFSA et al., 2020, 2021, 2022). While the farmer questionnaire template includes a generic question that aims to characterise the weed pressure in maize MON 810 fields, it does not mention teosinte explicitly as a relevant emerging weed. Explicitly mentioning teosinte may help to gather more targeted information on both the occurrence of teosinte and corresponding levels of infestation in maize MON 810 fields, as recommended previously by EFSA et al. (2020, 2021, 2022).

The literature searches (see Section 3.2.3) identified a single scientific publication on teosinte (i.e. Lohn et al., 2021) that is relevant for the ERA and risk management of maize MON 810 cultivation. EFSA previously assessed this scientific publication, and refers to EFSA et al. (2022) for the outcomes of its assessment. No other evidence on teosinte relevant for the ERA and risk management of maize MON 810 cultivation was retrieved through the literature searches. EFSA notes that the search strategy followed by the consent holder is tailored to identify scientific publications that are specific to maize MON 810. Yet, the strategy may overlook evidence that is not specific to maize MON 810, but relevant for the ERA and risk management of maize MON 810, as it could be used to further test specific risk hypotheses of the devised pathway to harm and confirm previously made ERA and risk management assumptions. To ensure that all new scientific evidence on (EU) teosinte relevant for the ERA and risk management of maize MON 810 is considered, the consent holder should explicitly include teosinte in the search strategy.

The reports supplied by the Competent Authority of Spain (ES) in June 2023 upon request of the European Commission (Directorate-General for Health and Food Safety) suggest that:

- Weed management measures continue to be employed in infested agricultural areas to monitor, control and/or eradicate teosinte, and restrict the cultivation of maize MON 810 in fields where the incidence of teosinte plants exceeds regional infestation thresholds;
- No hybridisation was observed between maize MON 810 and teosinte plants, where they co-occurred. Since insufficient details are reported on the materials and methods used to gather and analyse the hybridisation data, it is not possible to appraise the quality of the evidence on hybridisation reported.

3.2.4.3 | *Conclusions on teosinte*

EFSA re-iterates its previous recommendations on teosinte (EFSA, 2022) urging the consent holder to implement them from the 2022 growing season of maize MON 810 onwards:

1. The consent holder should revise the farmer questionnaire template to include the reporting of both the occurrence of teosinte and corresponding levels of infestation;
2. The consent holder should explicitly include teosinte in the literature search strategy to identify and retrieve all new scientific evidence on teosinte relevant for the ERA and risk management of maize MON 810 (including evidence that enables to test specific risk hypotheses of the devised pathway to harm, and confirm ERA and risk management assumptions);
3. The consent holder and the Competent Authorities share relevant information on teosinte for regions where maize MON 810 cultivation may co-occur with teosinte.
4. EFSA encourages the ES Competent Authorities to continue employing comprehensive weed management measures to monitor, control and/or eradicate teosinte in infested agricultural areas, and restrict the cultivation of maize MON 810 in fields where the incidence of teosinte plants exceeds regional infestation thresholds. The monitoring, control and eradication measures put in place in ES (especially in Aragón and Cataluña where maize MON 810 is widely grown) contribute to further reduce the low potential of vertical gene flow between GM maize and teosinte, and thus the likelihood of environmental harm to occur through the postulated pathway to harm.

3.2.5 | Non-target Lepidoptera

A potential risk associated with the cultivation of lepidopteran-active *Bt*-maize is the ingestion of harmful amounts of *Bt*-maize pollen deposited on host/food plants of non-target lepidoptera in or near *Bt*-maize fields, by some non-target lepidoptera. This risk has been quantified for the *Bt*-maize events 1507, MON 810 and Bt11 by EFSA's GMO Panel (see EFSA, 2009; EFSA GMO Panel, 2011c, 2012b, 2015c). The GMO Panel used the mathematical models developed by Perry et al. (2010, 2011, 2012, 2013) to derive estimates of larval mortality.

