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Annual post-market environmental monitoring (PMEM) report on the cultivation of genetically modified maize MON 810 in 2014 from Monsanto Europe S.A.

EFSA Panel on Genetically Modified Organisms (GMO)

Abstract

Following a request from the European Commission, the Panel on Genetically Modified Organisms of the European Food Safety Authority (GMO Panel) assessed the annual post-market environmental monitoring (PMEM) report for the 2014 growing season of maize MON 810 provided by Monsanto Europe S.A. The GMO Panel concludes that the insect resistance monitoring data do not indicate a decrease in susceptibility of field Iberian populations of corn borers to the Cry1Ab protein over the 2014 season. However, as the methodology for insect resistance monitoring remained unchanged compared to previous PMEM reports, the GMO Panel reiterates its previous recommendations for improvement of the insect resistance management plan. The GMO Panel considers that the farmer alert system to report complaints regarding product performance could complement the information obtained from the laboratory bioassays, but encourages the consent holder to provide more information in order to be in a position to appraise its usefulness. The data on general surveillance activities do not indicate any unanticipated adverse effects on human and animal health or the environment arising from the cultivation of maize MON 810 cultivation in 2014. The GMO Panel reiterates its previous recommendations to improve the methodology for the analysis of farmer questionnaires and conduct of the literature review in future annual PMEM reports on maize MON 810. The GMO Panel urges the consent holder to consider how to make best use of the information recorded in national registers to optimise sampling for farmer questionnaires, and requests to continue reviewing and discussing relevant scientific publications on possible adverse effects of maize MON 810 on rove beetles. Also, the GMO Panel encourages relevant parties to continue developing a methodological framework to use existing networks in the broader context of environmental monitoring.

Keywords: Cry1Ab, case-specific monitoring, farmer questionnaires, general surveillance, insect resistance management, *Zea mays*

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Correspondence: gmo@efsa.europa.eu

Panel members: Andrew Nicholas Birch, Josep Casacuberta, Adinda De Schrijver, Achim Gathmann, Mikolaj Gralak, Philippe Guerche, Huw Jones, Barbara Manachini, Antoine Messéan, Hanspeter Naegeli, Elsa Ebbesen Nielsen, Fabien Nogué, Christophe Robaglia, Nils Rostoks, Jeremy Sweet, Christoph Tebbe, Francesco Visioli and Jean-Michel Wal.

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Summary

Following a request from the European Commission, the Panel on Genetically Modified Organisms of the European Food Safety Authority (GMO Panel) assessed the annual post-market environmental monitoring (PMEM) report on the cultivation of maize MON 810 during the 2014 growing season provided by Monsanto Europe S.A.

The 2014 case-specific monitoring (CSM) dataset on maize MON 810 consists of a survey on compliance with non-*Bacillus thuringiensis* (non-Bt) refugia in Spain and Portugal, and dose–response and diagnostic dose bioassays to monitor for changes in susceptibility to Cry1Ab in target pests (European and Mediterranean corn borer) collected from Iberian populations. The 2014 PMEM report shows partial non-compliance with the implementation of non-Bt refugia in Spain as observed in previous years. Therefore, the GMO Panel recommends that the consent holder consolidates its efforts to increase the level of compliance, especially in regions of high maize MON 810 uptake, where such compliance is crucial to ensure the effectiveness of the high-dose refuge strategy. The analyses of the bioassays do not indicate a decrease in susceptibility to the Cry1Ab protein in the tested target pests from the populations monitored in 2014.

The methodology for insect resistance monitoring remained unchanged compared to previous PMEM reports. The GMO Panel reiterates its previous recommendations to improve of the insect resistance management plan of maize MON 810, in particular, the recommendations to: (1) set a minimum detection limit for resistance allele frequency at 3%, meaning that the minimum number of field larvae tested should be above *ca*. 1,000 in a given sampling area in those regions where the adoption rates of maize MON 810 are above 60%; (2) implement annual monitoring of bi-/multivoltine populations of both target pests in areas where adoption rate of maize MON 810 is at least 60%; and (3) monitor target pest populations exclusively from North East Iberia (i.e. the Ebro valley), where field resistance to Cry1Ab is more likely to evolve.

The consent holder put a farmer alert system in place allowing farmers to report complaints regarding product performance (including unexpected field plant damage caused by target pests). The GMO Panel cannot appraise the usefulness of the farmer alert system, which could complement the information received from the laboratory bioassays, and considers that more information should be provided to determine whether appropriate communication mechanisms and fit-for-purpose educational programmes are implemented that ensure the timely and effective reporting of farmer complaints.

The 2014 general surveillance (GS) dataset on maize MON 810 consists of a survey based on 261 farmer questionnaires, peer-reviewed publications relevant to the risk assessment and/or management of maize MON 810 (published between June 2014 and June 2015), and alerts on environmental issues issued by regulatory authorities and existing surveillance networks. To identify relevant publications not reported by the consent holder, the GMO Panel performed a literature search and assessed the relevance of retrieved scientific publications for the safety of maize MON 810. The available data do not indicate any unanticipated adverse effects on human and animal health or the environment arising from the cultivation of maize MON 810 during the 2014 growing season. Therefore, the GMO Panel considers that its previous conclusions on the safety of maize MON 810 remain valid and applicable. The consent holder is requested to continue screening, reviewing and discussing relevant scientific publications on possible adverse effects of maize MON 810 on rove beetles.

