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STATEMENT

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Assessment of the 2022 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 2022 post-market environmental monitoring (PMEM) report on the cultivation of Cry1Ab-expressing maize event MON 810. Overall, the 2022 PMEM report provides no evidence of adverse effects of maize MON 810 cultivation. It shows a high level of compliance with refuge requirements by Spanish and Portuguese farmers growing maize MON 810, but uncertainty remains on compliance in areas where the clustered surface of maize MON 810 farms exceeds 5 ha. There are no signs of practical resistance to Cry1Ab in the field in corn borer populations collected in north-eastern Spain in 2022, although a decrease in Cry1Ab susceptibility in Mediterranean corn borer populations from this area cannot be excluded. Information retrieved through farmer questionnaires in Spain and from the scientific literature reveals no unanticipated adverse effects on human and animal health or the environment arising from the cultivation of maize MON 810. Uncertainties remain on whether 'very highly' and 'extremely' sensitive non-target lepidoptera are potentially exposed to harmful amounts of MON 810 pollen. EFSA notes that several recommendations made in the frame of the assessment of previous PMEM reports remain unaddressed and identified additional shortcomings in the 2022 PMEM report that require further consideration by the consent holder in future annual PMEM reports. Particularly, EFSA emphasises the urgent need to increase the sensitivity of the insect resistance monitoring strategy and implement mitigation measures to ensure that the exposure of non-target lepidoptera to maize MON 810 pollen is reduced to levels of no concern.

KEYWORDS

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

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SUMMARY

Following a request from the European Commission (Directorate-General for Health and Food Safety), the European Food Safety Authority (EFSA) assessed the 2022 post-market environmental monitoring (PMEM) report on the cultivation of the Cry1Ab-expressing maize event MON 810. This report presents the results of the 2022 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 assessment also considered: (1) the reports on the situation of teosinte in Spain provided to the European Commission by the Competent Authorities of Spain; (2) the publication Arias-Martín et al. (2024); and (3) a letter from the consent holder replying to the European Commission's inquiry on the measures in place to address the recommendations by EFSA on the PMEM of maize MON 810.

The case-specific monitoring data set consists of: (1) a farmer survey to assess the level of compliance with refuge requirements in areas of Spain where maize MON 810 was grown in 2022, as well as data on refuge compliance in Portugal reported by the Competent Authorities of that Member State; 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 expressed in maize MON 810.

Full compliance with refuge requirements is observed in Portugal, while the compliance levels with refuge requirements of Spanish farmers growing maize MON 810 was higher than in previous years. However, uncertainty remains on compliance in areas where clustered surface of small maize MON 810 farms exceeds 5 ha. 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.

Resistance monitoring data gathered through diagnostic bioassays with field-collected corn borers sampled during the 2022 maize growing season (which included populations collected in Girona for the first time) show that moult inhibition was lower than the expected >99% in the three MCB populations tested and in one of the two ECB populations tested. Owing to the unprecedented low values of moult inhibition recorded in the diagnostic assays, a decrease in Cry1Ab susceptibility cannot be excluded for MCB populations. Additional studies with plant material indicate 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 identified methodological and reporting shortcomings in resistance monitoring that need revision in future annual 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 2022, 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 test 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) consider the adequacy of the MCB laboratory strain as a comparator; (2) recalculate (and validate) the diagnostic concentration for MCB, and revise the range of concentrations used in concentration-response assays; (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.

No follow-up studies were performed in 2022 in the region of Girona where unexpected damage to maize MON 810 plants caused by MCB was reported in 2021. EFSA considers that extending annual resistance monitoring to Girona is insufficient to efficiently monitor resistance evolution in this area, as resistance alleles at levels capable of causing damage to MON 810 plants are potentially present. Additional monitoring activities should include the scouting of maize MON 810 fields for signs of MCB presence and damage, and the selection of a resistant population from individuals collected close to the location of unexpected damage. Additionally, the consent holder should strengthen grower education and communication activities in Girona. EFSA welcomes the disposition of the consent holder to revise the remedial action plan to address previous recommendations made by EFSA, including the clear definition of the events triggering the implementation of remedial measures and the specific actions included in the remedial action plan.

In EFSA's view, it is timely for the consent holder to perform an 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, an 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 allowing farmers to report complaints about product performance. According to the evidence provided in the 2022 PMEM report, no farmer complaints about product performance were reported through this system. Since the farmer complaint system has been implemented by the consent holder recently, 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 survey of Spanish farmers (based on 250 farmer questionnaires) and five relevant scientific publications published between June 2022 and May 2023. These 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 in Spain 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.
- The consent holder did not interview farmers growing maize MON 810 in Portugal, as the consent holder relied on the information reported by the Competent Authorities of Portugal, which is less tailored to the identification of potential unanticipated adverse effects. EFSA strongly encourages the consent holder to re-apply the use of the farmer questionnaire in Portugal. This will ensure that the same information on potential unanticipated adverse effects associated with the cultivation of maize MON 810 is collected in a harmonised manner across the two Member States.
- 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.
- To ensure that all new scientific evidence on (EU) teosinte that is relevant for the environmental risk assessment and risk management of maize MON 810 is retrieved and considered appropriately, the consent holder should integrate teosinte as an additional search term in the search strategy.

The 2022 PMEM report on the cultivation of maize MON 810 provides no new evidence on teosinte. Therefore, uncertainty remains about the plausibility of some of the assumptions about the completion of the postulated pathways to harm made in EFSA's 2016 technical report and 2022b statement on teosinte. While Arias-Martín et al. (2024) indicate that the hybridisation potential between maize MON 810 and teosinte found in Spain can be greater than assumed previously, more research is needed to assess this potential under real-life conditions. Likewise, additional investigations are needed to assess whether the acquisition of Cry1Ab by teosinte and its expression in teosinte/maize hybrids would increase the persistence and invasiveness potential of such plants and raise concerns for target organisms and non-target organisms. This evidence would enable to further test specific risk hypotheses of the devised pathways to harm and confirm previously made environmental risk assessment and risk management assumptions. To further reduce the likelihood of environmental harm to occur through the postulated pathways to harm, comprehensive weed management measures, including restrictions on the cultivation of maize (including maize MON 810), as implemented in Spain must continue to be applied in infested agricultural areas to control and/or eradicate teosinte.

Although requested by EFSA Guidance, information from existing environmental monitoring networks was not retrieved and analysed in the 2022 PMEM report. EFSA acknowledges that the integration of information from existing environment networks entails several methodological challenges. Therefore, it is important that the Competent Authorities in concerned EU Member States (mostly Spain and Portugal), the consent holder and representatives of environmental networks engage into a dialogue on how to develop and implement a methodological framework to identify and report unexpected adverse effects from the cultivation of maize MON 810 varieties.

Owing to the lack of information on the implementation of isolation distances between maize MON 810 fields and protected habitats, which are tailored to reduce the exposure of non-target lepidoptera to maize MON 810 pollen to levels of no concern, EFSA cannot conclude on whether non-target lepidopteran species in the 'very highly' and 'extremely' sensitive categories are exposed to harmful amounts of maize MON 810 pollen. Therefore, EFSA reiterates its recommendation to risk managers to consider the implementation of isolation distances that are proportionate to maize MON 810 adoption levels and consistent with the applicable protection goals in their jurisdictions.

Overall, none of the evidence reported in the 2022 PMEM report invalidates previous EFSA and GMO Panel evaluations on the safety of maize MON 810. However, uncertainties remain on: (1) whether 'very highly' and 'extremely' sensitive non-target lepidoptera are potentially exposed to harmful amounts of MON 810 pollen; and (2) whether Cry1Ab susceptibility has decreased in MCB populations from north-eastern Spain. Moreover, several of EFSA's recommendations made in the frame of the assessment of previous PMEM reports remained unaddressed, while EFSA identified additional shortcomings in the 2022 PMEM report that require further consideration by the consent holder in future annual PMEM reports. For example, EFSA notes that the insect resistance monitoring strategy in place lacks the required sensitivity. The sensitivity of the current strategy for assessing Cry1Ab susceptibility should be increased, which can be achieved by replacing it with a more sensitive test method, such as F_2 screens. Both the unprecedented low Cry1Ab susceptibility recorded in MCB populations from north-eastern Spain, and the unexpected damage by MCB to maize MON 810 reported in 2021 in Girona reinforce the urgency to use a more sensitive monitoring method and amend the remedial action plan. Therefore, the consent holder is strongly encouraged to strive to meet EFSA's recommendations made in previous and the current assessment(s).

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 2022, maize MON 810 was cultivated in Spain (67,620 ha) and Portugal (2290 ha) over a total area of 69,910 ha (DGAV, 2022a; MAPA, 2022). This implies a decrease of around 30% compared to the 100,927 ha of maize MON 810 grown in the previous season.

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 EU 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). This IRM plan was updated in 2023 (Appendix 6).[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, resistance evolution and refuge compliance are typically monitored 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 strategy for resistance monitoring. This strategy 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 adverse effects associated with the commercial use of GM plants that were not anticipated in the environmental risk assessment (ERA). 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 activities (including insect resistance monitoring) 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 2009–2021 growing seasons (EFSA, 2018, 2019a, 2020, 2021, 2022a, 2023; EFSA GMO Panel, 2011a, 2012a, 2013, 2014a, 2015a, 2015b, 2016, 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 that of conventional maize. However, EFSA noted

[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 1 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) not very low initial frequency of resistance alleles (> 0.001); and (6) lack of fitness costs/recessive fitness costs of the resistance (Huang, 2021).

several shortcomings in the methodology for both case-specific monitoring and general surveillance, and made several recommendations to improve PMEM activities and reports on maize MON 810 (see also EFSA, 2015a for further recommendations on IRM). Some of EFSA's recommendations on insect resistance monitoring were included in the updated IRM plan (Appendix 6).

1.1 | Terms of Reference as provided by the requestor

On 16 November 2023, the European Commission (Directorate-General for Health and Food Safety) received from the consent holder the annual PMEM report for the 2022 growing season of maize MON 810 (hereafter referred to as the '2022 PMEM report'). The reporting period of the 2022 PMEM report covers July 2022 until June 2023.

On 30 January 2024, the European Commission (Directorate-General for Health and Food Safety) mandated that EFSA *'evaluates 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.'*

On 4 April 2024, the European Commission (Directorate-General for Health and Food Safety) requested EFSA to consider the reports submitted by the Spanish Competent Authority on the situation with teosinte in Spain in 2023 in the frame of this mandate.

On 28 May 2024, the European Commission (Directorate-General for Health and Food Safety) asked EFSA to consider a new publication on the potential hybridisation between maize MON 810 and teosinte found in Spain (i.e. Arias-Martín et al., 2024) and the clarifications on the measures taken by the consent holder to address the recommendations made in EFSA (2023) in the frame of this mandate.

2 | DATA AND METHODOLOGIES

2.1 | Data

In delivering this statement, EFSA considered the information provided in the 2022 PMEM report,[6] comments submitted by the EU Member States, the reports submitted by Spain on the situation of teosinte in Spain in 2023, the publication of Arias-Martín et al. (2024) and the letter from Bayer replying to the European Commission's request for information on the measures in place for the implementation of the recommendations in EFSA (2023). Additional information on alerts on environmental issues, literature searches and case-specific monitoring was provided by the consent holder upon EFSA's request.

2.2 | Methodologies

Following Annex VII of Directive 2001/18/EC and the terms of reference of the mandate, EFSA assessed the evidence contained in the 2022 PMEM report and appraised the methods used for the monitoring activities.

EFSA considered the principles described in its guidelines for the PMEM of GM plants (EFSA GMO Panel, 2011b). EFSA also assessed the consent holder's systematic literature search following the relevant principles and criteria outlined in EFSA (2010) and the recommendations given in EFSA (2019b).