In EFSA GMO Panel (2011c, 2012b, 2015c), the GMO Panel concluded that some lepidopteran species (i.e. those in the 'very highly' to 'extremely' sensitive categories) can be at risk when they ingest harmful amounts of maize MON 810 pollen, while emphasising that no actual species had yet been recorded with that degree of sensitivity and that the species at potential risk were therefore hypothetical. Despite this, the GMO Panel considered this worst-case scenario to ensure the inclusion of all potential species sensitivities within the modelling exercises, in order to study the possible implications of exposure to maize MON 810 pollen for all lepidopteran species. Based on the model estimates, the GMO Panel recommended risk managers to implement an isolation distance of 20 meters between protected habitats, where sensitive NT Lepidoptera can be found, and the nearest maize MON 810 field. This recommendation to risk managers is still valid wherever considered necessary and proportionate, in connection with the protection goals for non-target lepidoptera and the levels of maize MON 810 uptake in a given region.

4 | CONCLUSIONS

The evidence from the 2021 PMEM report, which was integrated following a weight of evidence approach (Appendix G), does not indicate any adverse effects on human and animal health and the environment arising from the cultivation of maize MON 810 during the 2021 growing season. Previous evaluations on the safety of maize MON 810 (EFSA, 2009; EFSA GMO Panel, 2012c,d) remain valid.

5 | RECOMMENDATIONS

As it was the case for previous annual PMEM reports on maize MON 810 cultivation, EFSA identified methodological and reporting limitations in the approach followed for case-specific monitoring and general surveillance. In the assessment of past PMEM reports, EFSA has provided a list of recommendations for the consent holder to address with the goal to improve both case-specific monitoring and general surveillance. Several of these recommendations remain unaddressed in the 2021 PMEM report, and they should be considered in future annual PMEM reports. Additionally, EFSA reiterates its recommendation to risk managers to consider the implementation of risk mitigation measures to reduce the exposure of non-target lepidoptera to maize MON 810 pollen.

New recommendations emerged from the assessment of the PMEM report for the 2021 growing season. The recent report of field damage to maize MON 810 plants caused by MCB in the province of Girona confirms the need to further improve the IRM approach currently followed by the consent holder. The region where unexpected damage was observed must be monitored closely for signs of decreased susceptibility to Cry1Ab and failure of maize MON 810 to control MCB, so that further remedial actions can be implemented to contain resistance evolution and spread. Additionally, the consent holder should provide an effective remedial action plan that clearly defines the events triggering its implementation and describes specific measures to be put in place.

A full list of recommendations made by EFSA in the frame of the assessment of annual PMEM reports for maize MON 810 cultivation is provided in Table 6 below.

TABLE 6 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 (3.1.1.2)	– To take relevant actions, in order to achieve full compliance of refuge requirements, especially in regions of high maize MON 810 adoption – Be more explicit in the information provided to farmers on how non-compliance with refuge requirements may speed up resistance evolution, especially 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 integrating all relevant stakeholders to ensure that structured refuges are planted in areas where the clustered cultivation of maize MON 810 exceeds 5 ha	– Consent holder – Competent Authorities of concerned EU Member States
	ECB/MCB resistance monitoring (3.1.2.2)	Monitoring strategy – To increase the sensitivity of the monitoring strategy so that it achieves a detection level of 3% resistance allele frequency in target pest populations (see below on 'testing')	– Consent holder and other relevant stakeholders
		Testing – To recalculate and validate the diagnostic concentration for MCB – To include a reference laboratory population in the leaf-tissue assays with ECB – To follow the stepwise 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 screens on ECB and MCB populations in north-eastern Spain)	– Consent holder
		Reporting – To consider recommendations outlined in Appendix F of this statement when preparing the reports of bioassays	– Consent holder
		Investigation of MCB unexpected damage on MON 810 in a field trial – To monitor resistance evolution in MCB populations of the area of Girona – To define trigger points for the implementation of remedial actions – To describe the range of remedial actions to be implemented	– Consent holder
	Farmer complaint system (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 programs exist ensuring the prompt and effective reporting of farmer complaints. – Competent Authorities in concerned EU Member States and the consent to collaborate to use the relevant data collected in Pest Monitoring Schemes to inform the PMEM	– Consent holder – Competent authorities of concerned EU Member States
General surveillance	Farmer questionnaires (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) – To include a question to report on the occurrence of novel pests	– Consent holder
	Existing environmental networks (EENs) (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 EENs in the broader context of environmental monitoring – Competent Authorities in concerned EU Member States, the consent holder and representatives of EENs 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 through the use of these EENs.	– Consent holder – Competent authorities of concerned EU Member States – Environmental networks active in the area of cultivation of maize MON 810.
	Literature searches (3.2.3.2)	– Explain and list the criteria which were used for assessing the reliability of scientific publications identified in the literature search. – Further information should be sought, if feasible (e.g. by contacting the authors), to enable publications to be included or excluded. – Provide a more detailed justification for the reasons of discarding papers from further assessment. – Include relevant information on teosinte in the literature search.	– Consent holder
	Non-target Lepidoptera (3.2.5)	– Implement mitigation measures to reduce the exposure of non-target lepidoptera to maize MON 810 pollen	– Risk managers