Methodological shortcomings similar to those found in previous annual PMEM reports on maize MON 810 were identified in the analysis of farmer questionnaires and conduct of the literature review. The GMO Panel therefore strongly reiterates its recommendations to provide more detailed information on the sampling methodology, reduce the possibility of selection bias in farmer questionnaires and ensure that all relevant scientific publications are identified. To improve the sampling frame of the farmer survey, the GMO Panel reiterates the importance of national GMO cultivation registers and its recommendations to consent holders to consider how they may make best use of the information recorded in national registers and foster dialogue with those responsible for the administration of these registers where maize MON 810 is cultivated.

No information collected from existing monitoring networks in the European Union (EU) was provided by the consent holder. However, the GMO Panel notes that initiatives have been taken to develop a methodological framework to use existing networks in the broader context of environmental monitoring and encourages relevant parties to continue to use these.

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1. Introduction

The transformation event MON 810 has been introduced into a wide range of maize varieties that have been cultivated in the European Union (EU) since 2003. Maize MON 810 produces the insecticidal protein Cry1Ab from *Bacillus thuringiensis* (Bt), which confers resistance to certain lepidopteran pests, such as the European corn borer (ECB), *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) and the Mediterranean corn borer (MCB), *Sesamia nonagrioides* (Lefebvre) (Lepidoptera: Noctuidae). In 2014, maize MON 810 was grown in Spain (137,537 ha), Portugal (8,542 ha), the Czech Republic (1,754 ha), Romania (711 ha) and Slovakia (411 ha) over a total area of approximately 143,015 ha.

According to Articles 13 and 20 of Directive 2001/18/EC[1], each notification for placing on the market of a genetically modified organism (GMO) shall contain a plan for monitoring in accordance with Annex VII of the Directive. Similarly, according to Articles 5.5(b) and 17.5(b) of Regulation (EC) No 1829/2003[2], each application for the placing on the market of a GMO or food/feed containing or consisting of that GMO shall be accompanied by a monitoring plan for environmental effects conforming with Annex VII to Directive 2001/18/EC. Annex VII was supplemented by notes providing guidance on the objectives, general principles and design of the monitoring plan.[3]

Results of post-market environmental monitoring (PMEM) activities on the cultivation of maize MON 810 in the EU are reported to the European Commission and the Member States on an annual basis by Monsanto Europe S.A. (hereafter referred to as the consent holder). Since 2010, the Scientific Panel on Genetically Modified Organisms of the European Food Safety Authority (hereafter referred to as GMO Panel) assesses these annual PMEM reports on the cultivation of maize MON 810 (EFSA GMO Panel, 2011a, 2012a, 2013, 2014a, 2015a,b).

1.1. Background and Terms of Reference provided by the requestor

The marketing of maize MON 810 (notification C/F/95/12-02) was authorised under Directive 90/220/EEC in the EU for all, other than food, uses by the Commission Decision 98/294/EC of 22 April 1998.[4] Consent was granted to the consent holder on 3 August 1998 by the Competent Authority of France. Food uses of maize derivatives were notified according to Article 5 of the Novel Food Regulation (EC) No 258/97 on 6 February 1998.

Following the request by the consent holder for the renewal of the authorisation for placing maize MON 810 on the market, the GMO Panel adopted a scientific opinion on the renewal under Regulation (EC) No 1829/2003 of maize MON 810 for: existing food and food ingredients produced from maize MON 810; feed consisting of and/or containing maize MON 810, including the use of seed for cultivation; and food and feed additives, and feed materials produced from maize MON 810 (EFSA, 2009). The GMO Panel concluded that *maize MON 810 is as safe as its conventional counterpart with respect to potential effects on human and animal health*, and that *maize MON 810 is unlikely to have any adverse effect on the environment in the context of its intended uses, especially if appropriate management measures are put in place in order to mitigate possible exposure of non-target (NT) Lepidoptera*. The GMO Panel recommended that *especially in areas of abundance of NT Lepidoptera populations, the adoption of the cultivation of maize MON 810 be accompanied by management measures in order to mitigate the possible exposure of these species to maize MON 810 pollen*. In addition, the GMO Panel advised that *resistance management strategies continue to be employed and that the evolution of resistance in lepidopteran target pests continues to be monitored, in order to detect potential changes in resistance levels in pest populations* (EFSA, 2009).

From 2005 onwards, the consent holder submitted to the European Commission its PMEM reports on the cultivation of maize MON 810 according to the provisions of Directive 2001/18/EC. These annual PMEM reports are composed of case-specific monitoring (CSM), to assess the efficacy of the 'high-dose/refuge' strategy, and general surveillance (GS), to detect unanticipated adverse effects caused by the cultivation of maize MON 810.

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[1] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 17.4.2001, p. 1–39.

[2] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23.

[3] Council Decision 2002/811 of 3 October 2002 establishing guidance notes supplementing Annex VII to Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 280, 18.10.2002, p. 27–36.