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

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

3 | ASSESSMENT

3.1 | Case-specific monitoring

3.1.1 | Compliance with refuge requirements[7]

3.1.1.1 | *Consent holder's assessment*

In 2022, 250 farmers from Spain completed a questionnaire that included the following question on compliance with the refuge strategy: *Did you plant a refuge in accordance to the technical guidelines?* All the maize MON 810-growing farmers

[6]The 2022 PMEM report is publicly available at https://food.ec.europa.eu/document/download/8319bc81-9113-44bc-b389-e769728b34c5_en?filename=gmo_rep-stud_mon-810_report-2022.pdf.

[7]2022 PMEM report: section 3.2.1.2; Appendix 1.

surveyed stated that they complied with refuge requirements, either because they implemented a refuge (183 farmers) or because they planted less than 5 ha of maize MON 810 and thus were not required to plant a refuge (67 farmers) (Appendix A).

Farmers growing maize MON 810 in Portugal were not interviewed by the consent holder in the growing season 2022 due to the low percentage of maize MON 810 grown (around 4% of the total maize area) and the availability of data on refuge compliance from other sources. The consent holder relied on the results of inspections performed by the Competent Authorities of Portugal in 21 farms where maize MON 810 was grown (out of the 69 maize MON 810 cultivation notifications registered in 2022) (DGAV, 2022b). Based on the results of these inspections, the Portuguese authorities concluded that there is full compliance with the implementation of refuge areas.

Based on the compliance monitoring data, the consent holder concluded that '*the results from the overall surveys (Portuguese authorities and Bayer) during the 2022 season 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*

Higher compliance with refuge requirements was reported in Spain compared to previous years, as all farmers growing over 5 ha of maize MON 810 did plant a refuge (Appendix A). However, uncertainty remains on compliance in areas where the clustered surface of maize MON 810 farms exceeds 5 ha. Approximately 27% of the surveyed Spanish farmers did not plant refuges due to the reduced area grown to *Bt* maize, but these farms might make part of areas where the clustered area of individual small farms is higher than 5 ha. For such areas, EFSA considers that refuge requirements also apply, irrespective of individual field and farm size (EFSA, 2009). Future annual PMEM reports should report whether small fields are in clustered areas where the total surface of maize MON 810 exceeds 5 ha and thus whether community refuges were implemented in those cases.

In Portugal, the consent holder interviewed no farmers. Data from inspections carried out by Portuguese authorities suggest full compliance with refuge requirements in Portugal, as observed in previous years. However, no information was collected via these surveys on the percentage of farmers that did not plant a refuge due to a surface cultivated to maize MON 810 being less than 5 ha. EFSA disagrees with the initiative of the consent holder to limit farmer questionnaires to farmers of Spain. It must be noted that, while the report published by the Competent Authorities of Portugal describes whether refuge areas have been grown, it does not report whether farmers would be exempt of planting a refuge due to cultivation of less than 5 ha of maize MON 810. EFSA considers that the same farmer questionnaire should be used to interview farmers growing maize MON 810 in both Spain and Portugal.

EFSA reemphasises the need to ensure full compliance with refuge requirements in areas where the uptake of maize MON 810 is high, such as north-eastern Spain, regardless of the size of individual fields. To this end, EFSA recommends that the consent holder, EU Member States where maize MON 810 is cultivated and other relevant stakeholders should strengthen farmers' awareness of refuge compliance and develop adequate information systems on GM crop cultivation (e.g. GMO registers) 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 2022 resistance monitoring activities focused on north-eastern Spain, around the Ebro basin, where the uptake of maize MON 810 was on average around 60% in the last years (Appendix B) and covered the two corn borer pests ECB and MCB. In 2022, Girona served as a sampling zone in the annual resistance monitoring activities for the first time. According to the consent holder, the decision to include Girona in the annual resistance monitoring activities was based on the high maize MON 810 uptake recorded in the region since 2016.

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 2022 PMEM report is presented in Table 1.

[8]2022 PMEM report: sections 3.2.1.3 and 3.2.1.4 and Appendixes 7 and 8; additional information provided on 17 May 2024.

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 2022 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 • 1536 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 • 3358 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 ($N=6805$) • 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 ($N=21{,}697$) • Neonates fed maize MON 810 leaves for 10 days • Susceptible reference population tested for comparison • Endpoint: Moult inhibition and survival (%)
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 ($N=27$) • 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 ($N=198$) • L_2 survivors fed maize MON 810 leaves for 10 days • L_2 survivors of susceptible reference population after DC assays tested for comparison ($N=28$) • 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 • 416 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 • 2260 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 ($N=18$) • 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. Nevertheless, the methodology that would have been followed in case larvae had moulted in Confirmatory assay Step I was not provided.

European corn borer monitoring

a. Field sampling and laboratory rearing

In 2022, 779 ECB late-instars from the last generation were collected at the end of the maize-growing season from three sampling sites (refuge areas or non-*Bt* maize fields) located in two zones across north-eastern Spain. Thirty-two additional sites were scouted, but either no ECB larvae were found or the minimum number of larvae established in the study protocol could not be reached for those 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 328 larvae reached the adult stage (42% of the field-sampled larvae) and were placed in 45 oviposition cages for mating. Thus, in the 2022 growing season, the estimated detection limit[9] for the recessive resistance alleles in ECB field populations was of 5.52%. 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 and the plant tissue 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;
4. Concentration-response assays with both susceptible reference populations (Table 1).

Diagnostic bioassay: The bioassay was conducted by exposing F_1 neonates to purified Cry1Ab protein at a diagnostic concentration of 28.22 ng Cry1Ab/cm^2 of diet surface area in an artificial diet overlay assay.[10]

In the bioassays for the 2022 growing season, 1536 neonates were tested against the diagnostic concentration. Two hundred and twenty 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, moult inhibition was below the expected 99% for Zone 1 (98.8%), whereas 99.28% of the larvae exposed to the discriminating concentration did not moult for Zone 2. On average, moult inhibition in NE Spain populations from NE Spain was of 99.04%. Moult inhibition in the control treatments was 3.65% and 0.69% for Zones 1 and 2, respectively (Table 2). These results are around the expected value of 99%, and they are slightly higher than those reported in the previous two growing seasons, but lower than those reported in the 2016–2019 growing seasons (Appendix C). For the two reference populations, moult inhibition at the diagnostic concentration was 100%, whereas at the control solution moult inhibition was 6.25% for ES Ref and 1.25% for G04.

TABLE 2 Moult inhibition of European corn borer (*Ostrinia nubilalis*) larvae at a diagnostic concentration of Cry1Ab protein: 2022 growing season [table based on data provided in the 2022 PMEM report]

		Treatment % Moult inhibition (Number of larvae tested)	
Population	**Sampling zone**	**Control**	**Cry1Ab[a]**
North-eastern Spain	**Huesca**	3.65 (144)	98.80 (896)
	Girona	0.69 (76)	99.28 (640)
	Total	2.17 ± 1.04[b,c] (220)	99.04 ± 0.17[b,d] (1536)
Laboratory reference strain	**ES Ref**	6.25 (80)	100 (160)
	G04	1.25 (80)	100 (160)

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

[b]Mean ± standard error.

[c]Of the 220 larvae tested, 3 larvae died and 2, 213 and 2 larvae moulted to the second, third and fourth instar, respectively.

[d]Of the 1536 larvae tested, 123 larvae died, 1386 larvae survived but did not moult to the second instar, and 27 larvae moulted to the second instar.

[9]A detection limit of 5.52% indicates that resistance alleles might not be detected in the susceptibility assays until they reached a frequency of 5.52% in the population.

[10]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, 2018). Batch 2d was used for the bioassays: 1.64 mg Cry1Ab/ml in 50 mM bicarbonate buffer; pH 10.25; 91% purity.

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, 6805 of the first instars not used in the diagnostic bioassays were fed maize MON 810 leaves (considered as a '*positive control*' of the diagnostic concentration bioassay). 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 and a mixture of agar and several components to prevent leaf degradation and microbial contamination (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 220 larvae fed non-*Bt* maize leaves placed on the same agar solution, was included in the study (considered as a '*negative control*' of the diagnostic concentration bioassay). Larvae from this control group were exposed individually to leaf discs.

All ECB larvae fed maize MON 810 leaves died within the exposure period. In the control group, 3.18% of the larvae died, whereas 96.82% of the larvae moulted to the second or third instar.

Confirmatory bioassay with maize MON 810 leaves: A follow-up study using maize MON 810 leaves was conducted with the 27 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 on maize MON 810 leaf discs. All larvae died within 7 days, but no information was provided by the applicant on whether any of the larvae moulted further in this period.

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). Larval mortality and moult inhibition were 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 2022 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 2022, 1967 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 23 additional sites, but the minimum number of larvae established in the IRM study protocol could not be reached for those 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 1199 larvae reached the adult stage (61% of the field-collected larvae) and were placed in 117 oviposition cages for mating. Emerging adults from the different sampling zones were kept separately. One hundred and seventeen cages, containing 1182 adults, were used to obtain F_1 progeny for the diagnostic bioassay (i.e. 60% of the field-collected larvae), which would represent a detection limit of 2.9%.

In addition, a population initiated from 800 larvae collected in 2020 from Galicia (north-western Spain), where *Bt* maize has never been grown, was used as an additional comparator in the diagnostic concentration and plant bioassays. This population was reared in the laboratory without any exposure to maize MON 810 or the Cry1Ab protein.

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);
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.[11] The reference population was also tested with the diagnostic concentration.

In the 2022 assays, between 1112 and 1128 larvae per sampling zone were exposed to 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.

[11]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%.

In the three sampling zones, corrected moult inhibition was lower than the expected 99% (89.16%–96.14%). In the control treatments, moult inhibition ranged between 5.10% and 12.84%. Corrected moult inhibition observed in the reference population was 96.77%, which is also below the expected 99% (see Table 3).

Average moult inhibition of the progeny of field-collected larvae (93.66 ± 2.25%) was significantly lower than the expected 99%, but the consent holder considers that such low moult inhibition is not indicative of resistance evolution as it is not significantly different from the values in the laboratory strain or from the expected 99%.

TABLE 3 Moult inhibition of Mediterranean corn borer (*Sesamia nonagrioides*) larvae at a diagnostic concentration of Cry1Ab protein: 2022 growing season [table based on data provided in the 2022 PMEM report].

		Treatment % Corrected moult inhibition (number of larvae tested)	
Population	**Sampling zone**	**Control**	**Cry1Ab**[a]
North-eastern Spain	**Huesca**	12.84 (148)	96.14 (1128)
	Girona	6.59 (167)	95.67 (1112)
	Navarra	5.10 (255)	89.16 (1118)
	Total	8.18 ± 2.37[b] (476)	93.66 ± 2.25[b] (3358)
Laboratory reference population		17.31 (104)	96.77 (1048)

Notes: Statistically significant differences were observed between the north-eastern population and the expected value of 99% ($t = 5.10$; $df = 2$; $p = 0.018$). No statistically significant differences were observed between the north-eastern population and the reference population ($t = 1.69$; $df = 2$; $p = 0.116$).

[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, 21,697 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 1120 larvae fed non-*Bt* maize leaves (approximately 10 larvae per oviposition 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 and 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 93.85% and 97.73%, whereas it was 96.92% in the reference population (see Table 4).

TABLE 4 Moulting to second instar of Mediterranean corn borer (*Sesamia nonagrioides*) neonates feeding on *Bt* (MON 810) or non-*Bt* maize leaves: 2022 growing season [table based on data provided in the 2022 PMEM report].

		Treatment % Moulting (number of larvae tested)	
Population	**Sampling zone**	**Non-*Bt***	**Bt**
North-eastern Spain	**Huesca**	93.85 (260)	0.0 (4980)
	Girona	95.71 (402)	0.0 (8318)
	Navarra	97.73 (430)	0.0 (8399)
Laboratory reference population		96.92 (260)	0.0 (5200)

Confirmatory bioassays: Experiments using maize MON 810 leaves were conducted with the 198 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. Six larvae from the three zones moulted to at least third instar and survived 10 days feeding on *Bt* maize (four of them from Huesca and one from each Girona and Navarra). When these larvae continued feeding on *Bt* leaves, two larvae from Huesca reached the fourth instar, but only one of them moulted to fifth instar upon further feeding with *Bt* maize. None of the larvae completed the larval stage and pupated.