Abbreviations: 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 6 February 2023, requesting EFSA to assess the annual PMEM report on the cultivation of maize MON 810 during the 2021 season provided by the consent holder.
2. Comments from the EU Member States on the 2021 PMEM report.
3. Additional information provided by the consent holder, dated 22 June 2023.
4. Additional information provided by the consent holder, dated 12 October 2023.

ABBREVIATIONS

CI	confidence interval
DC	Diagnostic concentration
ECB	European corn borer
FQ	farmer questionnaires
GLP	Good laboratories practices
GM	genetically modified
MCB	Mediterranean corn borer
MI	moult inhibition
PMEM	post-market environmental monitoring

ACKNOWLEDGEMENTS

CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

REQUESTOR

European Commission

QUESTION NUMBER

EFSA-Q-2023-00102

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Annex 1: Replies to EU Member States' comments.

APPENDIX A

Farmer compliance with refuge requirements in Spain between 2004 and 2021 (Table based on data provided in 2004–2021 PMEM reports on maize MON 810)

| Growing season | N farmers surveyed | N farmers planting structured refuges | N 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
2021	239	212	21	6	97.6	FQ

Note: Shaded row corresponds to the annual PMEM report under assessment. In the surveys conducted by Antama, all farmers were from north-eastern Spain.

Abbreviations: Antama, Study sponsored by Spanish foundation supporting the use of new technologies in agriculture; FQ, farmer questionnaires; NR, not reported.

[a]Farmers planting < 5 ha of maize MON 810 in the farm are not required to plant a refuge. In the FQ, only farmers who are required to plant a refuge based on the total hectares of maize MON 810 sown were considered for the calculation of non-compliance with refuge requirements.

APPENDIX B

Growing area and adoption rate of maize MON 810 in north-eastern, central and south-western Spain between 2016 and 2021

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	51.5
2021	82,275	165,435[c]	49.7
Mean 2016–2021	89,242	154,364	**58.1**
Central Spain (Albacete)			
2016	4388	9600	45.7
2017	3903	8700	44.9
2018	2406	7092	33.9
2019	3193	7300	43.7
2020	2084	7475	27.9
2021	2683	9021[c]	29.7
Mean 2016–2021	3110	8198	**37.6**
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	26.0
2021	10,668	51,599[c]	26.7
Mean 2016–2021	17,869	60,663	**30.1**

[a]Source: https://www.mapa.gob.es/es/agricultura/temas/biotecnologia/estimacionsuperficietotalomgespana2021_tcm30-577952.pdf (Accessed 5 May 2023).

[b]Avances de superficies y producciones de cultivos: https://www.mapa.gob.es/es/estadistica/temas/estadisticas-agrarias/agricultura/avances-superficies-producciones-agricolas/ (Accessed 5 May 2023).

[c]Provisional data.