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

Since 2010, the European Commission requested the GMO Panel to assess the annual PMEM reports on the cultivation of maize MON 810 submitted by the consent holder. The GMO Panel therefore adopted scientific opinions on the 2009, 2010, 2011, 2012, 2013 and revised 2013 annual PMEM reports (EFSA GMO Panel, 2011a, 2012a, 2013, 2014a, 2015a,b). From the data provided in the previous annual PMEM reports, the GMO Panel did not identify adverse effects on human and animal health and the environment resulting from the cultivation of maize MON 810. However, the GMO Panel noted shortcomings in the methodology for CSM and GS, and made recommendations to improve future annual PMEM reports on maize MON 810.

On 22 May 2012, the European Commission requested EFSA to compile an inventory of existing environmental surveillance networks at the European level and at the National level, and develop a set of assessment criteria to support the selection of such networks for PMEM of GM plants. Following this request, an external open call was launched by the EFSA Unit for Assessment and Methodological Support (hereafter referred to as AMU Unit). The external report reviewed statistical methods used in the analysis of ecological and environmental datasets; provided an inventory of statistical approaches in ecological and environmental monitoring and identification of data requirements for the items in the inventory; provided an inventory of European, National and Regional existing surveillance networks/programmes; and gave recommendations of the most appropriate analysis methodologies for PMEM of agroecosystems (Centre for Ecology and Hydrology et al., 2014).

On 24 March 2015, the European Commission requested EFSA to assess the concerns raised by the consent holder about the GMO Panel recommendations on the insect resistance management (IRM) strategy for maize MON 810 (EFSA GMO Panel, 2011a, 2012a, 2013, 2014a, 2015a). EFSA concluded that the previous conclusions and recommendations by the GMO Panel remain valid (EFSA, 2015a).

On 2 September 2015, the European Commission received from the consent holder the monitoring report for the 2014 cultivation season of maize MON 810.

On 26 October 2015, the European Commission requested the GMO Panel to assess the 2014 monitoring report and, in particular, *to evaluate the findings of the monitoring activities, taking into consideration the comments received from the Member States and to assess the appropriateness of the methodology if this is found to differ compared to the previous season*.

On 18 December 2015, the National Committee of Biosafety of the Spanish Competent Authority sent in several considerations about EFSA's recommendations on the IRM plan for maize MON 810.[5]

On 21 January 2016, the consent holder was invited as a hearing expert to a meeting of the PMEM Working Group to provide clarifications on the methodology of the monitoring activities which are part of the IRM strategy of maize MON 810 and are described in the 2014 monitoring report.[6]

On 3 February 2016, the European Commission requested the GMO Panel to assess an additional scientific publication (Zeljenkova et al., 2014) forwarded by the consent holder to be part of the annual 2014 PMEM report of maize MON 810.

2. Data and methodologies

2.1. Data

In delivering this scientific opinion, the GMO Panel took into account the information on CSM and GS activities provided by the consent holder:

- an IRM plan,[7] which is based on the 'high-dose refuge strategy', and that includes (1) surveys on farmers' compliance with non-Bt refugia, (2) the monitoring for changes in baseline susceptibility of target pests and diagnostic dose[8] assays,[9] (3) a plan for communication with farmers, and (4) a remedial action plan in the event of any confirmed evolution of pest resistance;
- a survey based on 261 questionnaires received from farmers in two European countries:[10] 213 in Spain and 48 in Portugal;

[5] http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2015-00650 (Accessed: 4 March 2016).
[6] http://www.efsa.europa.eu/sites/default/files/gmopmemreports2016.pdf (Accessed: 4 March 2016).
[7] Annual 2014 PMEM report, Appendix 6.
[8] The diagnostic dose is the dose that causes 99% of moulting inhibition (MIC_{99}) to first instars.
[9] Annual 2014 PMEM report, Appendixes 7 and 8.
[10] Annual 2014 PMEM report, Appendix 1.

- an assessment of 26 peer-reviewed publications relevant to the risk assessment and/or management of maize MON 810, which were published between June 2014 and the beginning of June 2015;[11]
- company stewardship activities;[12] and
- alerts on environmental issues by regulatory authorities and existing surveillance networks.

In addition, the GMO Panel assessed additional relevant peer-reviewed papers published between June 2014 and the beginning of June 2015 that were not included in the annual 2014 PMEM report supplied by the consent holder.

2.2. Methodologies

Following the terms of reference of the EC mandate, the GMO Panel considered whether the methodology applied to monitor maize MON 810 during the 2014 growing season differs from that followed in the previous PMEM reports on maize MON 810 (EFSA GMO Panel, 2011a, 2012a, 2013, 2014a, 2015a,b).

The GMO Panel assessed the 2014 CSM and GS dataset on maize MON 810 (see Section 3).

In addition, the GMO Panel performed a systematic literature search to identify relevant scientific publications not reported by the consent holder, and subsequently assessed their relevance for the risk assessment and/or risk management of maize MON 810.