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

- In the diagnostic concentration bioassay, 416 F_2 larvae were tested, with 18 larvae reaching the second instar (95.7% moult inhibition). These larvae did not survive after subsequently being fed maize MON 810 leaves for 10 days;
- In the maize leaf bioassays, none of the 2260 F_2 first instars moulted after feeding on maize MON 810 leaves for 10 days, while 99% 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). Moult 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 2022 and its CI 95% were 28 (20–38), which fall within the range of values estimated in previous years. Historical results of the concentration assays with the reference population are given in Appendix D.

Farmer complaint system

The consent holder states that, during the 2022 growing season, no complaints about the loss of efficacy of maize MON 810 against target pests were received via the farmer complaint system.

The consent holder refers to the outcome of a survey conducted by member companies of the National Breeder Association in Spain[12] selling maize MON 810 varieties, as they have the full overview of the farmer complaint schemes. None of the 614 complaints received by these companies in 2022 was attributed to the loss of efficacy of the *Bt* maize by corn borers.[13]

The consent holder also refers to regional monitoring networks that Spanish regional authorities have implemented for integrated pest management (IPM) (e.g. the Red Fitosanitaria Aragón,[14] north-eastern Spain; @RAIF_noticias in Andalucía,[15] southern Spain). These networks monitor and alert on incidence/outbreaks of agricultural pests and plant health issues, and they inform about IPM practices and resistance management. Such networks represent another tool that can be used by farmers and agronomic advisors to report any unusual observation, including product failure, which could be indicative of resistance evolution.

Follow-up on the report of MCB unexpected damage on MON 810 in a field trial

Unexpected damage to maize MON 810 plants caused by MCB was observed in October 2021 in a field trial in the province of Girona (north-eastern Spain). In the annual PMEM report corresponding to that growing season, the consent holder indicated that the observation was investigated following the steps outlined in CropLife Europe's IRM plan (Appendix 6), and concluded that, while population-level resistance had not evolved, there were signs of the presence of resistance alleles in the area of Girona. Moreover, the consent holder indicated that further studies with a larger population would be needed to confirm the preliminary results. The report of unexpected damage was considered an instance of suspected resistance, and as such it triggered the implementation of remedial measures in the area, including the addition of the area of Girona to the annual resistance monitoring plan (for more details see EFSA, 2023).

The information provided in the 2022 PMEM report confirms that Girona served as a sampling zone in the resistance monitoring activities of that growing season (Appendix 7). Moreover, upon EFSA's request, the consent holder confirmed that four MCB populations were collected from maize fields located 10 km within the area where unexpected damage was reported in 2021. Since no signs of resistance were detected in these populations when tested in diagnostic assays, the consent holder did not select a resistant population from this area. The consent holder indicated that additional recommendations to scout their *Bt* fields for signs of corn borer damage were not provided to farmers growing maize MON 810 in Girona, beyond the indication already contained in the Technical User Guide (TUG) for farmers to monitor their *Bt* fields. The only measure implemented to monitor resistance evolution in this region was the inclusion of Girona in the stewardship activities carried out by the Asociación Nacional de Obtentores Vegetales (ANOVE) in agreement with the consent holder, which focus on highlighting the importance of following good cultivation practices, including the cultivation of refuge areas.[16]

[12] Asociación Nacional de Obtentores Vegetales (ANOVE): https://anove.es/.

[13] One of the 614 complaints received in 2022 was initially reported to be related to maize MON 810 efficacy. This complaint was a misunderstanding, as it was confirmed that it was due to a mixing of conventional and MON 810 maize seeds.

[14] Red de avisos Fitosanitarios de Aragón: https://web.redfara.es/.

[15] https://twitter.com/raif_noticias?lang=en.

[16] Additional information provided on 17 May 2024.

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 that the two strains collected in 2019 and 2020 have similar susceptibility to Cry1Ab, as indicated by the results of separate susceptibility assays performed in 2021 with the same toxin batch.

In the 2021 PMEM report, the consent holder reported high MIC_{90} and broad 95% confidence interval values in the MCB reference strain. This, 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, indicates 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 have 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). In 2022, the MIC_{90} and its CI 95% obtained in the dose–response susceptibility bioassay of the MCB laboratory strain were within the historical range (Section 3.2 in Appendix 7). This points to lack of significant differences with historical susceptibility values. It must be noted that, due to the high variability historically reported in the MIC_{90} of the MCB reference strain, it is not unexpected that no significant statistical trend is observed over time. This could be attributed to the low statistical power of the test of difference. Consequently, during the assessment of the 2021 PMEM report, EFSA requested the consent holder to analyse the data using a test of equivalence instead of the test of difference. However, the consent holder did not address this recommendation in the 2022 PMEM report.

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.

Additionally, it must be noted that moult inhibition at the highest concentration tested in the dose–response susceptibility assays with the MCB laboratory population was around or below 90% in all three replicates. This compromises the fit of the regression line at the top end of the tested doses and adds uncertainty on the reliability of the MIC_{90} estimated. EFSA recommends revising the range of Cry1Ab concentrations used in the concentration-response assays to include concentrations exerting >99% moult inhibition in the laboratory population.

b. Field sampling and laboratory rearing

In line with the sampling scheme of the IRM plan implemented in the EU, the consent holder collected ECB and MCB larvae exclusively from two and three sampling zones in north-eastern Spain, respectively, in 2022. North-eastern Spain currently represents the only hotspot for resistance evolution in the EU, since around 60% of the total maize acreage of the area was cropped to maize MON 810 hybrids in the last years (Appendix B), and corn borer populations complete two generations annually in this area (Alfaro, 1972; Cordero et al., 1998).

Two of the sampling zones considered in 2022 (Huesca and Navarra) had been commonly sampled in the previous growing seasons, including fields located in the municipalities of Candasnos, Lanaja and Sariñena (both in Huesca) and Mendigorría (Navarra). Additionally, populations of the two target pests were collected in Girona (Cataluña) for the first time as part of the annual resistance monitoring programme. EFSA welcomes the consent holder's initiative to sample MCB and ECB populations in Girona due to the consistently high uptake of maize MON 810 in the region and the signs of the presence of resistance alleles in the MCB population collected in 2021 from damaged maize MON 810 plants in this area. Additionally, EFSA reiterates its recommendation to collect MCB populations from the zone where a resistance allele was detected in an F_2 screen performed in 2016 (Camargo et al., 2018).

In 2022, ECB and MCB populations were collected from non-*Bt* maize fields located within 10 m from the nearest maize MON 810 field, for those fields for which information on location is available (Appendix 7. Tables 3 and 4). In 32 out of the 35 (91%) and 23 out of the 35 (66%) sampling sites inspected in 2022, 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 1967 late-instar larvae collected in the 2022 growing season. For ECB, the target sample size could not be reached, as 779 larvae were collected.

Overall pre-imaginal mortality values during the laboratory rearing of field-collected individuals were high for both target pests: 58% and 39% 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 limit of 3% (recessive) resistance allele frequency in ECB, which is needed to detect a possible insurgence of field resistance timely. In 2022, the estimated upper bound of resistance allele frequency for ECB was 5.52%. 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 limit of 3% for the second year in a row (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) have succeeded to mate and produce viable offspring, and that offspring descending from all adults has been tested in the susceptibility assays, which cannot be demonstrated. To accurately determine the detection limit for resistance allele frequency, offspring descending from single pair crosses must be used in the susceptibility bioassays.

Additionally, as indicated in previous assessments, missing details about the rearing of the ECB field populations 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 7 in Appendix 7).

Once more, the target sample size of 2000 genomes was not reached for ECB, and ECB larvae were sampled from only two out of the three zones indicated in the CropLife Europe IRM plan (Appendix 6). EFSA acknowledges that it is a challenge to collate large amounts of larvae in practice 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 limit of 3%, which, in turn, would allow the early detection of signs of resistance evolution. To ease the detection of fields infested with ECB, the consent holder is strongly encouraged to: (1) strengthen the collaboration with field technicians from seed companies who scout maize areas, including areas that were not considered previously; (2) liaise more closely with the Competent Authorities of the concerned regions in Spain to optimise 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[17] and Gencat[18]; and (3) explore the potential use of the information gathered through the farmer complaint systems and the farmer questionnaires (e.g. in Appendix 1, six farmers reported high ECB infestation). Currently, the 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 (Appendix 3.1) does not ask farmers to report unexpected corn borer damage. Therefore, EFSA recommends the consent holder to encourage farmers from both countries where maize MON 810 is grown to actively and regularly inspect maize MON 810 fields to detect any potential unexpected damage to maize MON 810 plants caused by ECB and/or MCB as part of their communication and grower education activities. On this point, it is noteworthy to mention that the results of the farmer questionnaire (Appendix 1, section 3.1.4.2 Corn borer pressure) indicate there is a need to improve grower education, as most farmers reporting high corn borer pressure could not correctly tell apart the two target pest species (100% in the first wave 19/19, and 78.4% in the second wave 29/37).

Owing to the increasing difficulties to locate maize fields infested with the target pests in north-eastern Spain, EFSA reiterates the need to follow and implement an alternative more sensitive monitoring strategy (see Section '*Additional test methods*' below for more details).

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 strategy, but it expressed reservations on the actual implementation of this strategy 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, 2018, 2019a, 2020, 2021, 2022a, 2023). While the consent holder has been repeatedly invited to improve the IRM plan accordingly, and consider alternative test methods, the consent holder has implemented part of EFSA's recommendations only.

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 acknowledges the inclusion of susceptible reference populations in leaf tissue bioassays with ECB, a long-standing recommendation addressed for the first time in the 2022 PMEM

[17]https://web.redfara.es/?page_id=5808.

[18]https://agricultura.gencat.cat/ca/ambits/agricultura/dar_sanitat_vegetal_nou/avisos-fitosanitaris/.

report. As explained in previous statements (EFSA, 2022a 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%. No statistical comparison is needed, though, given that the DC was estimated as the upper limit of the 95% confidence interval of the MIC_{99}, and thus, it already covers the statistical variability (see proposed testing approach in Appendix E). This is further supported by the 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 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 field-collected MCB populations have been consistently below the expected 99% since the diagnostic concentration was first tested in the 2016 growing season (Appendix C), whereas in the laboratory susceptible strain, moult inhibition has only surpassed 99% twice in this period (Appendix D). 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 can reliably discriminate between homozygous resistant and susceptible individuals. To overcome this issue, the consent holder should recalculate the diagnostic concentration for MCB by, for instance, using data from bioassays in which only > 80% moult inhibition values were observed. This should consider data for MCB populations from different areas where maize MON 810 has been grown and Cry1Ab susceptibility has been evaluated, and thus not only those collected in the hotspot area of north-eastern Spain. The new diagnostic concentration should be validated with a susceptible population to prove that > 99% moult inhibition values are obtained.

Testing strategy: 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 96.24%, 95.67% and 89.16%, respectively. These values are lower than the expected > 99%, as happens with the average moult inhibition (93.62%). In fact, the average moult inhibition recorded in 2022 for MCB populations from north-eastern Spain was the lowest since diagnostic tests were first adopted in the growing season of 2016, suggesting the Cry1Ab susceptibility of MCB populations from this area could have decreased.

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 strategy to confirm suspected resistance by the stepwise approach recommended by the US Environmental Protection Agency (US EPA) for confirming resistance of lepidopteran pests of *Bt* plants (US EPA, 2010, 2018) in the corn borer resistance monitoring programme (Appendix E). This strategy allows to assess whether resistance is heritable and field relevant. In this respect, EFSA considers that the current IRM plan of CropLife Europe should be updated in line with US EPA's strategy, with each step taken to confirm resistance in a suspected population being described thoroughly. Furthermore, EFSA recommends the consent holder to explore the feasibility of replacing the current plant assays, which use leaf tissue to confirm whether the resistance is field relevant, 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 for two reasons. First, 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). Second, it would take into consideration the lower toxin concentrations expressed in stalks compared to leaves in maize MON 810 across phenological stages (Székács et al., 2010).