APPENDIX C

Historical data on Cry1Ab susceptibility of *Ostrinia nubilalis* (ECB) and *Sesamia nonagrioides* (MCB) field populations from north-eastern Spain (Table based on data provided in the 2008–2021 PMEM reports on maize MON 810)

Target pest	Season	Larvae collected (N)	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[g,d] (NR)	2.93/5.35[g,d] (NR)	NP
	2009	509	1	6.40 (5.32–7.75)	13.68 (10.77–20.02)	1.75[g] (NR)	1.43 (NR)	NP
	2011	382	2	1.79 (1.54–2.07)	4.19 (3.45–5.48)	0.61[g] (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[g] (NR)	0.77 (NR)	NP
	2016	1111	2b	NP	NP	NP	NP	99.23
	2017	1111	2b	NP	NP	NP	NP	99.19
	2018	1144	2b	NP	NP	NP	NP	99.83
	2019	1110	2c	NP	NP	NP	NP	99.64 ± 0.13[f]
	2020	651	2c	NP	NP	NP	NP	89.61 ± 5.49[f]
	2021	811	2d	NP	NP	NP	NP	98.33 ± 0.01[f]
MCB	2004	424	B1	63 (34–99)	570 (333–1318)	3.5 (NR)	5.8 (NR)	NP
	2005	400	B1	9 (3–15)	76 (54–117)	0.5 (NR)[e]	0.8 (NR)[e]	NP
	2007	457	B1	14 (8–20)	99 (71–158)	0.9 (NR)	1.0 (NR)	NP
	2009[h]	489	B1	22 (16–28)	188 (138–277)	1.1 (0.8–1.7)	1.6 (NR)	NP
	2011[h]	564	B2-1	20 (14–27)	135 (91–232)	2.2 (1.6–3.0)[g]	2.0 (1.3–2.9)[g]	NP
	2013[h]	742	B2-2	19 (14–25)	163 (108–287)	2.6 (2.0–3.4)[g]	3.4 (2.2–5.2)[g]	NP
	2015[h]	529	B2-2	17 (13–21)	84 (63–124)	0.6 (0.5–0.8)[g]	1.3 (0.9–1.8)	NP
	2016	1364	B2-3	NP	NP	NP	NP	97.96 ± 0.71[f]
	2017	1452	B2-4	NP	NP	NP	NP	94.14 ± 1.40[f]
	2018	1490	B2-6	NP	NP	NP	NP	98.65 ± 0.40[f]
	2019	1644	B2-7	NP	NP	NP	NP	97.97 ± 0.36[f]
	2020	1569	B2-8	NP	NP	NP	NP	98.31 ± 0.39[f]
	2021	1699	B2-9	NP	NP	NP	NP	98.27 ± 1.02[f]

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

Abbreviations: NP, not performed; NR, not reported.

[a]Data provided by the consent holder confirmed that the Cry1Ab protein batches 1 and 2, 2 and 2a, 2b and 2c, 2c and 2d, 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 independent assays corresponding to the different sampling zones.

[g]Significant difference ($p < 0.05$) between the field population and the reference population was identified for that season.

[h]Susceptibility data from these populations were used to estimate the diagnostic concentration (1091 ng Cry1Ab/cm^2 of diet surface area).

APPENDIX D

Cry1Ab susceptibility of reference susceptible populations of *Ostrinia nubilalis* (ECB) and *Sesamia nonagrioides* (MCB) (Table based on data provided in the 2006–2021 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
		2021	2d	2.81 (1.91–3.88)[a]	8.63 (6.07–14.62)[a]	100
	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
		2021	2d	2.31 (1.22–3.79)	6.91 (4.16–18.66)	100
MCB	Population 1[d]	2004	B1	18 (11–25)	99 (66–208)	NP
		2007	B1	16 (11–22)	94 (69–147)	NP
		2008	B1	19 (10–30)	120 (76–255)	NP
		2010	B1	8 (5–11)	74 (51–117)	NP
		2011	B2-1	9 (6–13)	68 (45–127)	NP
		2012	B2-1	7 (5–10)	62 (41–107)	NP
		2013	B2-1	7 (5–10)	48 (31–88)	NP
		2013	B2-2	5 (3–9)	42 (26–87)	NP
		2014	B2-2	17 (11–25)	91 (57–209)	NP
		2015	B2-2	28 (21–36)	67 (50–110)	NP
		2016	B2-3	30 (24–38)	83 (62–132)	99.23
		2017	B2-4	24 (16–35)	162 (100–363)	97.69
	Population 2[e]	2018	B2-6	19 (13–26)	116 (76–224)	97.75
	Population 3[f]	2019	B2-7	27 (16–40)	233 (133–656)	97.02
	Population 4[g]	2020	B2-8	14 (10–19)	93 (59–180)	98.67
		2021	B2-9	25 (14–40)	292 (139–1336)	99.20

Note: Shaded rows correspond to values from the 2021 PMEM report.