In its assessment, the GMO Panel also considered the comments from the Member States on the annual 2014 PMEM report.[13]

3. Assessment

3.1. Implementation of non-Bt refugia

The GMO Panel analysed the results of the farmer questionnaires addressing the implementation of non-Bt refugia.[14] The consent holder asked 261 farmers from Spain and Portugal, the two main EU countries where maize MON 810 was cultivated in 2014, to complete a questionnaire on the planting of non-Bt refugia. In Spain, 178 out of 189 farmers (94%) growing maize MON 810 planted non-Bt refugia. The 11 farmers that did not plant a refuge provided the following two reasons for their non-compliance (as indicated in the survey): (1) *the farmer had no or not enough information about the technical guidelines* (seven farmers) and (2) *the sowing is complicated by planting a refuge* (four farmers). In Portugal, all maize MON 810-growing farmers surveyed (48) confirmed to have planted non-Bt refugia. Full compliance with refugia requirements by Portuguese farmers was also reported in previous years.

The results of the Spanish farmer's survey of 2014 could not be corroborated by a study carried out by Antama,[15] the Spanish Foundation supporting the use of new technologies in agriculture, as the Antama survey was only conducted until 2012 (see Table 1).

In 2014, the Portuguese authorities performed inspections at 81 farms (out of the 238 notifications received in 2014) cultivating maize MON 810 to check compliance with the requirements for the cultivation of GM varieties outlined in Portuguese law. Based on the performed inspections, the Portuguese authorities concluded that there was full compliance with refuge implementation.

The 2014 PMEM report shows partial non-compliance with the implementation of non-Bt refugia in Spain as observed in previous years (Table 1). Therefore, the GMO Panel recommends that the consent holder consolidates its efforts to increase the level of compliance, especially in regions of high adoption rate of maize MON 810,[16] where such compliance is crucial to ensure the effectiveness of the high-dose refuge strategy. In this context, the education and training programme of the farming community in managing maize MON 810 as proposed by the consent holder[17] is essential to delay resistance evolution in target pests.

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[11] Annual 2014 PMEM report, Appendices 5.1-revised and 5.2.

[12] Annual 2014 PMEM report, Appendices 3.1–3.5.

[13] Comments were received from Austria, Germany, Hungary, Italy, Spain and the Netherlands.

[14] The harmonised IRM plan states that no refuge is required for farmers planting less than 5 ha of maize MON 810 in the farm.

[15] Spanish foundation supporting the use of new technologies in agriculture: http://fundacion-antama.org (Accessed: 4 March 2016).

[16] The adoption rate of maize MON 810 is expressed as a fraction of total maize cultivation in the same geographical area.

[17] Annual 2014 PMEM report, Section 3.2.1.3.

Table 1: Non-Bt refugia compliance by Spanish farmers between 2009 and 2014 from two sources

Growing season	No. farmers surveyed[(a)]	Compliance (%)	Source[(b)]
2009	93	91	FQ
	100	81	Antama
2010	142	91	FQ
	100	88	Antama
2011	140	96	FQ
	100	93	Antama
2012	154	84	FQ
	100	93	Antama
2013	190	87	FQ
2014	189	94	FQ

(a): For the FQ, only farmers who were required to plant a refuge were considered for the calculation of non-Bt refugia compliance.

(b): FQ: farmer questionnaires; Antama: Study conducted by Spanish foundation supporting the use of new technologies in agriculture between 2009 and 2014.

3.2. Susceptibility of Iberian field populations of *Ostrinia nubilalis* and *Sesamia nonagrioides* to Cry1Ab

3.2.1. Field sampling of target pests and laboratory assays

In line with EuropaBio's harmonised IRM plan, which aims to collect target pest larvae from three areas (i.e. North East, Central and South West Iberia) every 2 years, the ECB and MCB larvae were collected in 2014 from refuges and fields of conventional maize adjacent to maize MON 810 fields in two different geographical areas of the Iberian Peninsula (i.e. Central and South West Iberia). In accordance with the EuropaBio's harmonised IRM plan, no samples were collected in 2014 from North East Iberia, although this is the area with the highest adoption level of cultivation of MON 810 (Table 2).

A total of 353 ECB last-instars were collected from two fields in South West Iberia and 479 and 644 MCB last-instars were collected from two fields in Central Iberia and from five fields in South West Iberia, respectively (Table 3).

Dose–response and diagnostic dose laboratory assays were conducted to assess the susceptibility to the Cry1Ab protein of the ECB and MCB populations, using neonate larvae of the subsequent generation reared under laboratory conditions (F_1). The neonates were exposed to purified Cry1Ab protein. In addition, all surviving MCB larvae from both bioassays (1,650) and those neonates that were not used in the bioassays (*ca.* 3,000) were fed MON 810 leaves ad libitum for 12 days and their survival was assessed.

Table 2: Area and adoption rate of maize MON 810 in North East Iberia (Aragón and Cataluña, i.e. Ebro valley), Central Iberia (Albacete) and South West Iberia (Extremadura and Andalucía) between 2010 and 2014 from two sources

Season	Area maize MON 810 (ha)[(a)]	Source			
		Avances[(b)]		ESYRCE[(c)]	
		Total maize (ha)	Adoption rate (%)	Total maize (ha)	Adoption rate (%)
North East Iberia					
2010	56,910	93,162	61.1	124,386	45.8
2011	71,000	113,299	62.7	125,697	56.5
2012	75,200	108,621	69.2	107,564[(d)]	69.9
2013	88,447	125,293	70.6	119,859[(d)]	73.8
2014	90,421	126,628[(e)]	71.4	141,218	64.0
2010–2014	–	–	**67.0**	–	**62.0**