As previously stated, the monitoring strategy followed by the consent holder is not sensitive enough to allow for the detection of the recommended 3% resistance allele frequency. This underlines the need to increase the sensitivity and precision of the monitoring strategy. Increased sensitivity and precision are key for a timely implementation of remedial measures to delay resistance evolution for both target pests. As indicated in EFSA (2019a), 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 test methods. The consent holder has repeatedly highlighted that it is challenging to find sampling sites with enough corn borer larvae, and to reduce the mortality of field-collected individuals before laboratory testing, despite the results achieved for MCB this year. Therefore, in EFSA's view, using a more sensitive method would be the best way forward to increase the sensitivity of the monitoring strategy (see below *Alternative test methods*).

Bioassays with plant tissue: The consent holder conducted supplementary bioassays with ECB and MCB larvae surviving the diagnostic concentration and moulting to second instar, and with neonates that were not used in the bioassays. These larvae and neonates were fed maize MON 810 leaves. These assays aim to verify whether resistant individuals are present in the field-collected populations. It must be noted that the methodology provided by the applicant for the leaf tissue confirmatory assays with ECB populations is incomplete, as it does not specify the endpoint considered and the duration of the bioassay. These missing details must be provided. EFSA recognises the value of conducting studies with plant material, but it considers that such bioassays 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 for confirming resistance is to test whether a detected reduction in susceptibility (e.g. susceptibility lower than expected) is reproducible and heritable. To this end, 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 strategy only assesses susceptibility in the offspring of the siblings of those individuals that moulted when exposed to the diagnostic concentration and that managed to moult again when subsequently fed with maize MON 810 leaves, despite being greatly weakened. This strategy does not enable confirming whether a suspected decrease in susceptibility is heritable and may underestimate the presence of resistant individuals. In spite of these limitations, heritable lower susceptibility to Cry1Ab was observed in MCB in 2022, as average moult inhibition of all three sampling zones was below 99% in F1 and F2 offspring of the MCB field-collected populations (93.62% in F1 offspring and 95.70% in F2 offspring of those F1 individuals moulting in diagnostic concentration assays).

Alternative test methods: EFSA advocates replacing the current monitoring strategy (which is primarily based on diagnostic concentration assays) by a more precise and sensitive test 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), could 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 strategy would yield more accurate estimations of the Cry1Ab susceptibility in field populations. To obtain adequate sensitivity for detecting Cry1Ab resistance alleles before they become widespread in target pest populations and lead 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, as: (1) 8 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 an 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. It must be noted that ECB populations with low but statistically significant Cry1Ab resistance have been recently identified in Canada for the first time (Smith & Farhan, 2023). New simulations with resistance evolution models must be run after each F_2 screen, 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, which, in turn, will help to decide when to conduct the next F_2 screen.

As indicated previously (EFSA, 2023), the consent holder could also consider the adequacy of conducting a modified F_2 screen (Santiago-González et al., 2023) to assess the frequency of resistance alleles to Cry1Ab in the target pests. This could be particularly interesting in case an MCB-resistant population is selected in the laboratory.

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 IRM 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. Unfortunately, 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. The consent holder is strongly recommended to provide maize MON 810 growers with a clear threshold of ECB/MCB damage that should trigger reporting of unexpected damage, as well as to incentivise the reporting of these instances. 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. While EFSA has previously requested the consent holder to give more details on the communication mechanisms and grower education programs that make part of the farmer complain system, no further detail has been provided. Therefore, EFSA reiterates its former recommendation for the consent holder to provide more detail on the farmer complaint system, as this may enable EFSA to evaluate whether farmers have enough information to efficiently detect and communicate in a timely manner any potential unanticipated adverse effect of maize MON 810.

EFSA also notes that most Spanish farmers who reported high corn borer pressure in their field(s) in the famer questionnaires could not distinguish between the two target pests. This underlines the need to improve grower education to increase the reliability of the farmer alert system for insect resistance monitoring. It is therefore key that adequate communication mechanisms and educational programs (e.g. field scouting techniques, identification of the two main target pests and characterisation of the damage caused by corn borers) are implemented to ensure the prompt and effective reporting of farmer complaints relevant for resistance monitoring.

While the regional monitoring networks mentioned by the consent holder currently do not address resistance evolution in target pests, the data gathered and published could be useful 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 used for resistance monitoring.

Follow-up on the report of MCB unexpected damage on MON 810 in a field trial

After the first notification of unexpected damage on maize MON 810 plants by MCB in Girona in 2021, EFSA suggested to select a resistant population from those individuals moulting and surviving the leaf tissue and confirmatory assays as a first step of the further studies indicated by the consent holder as necessary to confirm the presence of resistance alleles (EFSA, 2023). Regrettably, the consent holder confirmed that no follow-up studies whatsoever were performed with the MCB population collected from maize MON 810 plants in 2021, and the only follow-up activity to this incidence of suspected resistance was the inclusion of Girona in the annual resistance monitoring plan as Zone 2, including the collection of four MCB populations from maize fields within 10 km of the field where unexpected damage was reported.

As previously discussed, moult inhibition of MCB populations from Girona was below the expected 99% when tested in diagnostic assays. Since no information is available on the Cry1Ab susceptibility of MCB populations from this area before 2021, it is not possible to establish whether the high maize MON 810 uptake in the region over the last years has led to a decrease in the susceptibility of MCB populations to Cry1Ab in Girona.

EFSA strongly supports the inclusion of Girona in the annual resistance monitoring programme and welcomes the broadening of the geographic scope of ANOVE's stewardship activities to include farmers from this area. However, EFSA considers that the monitoring and communication activities reported by the consent holder are insufficient to efficiently monitor the situation of potential resistance evolution in the area. Monitoring activities should not be limited to the sampling of MCB populations from conventional maize fields in Girona. They should include the development and implementation of a protocol to collect MCB populations close to the field where unexpected damage was reported, considering both conventional maize fields and active scouting for signs of MCB presence and damage in maize MON 810 fields. Additionally, EFSA recommends the consent holder to increase efforts to select an MCB-resistant population from the area where unexpected damage was reported in Girona. EFSA also considers it insufficient to limit communication activities to the instructions provided to farmers as part of the TUG and the routine communication campaigns in which farmers are reminded of the importance of refuge implementation and encouraged to monitor *Bt* fields. EFSA recommends the consent holder to further alert the farmers in 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. Moreover, farmers must be instructed to regularly inspect maize MON 810 fields for signs of MCB damage, which in turn, should be promptly notified.

Crop Life Europe's IRM plan (Appendix 6) 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 IPM 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, none of the remedial measures taken in 2021 as a response to the report of unexpected damage in Girona were tailored to reduce the spread of resistance in the field. According to the consent holder, there was no need to reduce the spread of resistance in the field, mainly due to the lack of population level resistance and because the surrounding maize fields had already been harvested when the unexpected damage was reported.

EFSA considers that CropLife Europe's IRM plan should be amended, so that it clearly defines what is meant by 'suspected resistance' and proposes 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 they could be implemented already during the investigation of an incident of suspected resistance. Remedial or mitigation actions could include: stopping the sales/growing of maize MON 810 in the region where resistance has evolved/is evolving; intensifying grower education activities; implementing alternative pest control measures; and increasing refuge sizes or increasing insect resistance monitoring in the affected area (US EPA, 2010). EFSA welcomes the consideration by the consent holder of these recommendations in their ongoing evaluation of CropLife Europe's remedial action plan for its potential improvement.

3.1.2.3 | *Conclusions on insect resistance monitoring*

Diagnostic concentration bioassays with the progeny of the field-collected corn borer populations resulted in moult inhibition values lower than the expected > 99% in one of the two ECB populations and in 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. Since these additional studies mimic worst-case exposure conditions, it is unclear whether some of the individuals tested could have completed their larval cycle under realistic exposure (field) conditions. Therefore, a decrease in susceptibility to the toxin Cry1Ab in MCB populations from north-eastern Spain cannot be excluded, especially in the light of the incident of unexpected damage to maize MON 810 plants reported in north-eastern Spain in 2021.

Following the observation of unexpected damage to MON 810 plants by MCB in 2021 in Girona, resistance evolution was monitored in 2022 in MCB populations of this area exclusively by including Girona as part of the annual resistance monitoring. Monitoring activities must be continued and supplemented by notifying farmers growing maize MON 810 in the area of the need to closely and regularly inspect their *Bt* fields for signs of damage, to allow for an early detection and investigation of any additional instances of suspected resistance. Additionally, EFSA strongly encourages the consent holder to increase efforts to select a resistant population from MCB populations collected in this area, and to strengthen communication and grower education to increase awareness in farmers growing maize MON 810 in Girona.

The US EPA recommends following a stepwise approach for confirming resistance of lepidopteran pests of *Bt* plants. The consent holder is strongly encouraged to implement a similar approach and update the harmonised IRM plan accordingly.

Whenever resistance evolution is suspected, the consent holder must implement remedial measures that limit further spread of resistance.

Based on the estimated numbers of ECB and MCB field-collected larvae represented in the diagnostic concentration bioassays, the monitoring strategy implemented in the 2022 growing season was not sensitive enough to allow for detection of 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, which may have enabled to reach the detection limit in MCB diagnostic concentration assays. It is also noted that reaching the 3% detection limit in a consistent manner is challenging. This emphasises the need to use an alternative, more sensitive test 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 an 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 unprecedented low values of Cry1Ab susceptibility recorded in MCB populations from north-eastern Spain collected in 2022 reinforce the urgency to implement a more sensitive monitoring strategy.

3.1.3 | Assessment of the consent holder communication on the implementation of the recommendations on case-specific monitoring made in EFSA (2023)

In a letter sent to the European Commission, the consent holder rejects the viability of the options suggested by EFSA to increase the sensitivity of the resistance monitoring strategy (i.e. increasing the size of the populations tested in diagnostic assays, performing F_2 screens). The consent holder argues that it is practically not feasible to increase the sensitivity of diagnostic concentration assays by collecting larger field populations, and that performing F_2 screens would be disproportionate. EFSA notes that the consent holder does not elaborate on why F_2 screens are considered disproportionate. As previously discussed, the current strategy is not sensitive enough to allow for early detection of resistance evolution, on time for preventive measures to be put in place to avoid field resistance, which makes it necessary and urgent to implement a more sensitive approach. EFSA considers that in case it can be proved unambiguously that none of the options suggested above is feasible, the consent holder should propose an alternative viable method that allows to reach the required sensitivity. Regretfully, no alternatives have been proposed by the consent holder.

Additionally, the consent holder emphasises the value of the farmer complaint system as a tool to assist insect resistance monitoring. In this respect, the consent holder confirms its commitment to '*continue providing to EFSA a detailed information on the tool, including how proper communication mechanisms is established and fit-for-purpose growers educational programs are provided (…)*'. EFSA notes that further details about the tool had been asked by EFSA previously, but that this recommendation has not been followed up by the consent holder so far. The lack of additional information prevents EFSA from concluding on the usefulness of the farmer complaint system for both resistance monitoring and the detection of unanticipated adverse effects.

As previously indicated, EFSA considers that the incidence of suspected resistance in Girona requires additional follow-up actions that go beyond the inclusion of the area in annual resistance monitoring and the continuation of the stewardship activities to remind farmers growing maize MON 810 of the importance of refuge compliance (see EFSA's assessment of *Follow up on the report of MCB unexpected damage on MON 810 in a field trial*).

3.2 | General surveillance

3.2.1 | Farmer questionnaire[19]

3.2.1.1 | *Consent holder's assessment*

In the annual 2022 PMEM report, the consent holder provides a survey based on 250 farmer questionnaires completed by farmers in Spain, which accounted for most of the maize MON 810 grown in the EU in that year (96.7%). The consent holder considered unnecessary to interview farmers growing maize MON 810 in Portugal given that a small amount of maize MON 810 is grown there (4.3%), and that the Competent Authorities of Portugal carry out annual farmer surveys.

The 2022 PMEM report represents the 16th reporting year, with the completion of a total of over 4100 questionnaires since 2006.