Abbreviation: 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.

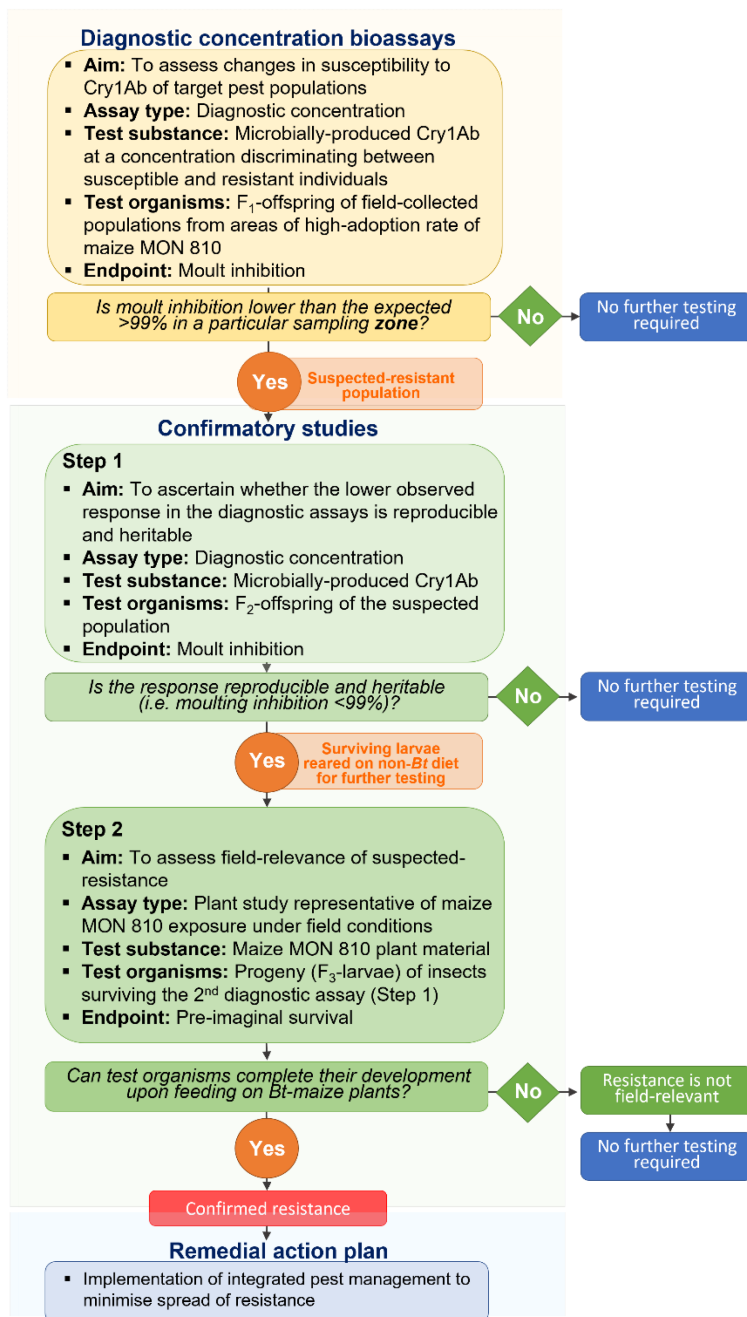[e]The population was established in 2018 from larvae collected from Galicia (Spain), where *Bt* maize has never been cultivated.

[f]The population was established in 2019 from larvae collected from Galicia (Spain), where *Bt* maize has never been cultivated.