Season	Area maize MON 810 (ha)[(a)]	Source			
		Avances[(b)]		ESYRCE[(c)]	
		Total maize (ha)	Adoption rate (%)	Total maize (ha)	Adoption rate (%)
Central Iberia					
2010	2,695	11,005	24.5	12,455[(d)]	21.6
2011	5,041	15,718	32.1	15,967[(d)]	31.6
2012	6,453	17,701	36.5	19,297[(d)]	33.4
2013	6,564	16,950	38.7	20,698[(d)]	31.7
2014	5,696	14,700[(e)]	38.8	16,585[(d)]	34.3
2010–2014	–	–	**34.1**	–	**30.5**
South West Iberia					
2010	11,543	65,030	17.8	73,910	15.6
2011	15,811	85,295	18.5	94,621	16.7
2012	26,313	101,649	25.9	118,039[(d)]	22.3
2013	31,058	113,437	27.4	123,097[(d)]	25.2
2014	24,507	114,358[(e)]	21.4	108,574	22.6
2010–2014	–	–	**22.2**	–	**20.5**

(a): Source:http://www.magrama.gob.es/es/calidad-y-evaluacion-ambiental/temas/biotecnologia/organismos-modificados-geneticamente-omg-/consejo-interministerial-de-ogms/superficie.aspx (Accessed: 4 March 2016).
(b): Avances de superficies y producciones de cultivos: http://www.magrama.gob.es/es/estadistica/temas/estadisticas-agrarias/agricultura/avances-superficies-producciones-agricolas/ (Accessed: 4 March 2016).
(c): Encuesta sobre superficies y rendimieto de cultivos (ESYRCE): http://www.magrama.gob.es/es/estadistica/temas/estadisticas-agrarias/agricultura/esyrce/ (Accessed: 4 March 2016).
(d): Data for maize as a second crop were not included.
(e): Provisional data.

Table 3: Field collection of *Ostrinia nubilalis* (ECB) and *Sesamia nonagrioides* (MCB) larvae in the 2014 growing season in different areas of the Iberian Peninsula

Target pest	Geographical area	Field (Province – Country)	No. larvae collected
ECB	South West Iberia	Mérida (Badajoz – SP)	246
		Elvas (PO)	107
		Total	353
MCB	Central Iberia[(a)]	La Gineta (Albacete – SP)	277
		Motilleja (Albacete – SP)	202
		Total	479
	South West Iberia[(b)]	Mérida (Badajoz – SP)	7
		Sanlúcar de Barrameda (Cádiz – SP)	216
		El Batán (Cáceres – SP)	248
		Logrosán (Cáceres – SP)	24
		Miajadas (Cáceres – SP)	149
		Total	644

PO: Portugal; SP: Spain.
(a): Two additional fields were inspected but larvae were not found.
(b): Five additional fields were inspected but larvae were not found.

3.2.2. Insect bioassays to assess Cry1Ab susceptibility

3.2.2.1. Dose–response bioassays

The consent holder monitored possible changes in baseline susceptibility to the Cry1Ab protein of the target pest populations of South West Iberia (for both ECB and MCB) and Central Iberia (for MCB only) by measuring the moulting inhibiting concentrations (MIC_{50} and MIC_{90}) in dose–response bioassays.

The MIC_{50} and MIC_{90} values reported in 2014 for the ECB populations are lower compared to previous years (Table 4), while those for the MCB populations are in the range of the MIC_{50} and MIC_{90} values obtained in previous years (Table 5).

The consent holder concluded that the *differences found in the susceptibility to the toxin are within the range of variability expected for field collections of these corn borers. Further, the analyses of historical series of susceptibility data of* S. nonagrioides *or* O. nubilalis *to Cry1Ab did not reveal signs of changed susceptibility to this toxin by field collections from the sampling the [sic] areas considered.*

Table 4: Susceptibility to Cry1Ab protein of South West Iberian field populations of *Ostrinia nubilalis* (ECB) collected in refuge areas and/or fields of conventional maize adjacent to maize MON 810 fields

Population	Growing season	No. larvae collected (no. fields)	Protein batch[(a)]	MIC_{50} (95 % CI)[(b)]	MIC_{90} (95 % CI)[(b)]	RR MIC_{50}[(c)]	RR MIC_{90}[(c)]
South West Iberia	2008	430 (3)	B1	3.39 (2.94–3.89)	6.90 (5.79–8.89)	4.24	2.38
	2010	548 (3)	B1	5.76 (4.38–7.84)	11.85 (8.53–23.52)	2.88	1.77
	2012	378 (2)	B2	4.08 (2.99–5.50)	8.69 (6.30–15.56)	3.71	3.48
	2014	353 (2)	B3	1.32 (0.94–1.74)	3.80 (2.78–6.21)	0.41	0.28

CI: confidence interval.
(a): Data provided by the consent holder showed that the Cry1Ab protein batches B1 and B2, and B2 and B3 have similar insecticidal activity.
(b): 50% and 90% moulting inhibition concentration (MIC_{50} and MIC_{90}) and their 95% confidence intervals (CI 95%) are expressed in ng Cry1Ab/cm^2.
(c): Resistance ratio (RR) between MIC values of the field-collected populations and of the susceptible laboratory strain for each growing season.