The surveys, which were completed in two waves in July 2022 and February 2023, were performed in Spain by an external company with experience in agricultural surveys. The response rate was 48.4% in Spain (Table 5).[20]

TABLE 5 Farmers surveyed and maize MON 810 area monitored in 2022 through questionnaires [Table based on data provided in the 2022 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	250[a]	18.2	4550	67,620	6.7

Note: One hundred and eighty-nine farmers were from Aragón/Cataluña, 24 from Navarra, 23 from Extremadura, 8 from Castilla la Mancha, 5 from Andalucía and 1 from Castilla y Leon.

The questionnaire used in 2022 was substantially modified compared to the one used in the previous years. According to the consent holder, the aim of the revision was to address several of the recommendations made by EFSA in its assessment of past PMEM reports and to simplify the questions in some areas for which no new information had been provided by farmers in all previous reporting seasons.

The revised farmer questionnaire collects information on four specific areas: (1) general information on maize cultivation, cultivation area and local insect pest and weed pressure; (2) typical agronomic practices used in maize MON 810 compared to the conventional maize grown in the same farm; (3) susceptibility of maize MON 810 to non-target insect pests, maize MON 810 growth, development and yield and unusual observations in maize MON 810; and (4) implementation of specific measures for 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 250 questionnaires from the survey of farmers cultivating MON 810 in Spain during the 2022 growing season did not reveal any adverse effects that could be associated with the genetic modification in MON 810. (…) In conclusion, the results from the overall surveys (Portuguese authorities and Bayer) during the 2022 season show no adverse effects of MON 810 to human or animal health, or the environment*'.

3.2.1.2 | *EFSA's assessment*

In the 2022 growing season, the consent holder updated the farmer questionnaires to identify unanticipated adverse effects potentially caused by the cultivation of maize MON 810. The revised questionnaire addresses some of the recommendations previously made by EFSA. For example, farmers are requested to indicate which maize MON 810 and conventional maize varieties they grow and select one of the MON 810 and one of the conventional maize varieties grown to describe any potential differences between them. Additionally, farmers are inquired on the number of years of maize MON 810 cultivation. Moreover, the consent holder also implemented the recommendation to conduct interviews at the beginning of the growing season, so that farmers know upfront which observations they must pay attention to throughout the entire growing season.

EFSA agrees that it is appropriate to condense questions whose relevance has not been demonstrated by the information retrieved over the las 15 years into more general questions. However, EFSA notes that the revised farmer questionnaire omits several relevant questions, such as information on the surrounding environment and on the occurrence of wildlife in maize MON 810 fields compared to conventional maize fields. Questions addressing those points should be reintroduced in the farmer questionnaire.

The revised questionnaire does not address a number of observations made by EFSA in its previous statements (EFSA, 2022a, 2023):

[19] 2022 PMEM report: section 3.1.3.1 and 3.1.5.1 and Appendixes 1 and 2.

[20] The questionnaire was completed by 250 out of the 517 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 2022 (86 farmers); (2) they grew MON 810 in 2022 but refused to answer the interview (76 farmers); (3) they did not grow maize in 2022 (68 farmers); (4) they were absent or could not be localised (twenty farmers); (5) they were retired (seventeen farmers).

- No additional questions are included to gain a better understanding of the uptake of maize MON 810 cultivation on the farm (frequency of maize MON 810 in crop rotations, possible presence of borers), using objective measurable outcomes, whenever possible;
- No questions are tailored to gather information on the occurrence of any novel/emerging pest in maize, which could arrive, for instance, due to climate change or commodity trade (e.g. *Spodoptera frugiperda*), and which might affect maize MON 810;

In 2022, the consent holder interviewed farmers growing maize MON 810 in Spain but not in Portugal. For Portugal, the consent holder used the data published by the Competent Authorities of Portugal, with the ambition to replace the farmer questionnaire by the farmer surveys and inspections conducted by the Competent Authorities of this EU Member State.

EFSA considers that the farmer questionnaire cannot be replaced by the farmer complaint system at present. Inspections and farmer surveys currently carried out by the Competent Authority of Portugal are not sufficient, as they do not capture most of the specific information compiled by the revised farmer questionnaire. The farmer survey used in Portugal is mainly designed to assess the compliance with coexistence measures, which are compulsory for maize MON 810 growers, and collect information on the economic and agronomic balance perceived by farmers growing maize MON 810. It contains only one general question asking farmers to comment on any adverse effects they perceive could be associated with the cultivation of maize MON 810 (i.e. '*Have you detected any negative effects that you think you can associate with the cultivation of GM maize? (e.g. surge of alternative pests and diseases, effects on bees, birds, allergies, etc.)*'). Other information regarded as highly relevant to determine any potential unanticipated adverse effects (e.g. insect or corn borer pest pressure, weed pest pressure, IPM practices in MON 810 and conventional maize, including whether corn borer control practices were applied in either type of maize, efficacy of maize MON 810 to control the target pests, susceptibility of maize MON 810 to other insect pests, specific IRM measures implemented) is not gathered.

3.2.1.3 | *Conclusions on the farmer questionnaire*

Based on the evidence gathered through the 2022 farmer questionnaire, no unintended effects associated with the cultivation of maize MON 810 could be identified. However, the farmer questionnaire was completed by Spanish farmers only. The consent holder relied on the farmer survey conducted by the Competent Authorities of Portugal to obtain feedback from Portuguese farmers. This farmer survey is not designed to identify any potential unanticipated adverse effects associated with the cultivation of maize MON 810. Therefore, EFSA considers that the same farmer questionnaire should be used to interview farmers in all areas where maize MON 810 is grown, independently of any information on the same aspects collected by national or regional Competent Authorities.

EFSA agrees with some of the modifications introduced in the revised farmer questionnaire by the consent holder. However, the updated version still presents several limitations associated with the sampling frame and the adequacy of some of the questions (see Section 3.2.1.2).

The consent holder suggested to replace the farmer questionnaire by the farmer complaint system, and thus discontinue them. However, since insufficient information is reported about the current farmer complaint system, it is unclear whether this system is adequate. Theoretically, a farmer alert system could support both 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). Together with the use of existing environmental monitoring networks (see 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. At present, EFSA is of the opinion that the farmer questionnaire must remain in place in Spain and be reapplied in Portugal.

3.2.2 | Existing monitoring networks[21]

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 it concludes that these networks cannot establish a cause-and-effect relationship, as none of the identified networks (Smets et al., 2014) measure 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 existing monitoring networks as an early warning system

[21] 2022 PMEM report: section 3.1.3.3 and 3.1.5.3.

in the context of general surveillance: '*(1) technical constraints (e.g. delayed publication of monitoring data); (2) lack of public availability of (raw) data; (3) harmonisation between networks (e.g. data collection and processing)… In addition, the EFSA has published a scientific opinion on the use of EENs for PMEM reports based on internal expertise and a report issued by a contracted consortium (Henrys et al., 2014). EFSA's opinion concluded that "In compliance with these assessment criteria, several existing ESNs have been identified as potentially suitable for GS of GMPs subject to further examination. However, the EFSA GMO Panel also identified several limitations pertaining to ESNs such as limited data accessibility, data reporting format and data connectivity with GMO registers" (EFSA, 2014a)*'.

3.2.2.2 | *EFSA's assessment*

EFSA acknowledges the challenges of using existing monitoring networks to identify possible 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 plant 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 for GM plants in each EU Member State or region is a significant obstacle.

However, there are existing monitoring networks adapted to such an exercise (e.g. the Catalan Butterfly monitoring scheme (Lee et al., 2020)). Also, the purpose of EENs is not to identify cause–effect relationships. Instead, EENs 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 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 notes that such a strategy should include other agricultural practices and thus go beyond the monitoring of maize MON 810 or any other GM crop only.

Additionally, existing monitoring networks could be useful to monitor non-target lepidopteran pests, including secondary and emerging maize pests (e.g. *Mythimna unipuncta, S. frugiperda*) which have shown lower susceptibility to maize MON 810 or have developed resistance to it (Eizaguirre et al., 2010; Omoto et al., 2016). These non-target species could evolve resistance to maize MON 810, even if their risk is lower than that of target pests (EFSA GMO Panel, 2012b). Information on unexpected damage to and survival on maize MON 810 plants by these species could be identified via General Surveillance and complemented by reports from regional pest monitoring systems. These data should trigger investigations to assess the potential need for an update of the IRM plan for maize MON 810 to include case-specific monitoring for particular NTL pests, as needed.

EFSA encourages the European Commission, the consent holder, the National Competent Authorities and relevant stakeholders to discuss how to make best use of existing monitoring networks. As a starting point, it is suggested that the consent holder liaises with the competent authorities of those regions where maize MON 810 is grown to compile a list of existing monitoring networks that are active in the areas where GM maize is cultivated, along with an evaluation of their suitability to support general surveillance (based on the assessment criteria outlined under point 3 in EFSA GMO Panel (2014b).

3.2.2.3 | *Conclusion on existing monitoring networks*

Overall, EFSA considers that existing monitoring networks could be a useful source of information to identify unanticipated adverse effects, including those related to secondary and emerging non-target lepidopteran pests. Given the current methodological limitations to the use of data gathered by these networks, 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 to inform on the specific impact of maize MON 810.

3.2.3 | Literature searches[22]

3.2.3.1 | *Consent holder's assessment*

The consent holder performed a systematic literature search to find scientific publications relevant to the food, feed and environmental safety assessment of maize MON 810 and the Cry1Ab protein published between 1 June 2022 and 31 May 2023.

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

[22]2022 PMEM report: section 3.1.5.5 and Appendix 5; additional information received on 17 May 2024.

Altogether, 407 scientific publications were retrieved (excluding duplicates); 393 publications were excluded after rapid assessment for relevance, eight were excluded after detailed assessment, while one publication was considered of unclear relevance because it was not possible to determine whether the event used in the study was MON 810. Therefore, after applying the predefined eligibility/inclusion criteria, the consent holder identified five publications as relevant for the assessment of food, feed and/or environmental safety.

The consent holder evaluated the reliability of all relevant scientific publications and the implications of the findings reported for the risk assessment of maize MON 810. Based on these analyses, the consent holder concluded that none of the evidence retrieved would invalidate the initial risk assessment and management conclusions on maize MON 810.

3.2.3.2 | *EFSA's assessment*

A modified version of EFSA's critical appraisal tool for assessing the quality of extensive literature searches (EFSA, 2015b) was used to evaluate the systematic literature search performed by the consent holder. The critical appraisal tool integrates the relevant principles and criteria outlined in EFSA (2010) and the recommendations provided in EFSA (2019b).

The consent holder excluded seven scientific publications identified through the literate search from further assessment. The rationale provided to justify exclusion was that '*It is not a safety study on MON 810*'. In EFSA's view, a more detailed explanation should be provided to substantiate exclusion (see also EFSA, 2023).

The relevance of one of the scientific publications retrieved could not be ascertained as, based on the available information, it was no possible to determine whether the transformation event used was MON 810. EFSA notes that the consent holder did not contact the authors of the publication to clarify which transformation event was used in the publication. EFSA considers that the consent holder should contact authors of unclear publications to obtain the necessary information to assess the relevance of the publication for risk assessment and risk management.

The consent holder did not provide sufficient details on how the reliability (internal quality) of the relevant publications was evaluated.

The literature searches identified no scientific publications on teosinte that are relevant for the ERA and risk management of maize MON 810 cultivation. 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 that is relevant for the food, feed and environmental safety assessment of maize MON 810, as it could be used to confirm previously made ERA and risk management assumptions. To ensure that all new scientific evidence on (EU) teosinte that is relevant for the ERA and risk management of maize MON 810 is retrieved and considered appropriately, the consent holder should integrate teosinte as an additional search term in the search strategy.

3.2.3.3 | *Conclusions on literature searches*

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.

While the overall quality of the literature searches performed by the consent holder is acceptable, the consent holder did not follow the recommendations listed in EFSA (2023) for improving the quality of the literature searches for maize MON 810. Therefore, EFSA reiterates its previous recommendations urging the consent holder to implement them in future annual PMEM reports:

- 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.
- Teosinte must be integrated as an additional search term in the search strategy.