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

APPENDIX E

Proposed stepwise approach for confirming resistance to *Bt* plants of suspected resistant populations (Adapted from US EPA [2010, 2018].[24] Once resistance is confirmed, the CropLife Europe IRM plan foresees the implementation of remedial actions)

Diagnostic concentration bioassays
- **Aim:** To assess changes in susceptibility to Cry1Ab of target pest populations
- **Assay type:** Diagnostic concentration
- **Test substance:** Microbially-produced Cry1Ab at a concentration discriminating between susceptible and resistant individuals
- **Test organisms:** F_1-offspring of field-collected populations from areas of high-adoption rate of maize MON 810
- **Endpoint:** Moult inhibition

Is moult inhibition lower than the expected >99% in a particular sampling zone? → **No** → No further testing required

Yes → Suspected-resistant population

Confirmatory studies

Step 1
- **Aim:** To ascertain whether the lower observed response in the diagnostic assays is reproducible and heritable
- **Assay type:** Diagnostic concentration
- **Test substance:** Microbially-produced Cry1Ab
- **Test organisms:** F_2-offspring of the suspected population
- **Endpoint:** Moult inhibition

Is the response reproducible and heritable (i.e. moulting inhibition <99%)? → **No** → No further testing required

Yes → Surviving larvae reared on non-*Bt* diet for further testing

Step 2
- **Aim:** To assess field-relevance of suspected-resistance
- **Assay type:** Plant study representative of maize MON 810 exposure under field conditions
- **Test substance:** Maize MON 810 plant material
- **Test organisms:** Progeny (F_3-larvae) of insects surviving the 2nd diagnostic assay (Step 1)
- **Endpoint:** Pre-imaginal survival

Can test organisms complete their development upon feeding on Bt-maize plants? → **No** → Resistance is not field-relevant → No further testing required

Yes

Confirmed resistance

Remedial action plan
- Implementation of integrated pest management to minimise spread of resistance

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

APPENDIX 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	4. Geographical area where the test organisms were collected[a] 5. Locations, number and type of fields (e.g. refuge areas, non-*Bt* maize field) per location where test organisms were collected (e.g. geographical coordinates, nearest municipality) 6. Sampling source (e.g. non-*Bt* maize field, refuge) and distance to the nearest *Bt* maize field
Test organism	7. Number and life stage of collected individuals (per sampling zone/field) 8. Sampling date(s) 9. Measures taken to avoid the collection of siblings 10. Diapause and health status of field-collected populations 11. Description of the laboratory rearing protocol (including environmental conditions during laboratory rearing of field-collected individuals) 12. Number of field-collected individuals reaching adulthood after laboratory rearing of field-collected individuals (pre-imaginal mortality) 13. Number, sex and location of adults placed in oviposition cages for obtaining F_1 larvae 14. Description of the use of susceptible/resistant laboratory reference population, including information on how the population was initiated and how it is maintained and invigorated
Test substance	15. Biochemical characterisation of the test substance (e.g. source, % purity, batch/lot used, nominal concentration, solvent/vehicle used) 16. Method used to quantify the concentration of the test substance (e.g. Bradford, ELISA, SDS-PAGE/densitometry) 17. Description of the storage conditions of the test substance 18. Biological activity (in case of new batch, comparison of biological activity to the former batch(es)) 19. Equivalence to the plant-expressed protein[b]
Study design	20. Study performed according to standardised guideline/peer-reviewed protocol 21. Study performed according to GLP or other standards 22. Description of control(s) 23. Preparation of stock solutions, including solvent concentrations in control(s) 24. Nominal concentration(s) of test substance and rationale for their selection 25. Administration of test substance (e.g. diet-overlay, mixed with artificial diet) 26. Age and generation of individuals tested (e.g. < 24 h-old larvae from F_1 generation) 27. Duration of the assay(s) 28. Description of measurement endpoints (e.g. mortality, moult inhibition) 29. Environmentally controlled conditions (e.g. temperature, humidity and light regime) 30. Validity criteria of the study (e.g. mortality in the control group < 20%) 31. Blinding of personnel
Statistical design	32. Number of replicates for control(s) and test concentration(s); set-up of replicates (to avoid pseudo-replication) 33. Number of individuals tested per replicate 34. Treatment design (e.g. block, randomised) 35. Statistical method used 36. Statistical software used
Results and discussion	37. Deviations from the protocol 38. Description of the response effects for each of the measurement endpoints followed 39. Control mortality and other observed endpoints, and comparison to validity criteria from protocol 40. Estimation of variability for measurement endpoints (if relevant, e.g. 95% confidence intervals for MIC_x values) 41. Comparison to laboratory reference population (i.e. use of resistance ratios in case of concentration/response assays) 42. Estimation of slope, Chi-square (for Probit analysis) 43. Relevance of the results (in the context of baseline susceptibility and natural variability to the test substance) 44. Availability of raw data

Abbreiations: GLP, Good laboratories practices; MIC_x, Cry1Ab concentration at which × % moult inhibition is observed.