Table 5: Susceptibility to Cry1Ab protein of South West Iberian field populations of *Sesamia nonagrioides* (MCB) collected in refuge areas and/or fields of conventional maize adjacent to maize MON 810 fields

Population	Growing season	No. larvae collected (no. fields)	Protein batch[(a)]	MIC_{50} (95% CI)[(b)]	MIC_{90} (95% CI)[(b)]	RR MIC_{50}[(c)]	RR MIC_{90}[(c)]
Central Iberia	2004	n.a.	B1	12 (5–22)	248 (143–588)	0.67	2.50
	2006	n.a.	B1	7 (1–17)	321 (157–1360)	n.a.	n.a.
	2008	320 (4)	B1	28 (18–38)	170 (124–259)	1.47	1.42
	2010	570 (3)	B1	10 (6–14)	119 (81–200)	1.25	1.61
	2012	544 (3)	B2	15 (8–25)	160 (79–608)	2.14	2.58
	2014	479 (2)	B3	15 (9–21)	138 (81–329)	0.88	1.52
South West Iberia	2005[(d)]	n.a.	B1	16[(e)]	30[(e)]	n.a.	n.a.
	2005[(f)]	n.a.	B1	8 (3–16)	152 (94–309)	n.a.	n.a.
	2007	717 (3)	B1	17 (10–25)	223 (153–385)	1.06	2.37
	2010	164 (2)	B1	16 (11–21)[(g)]	86 (60–141)[(g)]	2.00	1.16
	2012	243 (5)	B2	29 (19–1)	158 (101–339)	4.14	2.55
	2014	644 (5)	B3	31 (23–43)	236 (140–569)	1.82	2.59

n.a.: data not available; CI: confidence interval.
(a): Bridging studies showed that the Cry1Ab protein batches B1 and B2, and B2 and B3 have similar insecticidal activity.
(b): 50% and 90% moulting inhibition concentration (MIC_{50} and MIC_{90}) and their 95% confidence intervals (CI 95%) are expressed in ng Cry1Ab/cm^2.
(c): Resistance ratio (RR) between MIC values of the field-collected populations and the susceptible laboratory strain for each growing season.
(d): Larvae collected in Spain.
(e): 95% CI could not be estimated because the coefficient g was $>$ 0.5 at the 95% probability level.
(f): Larvae collected in Portugal.
(g): Bioassays were performed with the F_2 generation due to the low number of field larvae collected.

3.2.2.2. Diagnostic dose assays

The diagnostic dose assays were performed with both the ECB and MCB populations collected in 2014, respectively, using a dose of 28.22[18] and 726[19] ng Cry1Ab/cm^2.

Moult inhibition of the ECB larvae collected in South West Iberia was 100% as no single larvae survived after 7 days of exposure. For the MCB larvae, moult inhibition was 96 $\pm$ 2% and 96 $\pm$ 1% (mean $\pm$ SE) for Central and South West Iberian populations, respectively.

3.2.2.3. Survival of MCB larvae fed maize MON 810 leaves

None of the MCB larvae fed maize MON 810 leaves survived after 12 days of exposure.

The bioassay with MON 810 leaves was used by the consent holder to further prove the absence of resistant individuals in the progenies obtained from field-collected larvae. As maize MON 810 is expected to cause 100% mortality of heterozygotes, this bioassay reinforces the results obtained in the diagnostic dose assay. However, to correctly interpret the results, additional information (e.g. lethal time (LT_{50}), developmental stage at death, Cry1Ab protein expression in detached leaves) should be made available.

3.2.3. Conclusions of the assessment of Cry1Ab susceptibility

The GMO Panel did not identify changes in the methodology compared with the 2013 annual PMEM report. However, the GMO Panel recommends that further details on the methodology followed in the dose–response and diagnostic dose assays (e.g. preimaginal mortality of field-collected larvae, number of adults mated to obtain F_1 larvae, number of individuals screened and number of replicates used in the diagnostic dose assay) should be provided by the consent holder for an appropriate appraisal of the methodology and for a more precise estimation of the detection limit of the resistance allele frequency.

In the 2014 PMEM report, only MIC values were provided. The GMO Panel agrees that measurements of sublethal effects proved in some cases to be more sensitive than mortality for natural toxins (e.g. Schmutterer, 1990), including Cry proteins (e.g. Lovei et al., 2009). However, both lethal and sublethal measurements provide useful information for detecting possible adverse effects. Therefore, the GMO Panel reiterates its previous recommendation to provide both LC (lethal concentration) and MIC values in future PMEM reports (EFSA GMO Panel, 2012a).

The GMO Panel concludes that the analyses of the 2014 dataset provided by the consent holder do not indicate a decrease in susceptibility to the Cry1Ab protein in the tested target pests from those Iberian populations that were monitored in 2014. As the populations tested in 2014 came from areas of low adoption rate of maize MON 810 (Table 2) and, thus, of low selection pressure, this confirms the assumptions made during the initial risk assessment, i.e. that resistance is unlikely to evolve in situations of low adoption of maize MON 810. The GMO Panel reiterates its previous recommendation to focus sampling in areas of high adoption rate of maize MON 810 (EFSA, 2015a) (for further information, see Section 3.2.4).