3.2.4 | Teosinte

3.2.4.1 | *Consent holder's assessment*

In the 2022 PMEM report on the cultivation of maize MON 810, the consent holder concludes that the general surveillance, including the farmer questionnaire and literature searches, did not identify any adverse effects attributable to the potential presence of teosinte in maize MON 810 fields in the 2022 growing season.

The consent holder reported to have taken note of EFSA's recommendations (EFSA, 2022b) to revise the farmer questionnaire (i.e. inclusion of the reporting of both the occurrence of teosinte and corresponding levels of infestation, consideration of all new scientific evidence on teosinte relevant for the ERA and risk management of maize MON 810).

3.2.4.2 | *EFSA's assessment*

Risk concerns have been expressed that maize MON 810 may hybridise with teosinte and teosinte/maize hybrid progeny (referred to hereafter as teosinte) in regions where they co-occur, possibly 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 strategy 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, 2022b). EFSA concluded that the completion of the pathways to harm requires a succession of rare events, of which the combined probabilities are very low. Since part of EFSA's assessment relied on plausible assumptions that could not be confirmed or rejected by the evidence available at that time (EFSA, 2022b), EFSA recommended that:

- The consent holder revises the farmer questionnaire to include the reporting of both the occurrence of teosinte and corresponding levels of infestation (see also EFSA, 2020, 2021, 2022a, 2023);
- The consent holder explicitly considers all new scientific evidence on teosinte relevant for the ERA and risk management of maize MON 810;
- The consent holder and the competent authorities of Spain continue to share relevant information on teosinte for regions where maize MON 810 and teosinte co-occur;
- Research/monitoring activities pertaining to teosinte be continued and expanded, as this will be critical for the generation of empirical data on teosinte that could be used to further test specific risk hypotheses of the devised pathways to harm and confirm previously made ERA and risk management assumptions.

EFSA observes that no specific information on the occurrence of teosinte and corresponding levels of infestation was collected with the farmer questionnaire for the 2022 growing season of maize MON 810. This is partially because the farmer questionnaire has not been revised to address EFSA's recommendations on teosinte (see EFSA, 2020, 2021, 2022a, 2023). While the farmer questionnaire includes a generic question designed to characterise the weed pressure in maize MON 810 fields, it does not mention teosinte explicitly as a relevant emerging weed. Explicitly mentioning teosinte would 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 (EFSA, 2020, 2021, 2022a, 2023).

The literature searches (see Section 3.2.3) identified no scientific publications on teosinte that are relevant for the ERA and risk management of maize MON 810 cultivation. 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 that is 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 pathways to harm and confirm previously made ERA and risk management assumptions. To ensure that all new scientific evidence on (EU) teosinte that is relevant for the ERA and risk management of maize MON 810 is retrieved and considered appropriately, the consent holder should integrate teosinte as an additional search term in the search strategy.

In line with the request of the European Commission, EFSA assessed the findings reported in the publication on teosinte by Arias-Martín et al. (2024). In this publication, Arias-Martín et al. (2024) provide new empirical evidence on vertical gene flow between maize MON 810 and teosinte found in Spain, complementing the evidence previously reported by Arias-Martín et al. (2019, 2022) in proceeding abstracts (see Table 6 for a high-level summary). Despite the small number of plants tested (15 in total) and the fact that teosinte/maize hybrids were obtained only in one of the 3 years in which hybridisation was attempted, for four out of the five plants tested, the evidence indicates that the hybridisation potential between maize MON 810 and teosinte found in Spain can be greater than assumed previously (data obtained under experimental conditions without pollen competition (e.g. Lohn et al., 2021)). Arias-Martín et al. (2024) show that teosinte/maize hybrids can produce viable seeds and give rise to plants that may be more vigorous (e.g. taller, thicker stem, more leaves) than teosinte when grown in pots. It is unclear how the reduced number of secondary stems observed in teosinte/maize hybrids and the reduced time for teosinte/maize hybrids to reach the reproductive phase would affect their reproduction compared to teosinte. Arias-Martín et al. (2024) also suggest that teosinte/maize hybrids express the Cry1Ab protein at similar levels than maize MON 810. EFSA notes that no evidence was provided by Arias-Martín et al. (2024) on whether the acquisition of Cry1Ab by teosinte and its expression in teosinte/maize hybrids would increase the persistence and invasiveness potential of such plants and raise concerns for target organisms and non-target organisms. Further research would be needed to assess such aspects, as well as the hybridisation potential between maize MON 810 and teosinte under real-life conditions. This evidence would enable to further test specific risk hypotheses of the devised pathways to harm and confirm previously made ERA and risk management assumptions (see above).

TABLE 6 High-level summary of the publication on teosinte by Arias-Martín et al. (2024)

Topic	Materials and methods	Results and authors' conclusions
Hybridisation experiments	– Crosses were performed between maize MON 810 (hybrid DKC6451YG; serving as pollen donor; ♂) and teosinte (serving as pollen recipient; ♀) found in Spain – Teosinte seeds were gathered from plants found in fields in Zaragoza (Aragón) – In 2019, 2020 and 2021, five maize MON 810 and five teosinte plants were placed together (1:1) in a cage closed with double mesh curtains to foster free pollination with pollen competition – Plants were grown in pots (with a 30-cm diameter) – Teosinte/maize hybrid seeds (at least 20%) were collected from each teosinte plant and sown/grown in a growth chamber. Cry1Ab expression was measured at 2–3 leaf stage using ELISA	– Teosinte/maize hybrids were obtained in one (2022) of the 3 years in which hybridisation was attempted, for four out of the five plants tested – Of the 450 seeds tested for germination in 2022, 63.8% germinated – The percentage of hybridisation between maize MON 810 and teosinte calculated on the basis of the number of seeds that germinated varied between 28.6% and 72.2% from plant to plant, with a mean of 37.9 ± 26.5%. When the percentage of hybridisation was calculated on the basis of the total number of seeds tested, the estimated minimum hybridisation was 21.3 ± 13.4% (20%–35.6%) – Over the 3 years, for the 15 plants tested, the mean rate of hybridisation between maize MON 810 and teosinte was 12.6% – Hybridisation rates between maize MON 810 (♂) and teosinte (♀) (when observed) are higher than previously reported
Phenotypic characterisation	– Part of the teosinte/maize hybrids expressing the Cry1Ab protein was grown in pots and subject to phenotypic characterisation – Parent plants (maize MON 810 and teosinte) were sown on 13 May 2022 and 10 May 2023, while the teosinte/maize hybrids were transplanted between 10 and 15 days later, following their identification, in each year – The phenotypic endpoints measured included: plant height; stem area between 3rd and 4th leaves; number of leaves; number of secondary stems, number of days to tasselling and the emergence of stigmas. In addition, growing degree days were calculated – Differences in phenotypic endpoints of parent plants and teosinte/maize in vegetative phase were analysed using a multivariate linear model (fixed factor: generation, covariate: cumulated growing degree days)	– Teosinte/maize hybrids produce viable seeds and plants that may be more vigorous (e.g. taller, thicker stem, more leaves) than teosinte. However, the reduction in secondary stems may affect the seed production of teosinte/maize hybrids compared to teosinte – The time to reach the reproductive phase is shortened in teosinte/maize hybrids, which might make it easier for them to cross-breed with maize due to synchronous flowering
Cry1Ab quantification	– A sample (8–13 mg) of teosinte/maize hybrids and maize MON 810 plants was taken of the central part of leaves 6, 7 and 8 when maize MON 810 plants where at the V8–V11 stage. At least five plants per year and generation were sampled – Quantification was done in duplicate by DAS ELISA using Agdia's Bt-Cry1Ab/Cry1Ac ELISA kit – Differences in Cry1Ab concentration in maize MON810 and teosinte/maize were detected using a mixed linear model to analyse the genotype factors (fixed), year (random) and their interaction	– Teosinte/maize hybrids express the Cry1Ab protein at similar levels than maize MON 810, ranging between 36.4 and 48.8 ng Cry1Ab/mg fresh leaf, compared to 46.8–51.4 ng Cry1Ab/mg fresh leaf for maize MON 810 – Cry1Ab concentrations in teosinte/maize hybrids vary dependent on environmental conditions

The Competent Authority of Spain supplied to the European Commission reports on teosinte monitoring performed in Aragón and Cataluña, and on the measures taken to control and eradicate teosinte. These reports suggest that:

- Field surveys are performed in Aragón and Cataluña to monitor the occurrence of teosinte in infested plots (including those previously cropped to maize MON 810) and neighbouring areas;
- Teosinte is mostly found in conventional maize fields;
- No hybridisation has been observed between maize MON 810 and teosinte plants, where they co-occurred. However, 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 reported, including the conclusion on the lack of hybridisation observed between maize MON 810 and teosinte under real-life conditions;
- Weed management measures, including restrictions on the cultivation of maize (including maize MON 810), continue to be applied in infested agricultural areas to control and/or eradicate teosinte.

3.2.4.3 | *Conclusions on teosinte*

The 2022 PMEM report on the cultivation of maize MON 810 provides no new evidence on teosinte. Therefore, uncertainty remains about the plausibility of some of the assumptions about the completion of the postulated pathways to harm made in EFSA's 2016 technical report (EFSA, 2016) and 2022 statement on teosinte (EFSA, 2022b). While Arias-Martín et al. (2024)

indicate that the hybridisation potential between maize MON 810 and teosinte found in Spain (when observed) can be greater than assumed previously, more research is needed to assess this potential under real-life conditions. Likewise, additional investigations are needed to assess whether the acquisition of Cry1Ab by teosinte and its expression in teosinte/maize hybrids would increase the persistence and invasiveness potential of such plants and raise concerns for target organisms and non-target organisms. This evidence would enable to further test specific risk hypotheses of the devised pathways to harm and confirm previously made ERA and risk management assumptions. To further reduce the likelihood of environmental harm to occur through the postulated pathways to harm, comprehensive weed management measures, including restrictions on the cultivation of maize (including maize MON 810), as implemented in Spain must continue to be applied in infested agricultural areas to control and/or eradicate teosinte.

EFSA observes that the consent holder did not follow some of its recommendations on teosinte listed in EFSA (2023). Therefore, EFSA reiterates these previous recommendations urging the consent holder to implement them for the next PMEM reports:

- The consent holder should revise the farmer questionnaire template to include the reporting of both the occurrence of teosinte and corresponding levels of infestation;
- The consent holder should integrate teosinte as an additional search term 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. This must include all evidence that enables to test specific risk hypotheses of the devised pathways to harm, and confirm ERA and risk management assumptions.

EFSA welcomes the actions that the consent holder and the Competent Authorities of Spain take to share relevant information on teosinte for regions where maize MON 810 cultivation may co-occur with teosinte and recommends they be continued.

Moreover, EFSA encourages that the research/monitoring activities pertaining to teosinte be continued and expanded.

3.2.5 | Non-target lepidoptera

3.2.5.1 | *Consent holder's assessment*

No specific information relevant for the assessment of potential adverse effects of maize MON 810 cultivation on non-target lepidoptera (NTL) is provided in the 2022 PMEM report.

3.2.5.2 | *EFSA's assessment*

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 NTL. EFSA's GMO Panel used the mathematical models developed by Perry et al. (2010, 2011, 2012, 2013) to quantify this risk for the *Bt*-maize events 1507, MON 810 and Bt11, deriving estimates of larval mortality (see EFSA, 2009; EFSA GMO Panel, 2011c, 2012c, 2015c). The GMO Panel (EFSA GMO Panel, 2011c, 2012c, 2015c) concluded that some NTL 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, 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 m between protected habitats, where sensitive NTL can be found, and the nearest maize MON 810 field. While this recommendation to risk managers is still valid wherever considered necessary and proportionate (in connection with the protection goals for NTL and the levels of maize MON 810 uptake in a given region), EFSA notes that the 2022 PMEM report provides no information on the implementation of isolation distances between maize MON 810 fields and protected habitats.