[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.

APPENDIX G

Weight of evidence assessment

EFSA assembled, weighed and integrated the evidence provided in the 2021 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 et al., 2017).

The following table 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.

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 2021 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 2021 PMEM report submitted by the consent holder – Additional information on insect resistance management, farmer complaint system, literature searching, alerts on environmental issues and farmer questionnaires provided by the consent holder following EFSA's requests – Scientific comments submitted by EU Member States – Relevant scientific publications
	Lines of evidence (LoE)	A summary of the evidence provided is as follows: **Case-specific monitoring** – **LoE 1:** Farmer compliance with refuge requirements. Survey of 239 Spanish and 12 Portuguese farmers growing maize MON 810 *(Section 3.1.1)* – **LoE 2:** ECB and MCB resistance monitoring *(Section 3.1.2)*: – Sampling of 811 ECB and 1699 MCB larvae from two and three zones, respectively, 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 and descendants of their siblings – **LoE 3:** Farmer complaint system: complaints received from farmers growing maize MON 810 varieties during the 2021 growing season *(Section 3.1.2)* – **LoE 4:** An observation of unexpected damage of MON 810 plants by MCB in Girona, notified by industrial networks, and the results of the subsequent investigation of this event of suspected resistance *(Section 3.1.2)* **General surveillance** – **LoE 5:** Systematic literature search (1 June 2021–31 May 2022). Seven food and feed-, agronomic- and environmental- safety relevant publications were identified and assessed *(Section 3.2.3)* – **LoE 6:** Existing monitoring networks – **LoE 7: Farmer survey based on 251 questionnaires received from farmers in Spain and (239) 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** – **LoE 3:** Best professional judgement – **LoE 4**: The relevance and validity of the observations and the results of the bioassays was assessed by best professional judgement. 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 – **LoE 5:** The methodology of the search was assessed by best professional judgement considering the principles for literature searching laid down in EFSA (2010) and the recommendations given in EFSA et al. (2019); EFSA et al. (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 6:** Best professional judgement – **LoE 7:** 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, 2011b)

	Results	**Case-specific monitoring**
		– **LoE 1:** Partial compliance (97.6%) 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 788 complaints received in 2021 were attributed to loss of efficacy of maize MON 810 to provide protection against ECB/MCB damage.
		– **LoE 4:** The results of the diagnostic concentration assays indicated that the susceptibility of the MCB population damaging MON 810 plants in Girona was within the range of values reported in the last years in both MCB populations from north-eastern Spain and in the laboratory reference strains
		General surveillance
		– **LoE 5:** 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 6:** The consent holder indicated that the observation of unexpected damage by MCB larvae on MON 810 plants reported through professional networks was not indicative of reduced susceptibility to the Cry1Ab toxin in MCB populations of the region
		– **LoE 7:** 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 4 were integrated to conclude on resistance management strategies and insect resistance monitoring 2. LoE 5–LoE 7 were integrated to conclude on unexpected adverse effects due to the cultivation of maize MON 810 in the EU during the 2021 growing season
	Results	**Conclusions** *(Section 4)* – The monitoring strategy implemented in 2021 is not sensitive enough to detect the recommended 3% resistance allele frequency in ECB populations – The information reported in the 2021 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 2021 growing season – The consent holder reports for the first time MCB damage to MON 810 plants in an area of high and continuous maize MON 810 uptake – EFSA concludes that no new evidence has been reported in the context of the 2021 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. – The region of Girona where MCB damage to MON 810 plants was reported should be included in annual resistance monitoring, and remedial measures should be implemented to prevent further resistance evolution and spread – EFSA gives other practical recommendations on insect resistance monitoring, farmer questionnaires, existing environmental networks and literature searching that should be implemented by the consent holder in future reports *(Section 5)* – EFSA reiterates its previous recommendations to risk managers to implement mitigation measures to reduce exposure of NT lepidoptera to MON 810 pollen.

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