The GMO Panel is not aware of any scientific report on the detection of field resistance to Cry1Ab in the ECB and MCB populations in the EU.

3.2.4. Further considerations on the harmonised IRM plan

The purpose of the IRM plan is to maintain the effectiveness of Bt crops as an insect pest management tool by preventing or delaying the evolution of resistance to Bt traits in the target pests. For maize MON 810, the consent holder follows EuropaBio's harmonised IRM plan for cultivation of Bt-maize (single insecticidal trait) in the EU. For maize MON 810 grown in the EU, this plan foresees resistance monitoring of ECB and MCB populations in the Iberian Peninsula.

3.2.4.1. Detection of resistance allele frequency

The harmonised IRM plan states that the methodology followed by the consent holder to assess changes in susceptibility to Cry1Ab is able to detect resistance when resistance allele frequency

[18] The diagnostic dose was based on the MIC_{99} value obtained from data of the ECB larvae collected in fields from the Czech Republic, France, Germany, Italy, Panonia, Poland, Portugal, Romania and Spain between 2005 and 2012.

[19] The diagnostic dose was based on the MIC_{99} value obtained from data of the MCB larvae collected in fields from South West, Central and North East Iberia between 2008 and 2012.

reaches 1–5%. The level of resistance allele frequency that one can detect will determine the time left to implement remedial measures before field resistance occurs.[20]

In its technical report (EFSA, 2015a), EFSA conducted new model simulations for the ECB populations using the Populus model to estimate the number of generations required to reach a resistance allele frequency of 50% in the target insect population, once the allele frequency detected during CSM has reached 1%, 3% or 5% (Table 6).[21] In these simulations, two adoption rates of maize MON 810 (i.e. 60% and 80%) and three values for density-dependent mortality (0.3, 0.5 and 0.7) were considered. For instance, in a geographical area where adoption of maize MON 810 is 60%, a detection level of 3% resistance allele frequency will leave seven generations (i.e. 3.5 years for populations in the Ebro valley, see Velasco et al., 2007) before field resistance is achieved, considering a density-dependent mortality estimate of 0.7, which is the most conservative estimate used in the simulations (Table 6).

Table 6: Minimum number of generations before 50% resistance allele frequency (threshold for resistance in a population) in *Ostrinia nubilalis* (ECB) populations is reached from the detection of an allele frequency of 1%, 3% and 5%, according to model simulations by EFSA (2015a) using the Populus model. Calculations are reported for a density-dependent mortality estimate of 0.7

Adoption rate of maize MON 810	Minimum number of generations[(a)] before resistance occurs		
	Detection of resistance allele frequency		
	1%	3%	5%
60%	13	7	5
80%	12	6	5

(a): In the Ebro valley, *O. nubilalis* populations complete two generations per year (Velasco et al., 2007).

The GMO Panel acknowledges that all simulation exercises are subject to scientific uncertainty. The major sources of variability in the above predictions of the number of generations arise from assumptions made in the model structure and the incomplete availability of data. However, the GMO Panel notes that there is a widespread acceptance by all stakeholders that once allele frequency approaches close to 1%, the rate of evolution of resistance is very rapid and the implementation of mitigation measures becomes urgent if field resistance is to be delayed.

Therefore, based on the outcome of the model simulations, and considering the time needed to implement appropriate mitigation measures before pest populations become resistant (for more information, see EFSA GMO Panel, 2013), the GMO Panel considers that a detection level of 1% would be an appropriate threshold, as it allows more time to undertake measures and offers more options to mitigate the potential for field resistance to evolve than a level of 3%.

However, the GMO Panel acknowledges that limitations exist for sampling adequate numbers of target pests in the fields. For instance, when no resistant individual is observed in a diagnostic dose assay, it is necessary to screen *ca*. 10,000 larvae to conclude that the actual resistance allele frequency is below 1% in a target pest population, and it could be difficult to sample that many larvae in a given sampling area. It should also be considered that larvae must be reared and mated before testing (i.e. F_1 larvae are tested) and that several additional factors, like the preimaginal mortality during the laboratory rearing, need to be considered to determine the appropriate sample size of field-collected larvae. Therefore, the GMO Panel considers that a threshold of 3% is the maximum detection level that must be achieved in practice, meaning that the minimum number of field larvae tested should be above *ca.* 1,000 in each sampling area. If achievable, a lower detection limit should be aimed at by the consent holder. Although not currently used on a routine basis due to technical limitations, F_2 screening could be considered as an alternative testing approach, because it requires less larvae than the diagnostic dose assay (Andow and Ives, 2002).

The GMO Panel considers that a detection level of 5% resistance allele frequency would not leave sufficient time to undertake action to prevent the development of field resistance.

[20] A target pest population is considered resistant when the resistance allele frequency has reached 0.5 (or 50%).

[21] The parameters (and values) entered for the simulations with the Populus model were: adoption rate of maize MON 810 (60% and 80%); density-dependent mortality (0.3, 0.5 and 0.7); initial allele frequency (0.006, see Engels et al., 2010); fecundity (100); dominance (0.01 – almost fully recessive); preference for Bt-maize in the second generation (120%); overwinter survival (0.01): and survival of susceptible homozygotes on Bt (0.001).