3.2.5.3 | *Conclusions on non-target lepidoptera*

In the absence of information on implementation of isolation distances between maize MON 810 fields and protected habitats, there is uncertainty on whether the cultivation of maize MON 810 in the EU in 2022 led to the exposure of hypothetical NTL species in the 'very highly' and 'extremely' sensitive categories potentially present in protected habitats to harmful amounts of maize MON 810 pollen. To ensure that exposure remains at levels of no concern, EFSA reiterates its recommendation to risk managers to consider the implementation of isolation distances that are proportionate to maize MON 810 adoption levels and consistent with the applicable protection goals in their jurisdictions. Alternatively, in case this measure is considered too burdensome and/or disproportionate, the consent holder could gather data or provide evidence to verify that no NTL species in the 'very highly' and 'extremely' sensitive categories with a larval period overlapping with maize MON 810 pollen shed are present in protected habitats within 20 m of maize MON 810 fields.

3.2.6 | Assessment of the consent holder communication on the implementation of the recommendations on general surveillance made in EFSA (2023)

In its letter to the European Commission, the consent holder claims that EFSA's recommendations pertaining to general surveillance are not substantiated, as they disregard the low level of risk posed by maize MON 810, as evidenced in the annual PMEM reports compiled by the consent holder during the last 15 years of maize MON 810 cultivation. EFSA does not support this claim. It considers that general surveillance is an important pillar of the PMEM of maize MON 810 that can help to detect potential unanticipated adverse effects arising from the cultivation of this GM maize event. Hence, a robust methodology for general surveillance is needed to effectively detect changes in human or animal health and the environment not foreseen in the ERA and potentially connect such changes to the cultivation of maize MON 810. EFSA considers that the general surveillance approach currently in place is not sufficient to efficiently identify unforeseen adverse effects. Therefore, it is important that the consent holder considers EFSA's recommendations to improve the adequacy of general surveillance.

4 | CONCLUSIONS

EFSA assessed the evidence from the 2022 PMEM report following a weight of evidence approach (see Appendix G). Overall, there is no evidence of adverse effects of maize MON 810 cultivation in the 2022 PMEM report. However, uncertainties remain on:

- The susceptibility to Cry1Ab of MCB populations from north-eastern Spain. Considering the unprecedented low Cry1Ab susceptibility reported for MCB in diagnostic assays in 2022, as well as the unexpected damage by the pest on maize MON 810 reported the previous growing season, a decrease in Cry1Ab susceptibility in MCB populations from north-eastern Spain cannot be excluded;
- Whether the cultivation of maize MON 810 in the EU in 2022 led to the exposure of hypothetical NTL species in the 'very highly' and 'extremely' sensitive categories potentially present in protected habitats to harmful amounts of maize MON 810 pollen.

Moreover, EFSA identified several methodological and reporting issues (see Section 5) that require further consideration by the consent holder in future annual PMEM reports.

5 | RECOMMENDATIONS

Several of EFSA's recommendations made in the frame of the assessment of previous annual PMEM reports remained unaddressed, while EFSA identified additional shortcomings in the 2022 PMEM report that require further consideration by the consent holder in future annual PMEM reports. Owing to the critical nature of some of EFSA's recommendations, it is imperative for the consent holder to strive to meet EFSA's recommendations made in previous and the current assessment(s). Failure to do so may compromise the safety of maize MON 810 cultivation for human and animal health and the environment.

These recommendations include, among other aspects, the need to:

- Increase the sensitivity of the monitoring strategy to ensure early detection of changes in Cry1Ab susceptibility that could be indicative of resistance evolution in north-eastern Spain. This could be achieved by replacing the current diagnostic tests by periodic F_2 screens;
- Reinforce monitoring and communication efforts in Girona to ensure efficient monitoring of potential resistance evolution in the area;
- Amend the current remedial action plan so that it clearly defines the events triggering its implementation and describes the specific measures to be put in place;
- Implement isolation measures or gather data to ensure non-target lepidoptera in the upper categories of Cry1Ab sensitivity that could be present in protected habitats are not exposed to harmful amounts of MON 810 pollen;
- Use the same farmer questionnaire to interview farmers growing maize MON 810 in Portugal and Spain.

A full list of recommendations made by EFSA in the frame of the assessment of annual PMEM reports for maize MON 810 cultivation, containing both recurrent and new recommendations, is provided in Table 7 below.

TABLE 7 Summary of EFSA's recommendations for future PMEM reports on maize MON 810.

Area	Section	Recommendation[a]	Recommended since	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	– Recurrent since EFSA (2018)	– Consent holder – Relevant National Competent Authorities – Other relevant stakeholders (e.g. farmer associations)
		– 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	– Recurrent since EFSA (2021)	
		– 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	– Recurrent since EFSA (2018)	
	ECB/MCB resistance monitoring (3.1.2.2)	Monitoring strategy – To increase the sensitivity of the monitoring strategy so that it achieves a detection limit of 3% resistance allele frequency in target pest populations (see below on 'testing')	– Recurrent since EFSA GMO Panel (2016)	– Consent holder and other relevant stakeholders
		Testing – To revise the range of Cry1Ab concentrations used in the concentration-response assays to include concentrations exerting > 99% moult inhibition	– New recommendation	– Consent holder
		– To recalculate and validate the diagnostic concentration for MCB	– Recurrent since EFSA (2021)	
		– To follow the stepwise approach recommended by the US Environmental Protection Agency for confirming resistance of suspected resistant populations (see Appendix E)	– Recurrent since EFSA (2021)	
		– To compare the moult inhibition obtained at the diagnostic concentration assays with the expected > 99% for each zone independently	– Recommended in EFSA (2015a)	
		– To replace annual diagnostic assays by more sensitive test methods (periodic F_2 screens on ECB and MCB populations in north-eastern Spain)	– Recurrent since EFSA (2018)	
		Reporting – To consider recommendations outlined in Appendix F of this statement when preparing the reports of bioassays	– Recurrent since EFSA (2018)	
		Investigation of MCB unexpected damage on MON 810 in a field trial – To monitor resistance evolution in MCB populations of the area of Girona, including sampling of MCB populations in conventional maize fields and scouting for damage in MON 810 fields	– First appeared in EFSA (2023)	– Consent holder
		– To alert farmers in the area of Girona of the potential presence of resistance alleles in MCB populations, and instruct them to scout for damage in MON 810 fields and promptly notify it	– New recommendation	
		– To define trigger points for the implementation of remedial actions	– First appeared in EFSA (2023)	
		– To describe the range of remedial actions to be implemented	– First appeared in EFSA (2023)	
	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 programmes exist ensuring the prompt and effective reporting of farmer complaints	– Recurrent since EFSA GMO Panel (2016)	– Consent holder – Competent authorities of concerned EU Member States
		– 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	– First appeared in EFSA (2023)	

TABLE 7 (Continued)

Area	Section	Recommendation[a]	Recommended since	Responsible for implementation
General surveillance	Farmer questionnaires (3.2.1.2)	– To report the occurrence of teosinte and teosinte hybrid plants and the corresponding level of infestation	– Recurrent since EFSA (2021)	– Consent holder
		– 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)	– Recurrent since EFSA (2021)	
		– To include a question to report on the occurrence of novel pests	– First appeared in EFSA (2023)	
	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 GMO Panel (2014b)	– Recurrent since EFSA (2021)	– Consent holder – Competent authorities of concerned EU Member States – Environmental networks active in the area of cultivation of maize MON 810
		– To implement a methodological framework enabling the use of EENs in the broader context of environmental monitoring	– Recurrent since EFSA (2019a)	
		– 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	– Recurrent since EFSA (2021)	
		– Explore the potential usefulness of Pest Monitoring Schemes to monitor the impact of secondary or emerging non-target lepidoptera pests which could lead to changes in the PMEM plan	– New recommendation	
	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	– Recurrent since EFSA (2021)	– Consent holder
		– Further information should be sought, if feasible (e.g. by contacting the authors), to enable publications to be included or excluded	– First appeared in EFSA (2023)	
		– Provide a more detailed justification for the reasons of discarding papers from further assessment	– Recurrent since EFSA (2022a)	
		– Include relevant information on teosinte in the literature search	– Recurrent since EFSA (2021)	
	Teosinte (3.2.4)	– Include and explicitly consider in future annual PMEM reports all scientific evidence relevant for the environmental risk assessment and risk management of maize MON810 in relation to teosinte	– Recurrent since EFSA (2021)	– Consent holder – Competent Authorities of areas affected by teosinte – Other relevant stakeholders (e.g. academia, public funding bodies)
		– Comprehensive weed management measures as implemented in Spain must remain in place in infested agricultural	– First appeared in EFSA (2023)	
		– Research/monitoring activities pertaining to teosinte be continued and expanded	– New recommendation	
	Non-target Lepidoptera (3.2.5)	– Implement mitigation measures to reduce the exposure of non-target lepidoptera to maize MON 810 pollen	– First appeared in EFSA (2023)	– Risk managers

Abbreviation: ECB: European corn borer; MCB: Mediterranean corn borer.

[a]Further details are provided in the respective sections of this statement.

6 | DOCUMENTATION PROVIDED TO EFSA

1. Letter from the European Commission, dated 30 January 2024, requesting EFSA to assess the annual PMEM report on the cultivation of maize MON 810 during the 2022 season provided by the consent holder.
2. Comments from the EU Member States on the 2022 PMEM report.
3. Reports on the activities for the monitoring and control of teosinte in Spain in 2023 provided to the European Commission by the Competent Authorities of Spain, forwarded to EFSA on 4 April 2024 to be considered in the frame of this mandate.
4. Additional information provided by the consent holder, dated 17 May 2024.
5. Letter from the European Commission, dated 28 May 2024, requesting EFSA to consider the publication Arias-Martín et al. (2024) and the letter from the consent holder in response to the European Commission's letter requesting it to indicate what measures are in place to implement the recommendations provided in EFSA (2023) in the frame of this mandate.

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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

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SUPPORTING INFORMATION

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

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APPENDIX A

Farmer compliance with refuge requirements in Spain between 2004 and 2022

[Table based on data provided in 2004–2022 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
2022	250	183	67	0	100	FQ

Abbreviation: NR, not reported.

Note: Shaded row corresponds to the annual PMEM report under assessment.

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

[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 2022

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	157,939	52.1
2022	61,935	160,135[c]	38.7
Mean 2016–2022	85,341	154,118	**56.0**
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	7700	34.9
2022	944	9055[c]	10.4
Mean 2016–2022	2800	8132	**34.0**
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	52,004	20.5
2022	3708	26,026[c]	14.2
Mean 2016–2022	15,846	55,772	**27.0**

[a]Source: https://www.mapa.gob.es/es/agricultura/temas/biotecnologia/omg/registro-publico-omg/superficie_cultivada.aspx.
[b]Avances de superficies y producciones de cultivos: https://www.mapa.gob.es/es/estadistica/temas/estadisticas-agrarias/agricultura/avances-superficies-producciones-agricolas/ (Accessed 29 March 2024).
[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 2008–2022 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*[d] (NR)	2.93/5.35*[,d] (NR)	NP
	2009	509	1	6.40 (5.32–7.75)	13.68 (10.77–20.02)	1.75* (NR)	1.43 (NR)	NP
	2011	382	2	1.79 (1.54–2.07)	4.19 (3.45–5.48)	0.61* (NR)	0.67 (NR)	NP
	2013	452	2a	2.48 (2.03–3.02)	5.41 (4.27–7.61)	1.26 (NR)	0.82 (NR)	NP
	2015	376	2a	2.12 (1.75–2.55)	5.43 (4.36–7.29)	0.53* (NR)	0.77 (NR)	NP
	2016	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]
	2022	779	2d	NP	NP	NP	NP	99.04 ± 0.17[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[†]	489	B1	22 (16–28)	188 (138–277)	1.1 (0.8–1.7)	1.6 (NR)	NP
	2011[†]	564	B2-1	20 (14–27)	135 (91–232)	2.2 (1.6–3.0)*	2.0 (1.3–2.9)*	NP
	2013[†]	742	B2-2	19 (14–25)	163 (108–287)	2.6 (2.0–3.4)*	3.4 (2.2–5.2)*	NP
	2015[†]	529	B2-2	17 (13–21)	84 (63–124)	0.6 (0.5–0.8)*	1.3 (0.9–1.8)	NP
	2016	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]
	2022	1967	B2-10	NP	NP	NP	NP	93.66 ± 2.25[f]

(Continues)

(Continued)

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

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

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

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

[a]Data provided by the consent holder confirmed that the Cry1Ab protein batches 1 and 2, 2 and 2a, 2b and 2c, 2c and 2d, B1 and B2-1, and B2-1 and B2-2 have similar insecticidal activity.

[b]50% and 90% moult 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.

APPENDIX D

Cry1Ab susceptibility of reference susceptible populations of *Ostrinia nubilalis* (ECB) and *Sesamia nonagrioides* (MCB) [Table based on data provided in the 2006–2022 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)	8.63 (6.07–14.62)	100
		2022	2d	3.21 (2.36–3.92)	6.39 (5.18–8.98)	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
		2022	2d	2.16 (1.56–2.94)	5.03 (3.59–8.94)	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
		2022	B2-10	28 (20–38)	158 (103–321)	96.77

(Continues)

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Note: Shaded rows correspond to values from the 2022 PMEM report.

Abbreviation: NP, not performed.

[a]50% and 90% moult 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.

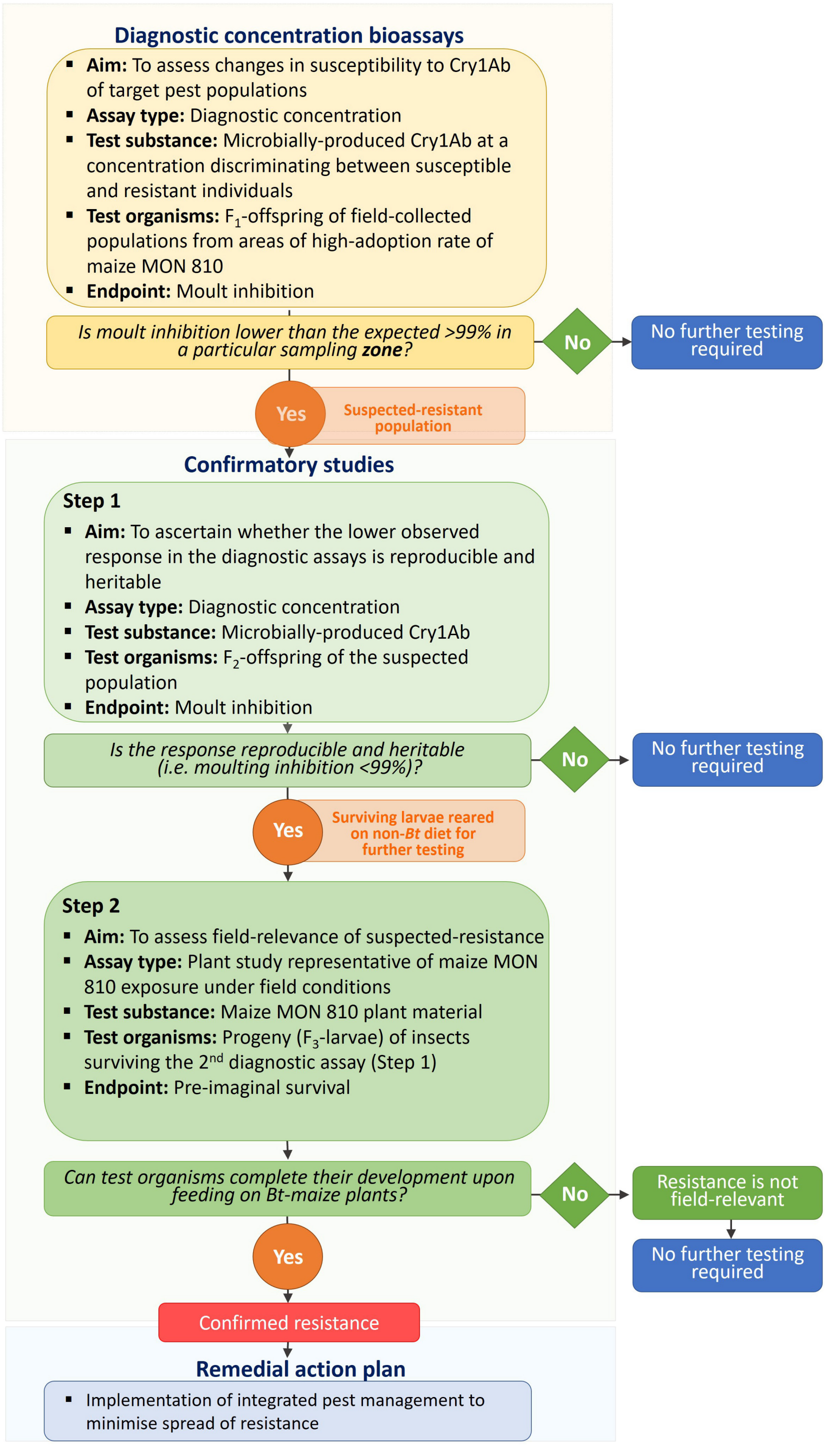
[f]The population was established in 2019 from larvae collected from Galicia (Spain), where *Bt* maize has never been cultivated.

[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).[23] Once resistance is confirmed, the CropLife Europe IRM plan foresees the implementation of remedial actions].



[23] 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. Environmental-controlled conditions (e.g. temperature, humidity and light regime) 30. Validity criteria of the study (e.g. mortality in the control group < 20%) 31. Blinding of personnel
Statistical design	32. Number of replicates for control(s) and test concentration(s); set-up of replicates (to avoid pseudo-replication) 33. Number of individuals tested per replicate 34. Treatment design (e.g. block, randomised) 35. Statistical method used 36. Statistical software used
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

Abbreviations: 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 2022 PMEM report, additional information provided by the consent holder on insect resistance management, farmer complaint system, literature searching, alerts on environmental issues and farmer questionnaires, comments provided by EU Member States, relevant scientific publications, reports on the situation of teosinte in Spain in 2023 and a letter from the consent holder replying to the request of the European Commission on the measures in place to implement recommendations in EFSA (2023) following a weight of evidence approach (EFSA Scientific Committee, 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 2022 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 2022 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 retrieved by the applicant – The scientific publication Arias-Martín et al. (2024) – Reports on the activities for the monitoring and control of teosinte in Spain in 2023 provided to the European Commission by the Competent Authorities of Spain – Letter from the consent holder replying to the request of the European Commission on measures in place to implement recommendations in EFSA (2023)
	Lines of evidence (LoE)	A summary of the evidence provided is as follows: **Case-specific monitoring** – **LoE 1:** Farmer compliance with refuge requirements. Consent holder surveys to 250 Spanish farmers growing maize MON 810 and 21 official inspections of the Competent Authorities of Portugal to Portuguese farmers growing maize MON 810 *(Section 3.1.1)* – **LoE 2:** ECB and MCB resistance monitoring *(Section 3.1.2)*: • Sampling of 779 ECB and 1967 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 2022 growing season *(Section 3.1.2)* – **LoE 4:** Follow-up on the observation of unexpected damage of MON 810 plants by MCB notified in 2021 in Girona *(Section 3.1.2)* **General surveillance** – **LoE 5:** Farmer survey based on 250 questionnaires received from farmers in Spain and 16 surveys to Portuguese farmers performed by the Competent Authorities of Portugal *(Section 3.2.1)* – **LoE 6:** Existing monitoring networks – **LoE 7:** Systematic literature search (1 June 2022–31 May 2023). Five food and feed, agronomic and environmental safety relevant publications were identified and assessed *(Section 3.2.3)* – **LoE 8:** Reports on the area infested by teosinte in Aragón and Cataluña, and identification of potential teosinte/maize hybrids – **LoE 9**: New evidence on potential for hybridisation between maize MON 810 and teosinte found in Spain, the phenology of teosinte/maize hybrids and the Cry1Ab expression in teosinte/maize hybrids
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**: Best professional judgement – **LoE 5:** The methodology of the revised 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) – **LoE 6:** Best professional judgement – **LoE 7:** The methodology of the search was assessed by best professional judgement considering the principles for literature searching laid down in EFSA (2010) and the recommendations given in EFSA (2019). A critical appraisal tool was used (EFSA, 2015b). The implications of each of the publications identified in the search were assessed by best professional judgement – **LoE 8:** Best professional judgement, considering the information on the situation of teosinte provided in previous growing seasons – **LoE 9**: Best professional judgement

(Continues)

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	Results	**Case-specific monitoring** – **LoE 1:** Full compliance with refuge requirements in Spain and Portugal was reported in the farmers questionnaires implemented in Spain and in the official inspections carried out by Portuguese Competent Authorities. No information is reported on compliance in areas where clustered surface of small maize MON 810 fields exceeds 5 ha – **LoE 2:** 1. ECB: MI of larvae tested against the DC was lower than the expected 99% in one of 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 the three sampling zones, and it was the lowest value ever recorded for MCB in north-eastern Spain. No resistant larvae were found in the follow-up/confirmatory bioassays with maize MON 810 leaves. – **LoE 3:** None of the 614 complaints received in 2022 were attributed to loss of efficacy of maize MON 810 to provide protection against ECB/MCB damage – **LoE 4:** No specific follow-up activities were carried out in the area of Girona beyond the inclusion of this zone in annual resistance monitoring and increasing communication to farmers on the importance of complying with refuge requirements **General surveillance** – **LoE 5:** No unanticipated adverse effects arising from the cultivation of maize MON 810 were identified in the assessment of the farmer questionnaires. The farmer surveys conducted by the CA of Portugal did not collect enough information to allow to conclude that cultivation of maize MON 810 did not cause any unanticipated adverse effects – **LoE 6:** No alerts were received through existing environmental networks – **LoE 7:** 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 8:** Monitoring and control efforts are routinely implemented in the Spanish areas affected by teosinte. The area affected by teosinte increased slightly in 2023 and no maize MON 810 – teosinte hybrids were identified in the field surveys – **LoE 9**: The results of a small-scale set of experiments indicate high rates of hybridization can occur between maize MON 810 and teosinte found in Spain under certain environmental conditions. Hybrids express Cry1Ab at levels comparable to the parental plant
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 9 were integrated to conclude on unexpected adverse effects due to the cultivation of maize MON 810 in the EU during the 2022 growing season
	Results	**Conclusions** *(Section 4)* – Overall, the information reported in the 2022 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 2022 growing season – The monitoring strategy implemented in 2022 is not sensitive enough to detect the recommended 3% resistance allele frequency in ECB populations from north-eastern Spain – A decrease in Cry1Ab susceptibility cannot be excluded for MCB populations from north-eastern Spain – The lack of data on the implementation of mitigation measures to limit exposure of non-target lepidoptera to harmful amounts of maize MON 810 pollen precludes EFSA from concluding on this point **Recommendations** *(Section 5)* – EFSA strongly recommends the consent holder to 1. Increase the sensitivity of the resistance monitoring plan 2. Perform a F_2 screen on European and Mediterranean corn borer populations from north-eastern Spain 3. Report all the relevant information on teosinte, including those derived from national monitoring programs, 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 carefully monitored for signs of resistance evolution, and remedial measures should be implemented to prevent further resistance evolution and spread – The farmers in this area of Girona should be alerted of the potential presence of resistance alleles in the area at levels capable of causing unexpected damage, and instructed to scout their maize MON 810 fields for signs of damage, which should be promptly reported – The harmonised IRM plan should be amended to clearly define the events triggering the implementation of remedial measures, and the specific actions included in the remedial action plan to be implemented – 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 – EFSA reiterates its previous recommendations to risk managers to implement mitigation measures to reduce exposure of NT lepidoptera in the upper level of Cry1Ab sensitivity to MON 810 pollen, or, alternatively, to gather data to confirm no such species occur in protected habitats close to MON 810 fields

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