3.2.4.2. Sampling frequency

The sampling frequency of the ECB and MCB populations proposed by the consent holder and recommended by the GMO Panel is determined by the adoption rate of maize MON 810 in a geographical area and the voltinism (i.e. number of generations in a year)[22] of the pests in that area.

Following its harmonised IRM plan, the consent holder proposed to sample target pest populations every 2 years in areas where adoption rate varies between 20% and 80% of the total maize cultivated area, and only to sample annually in case of multivoltine populations in areas where adoption rate is higher than 80%. Based on simulations using the Populus model developed by Alstad and Andow (1995)[23] (for more details see EFSA, 2015a), the GMO Panel recommends annual sampling of bi-/multivoltine target pests when adoption rate is higher than 60% in order to ensure an early detection of change in susceptibility of the ECB and MCB field populations (Table 7). Conversely, the GMO Panel considers that it is not necessary to monitor areas where adoption rate is lower than 20%.

Table 7: Sampling frequency of field populations of *Ostrinia nubilalis* (ECB) and *Sesamia nonagrioides* (MCB) recommended by the GMO Panel

Adoption rate of maize MON 810	Monovoltine populations(a)	Bi-/Multivoltine populations(b)
> 60–80%	Biennial	**Annual**
> 80%(c)	Annual	Annual

Current situation for adoption rate of maize MON 810 and voltinism of target pests in the Ebro valley is shown in bold.
(a): A monovoltine population is a population producing one generation in a year.
(b): A bivoltine population is a population having two generations in a year. This is the case for the ECB and MCB populations in the Ebro valley. A multivoltine population is a population having several generations in a year.
(c): In circumstances in which non-Bt-maize refugia have not been fully implemented.

The average adoption rate of maize MON 810 in the Ebro valley between 2009 and 2014 has been *ca.* 65% (Table 2). Because the ECB and MCB populations can complete two generations per year (i.e. they are both bivoltine) in north Spain (Velasco et al., 2007), the GMO Panel recommends that the consent holder samples both target pests annually in that particular geographical area.

3.2.4.3. Sampling areas and zones

In its harmonised IRM plan, the consent holder stated that *the sampling will be intensified in areas where high levels of Bt-maize adoption occur and where target pest pressure is higher*. Currently, the ECB and MCB populations are sampled in three different geographical areas of the Iberian Peninsula, i.e., North East, Central and South West Iberia. Instead, the GMO Panel reiterates its recommendation to focus the sampling effort exclusively in North East Iberia (i.e. the Ebro valley), where adoption rates of maize MON 810[24] have been the highest in the Iberian Peninsula since 2003,[25] because field resistance to Cry1Ab is more likely to evolve in areas where the selection pressure is the highest.

In its technical report on the IRM plan for maize MON 810 (EFSA, 2015a), EFSA recommended that, in order to capture variability in sensitivity, *the ECB and MCB larvae should be collected from three sampling zones of approximately 10 km × 10 km within the Ebro valley, where adoption rate of maize MON 810 is higher than 50% for at least three consecutive years*. The GMO Panel recognises that, in some cases, the collection of sufficient number of larvae in zones of 10 km × 10 km is not feasible. In these cases, samples can be collected from larger zones, provided that justification is given for this deviation.

The GMO Panel acknowledges that it may be difficult for the consent holder to acquire information on maize MON 810 cropping areas in the absence of a national GMO register that records all maize MON 810 cultivation. The GMO Panel therefore reiterates the importance for the Member States to implement registers of GM crop cultivation.

[22] The terms univoltine, bivoltine and multivoltine refer to insects having one, two or more than two generations per year, respectively.
[23] http://cbs.umn.edu/populus/overview (Accessed: 4 March 2016).
[24] At the time of adoption of this opinion, maize MON 810 is the only Cry1-expressing maize cultivated in the EU. However, the GMO Panel recommends that in future the consent holder takes into consideration the overall adoption rate of Cry1-expressing maize when identifying zones of high adoption for sampling target pests.
[25] Source:http://www.magrama.gob.es/es/calidad-y-evaluacion-ambiental/temas/biotecnologia/organismos-modificados-geneticamente-omg-/consejo-interministerial-de-ogms/superficie.aspx (Accessed: 4 March 2016).

In addition, the GMO Panel recommends that geographical coordinates of each sampling site are given in future annual PMEM reports.

In the current sampling protocol followed by the consent holder, samples from the three sampling zones within a geographical zone are pooled. However, the GMO Panel recommends that the data from samples taken in different sampling zones should be analysed separately in order to detect potential interpopulation variation in the susceptibility of target pest populations (EFSA GMO Panel, 2012a) and to make it more sensitive to possible resistance evolution over time in a given sampling zone.

3.2.4.4. Farmer alert system

The GMO Panel notes that the consent holder put a farmer alert system in place allowing farmers to report complaints regarding product performance (including unexpected field plant damage caused by target pests). The information gathered through this channel could complement the information obtained in the laboratory bioassays. However, due to lack of information on this alert system, the GMO Panel is not in a position to appraise its usefulness. The GMO Panel considers that the consent holder should provide more information on the farmer alert system in their future annual PMEM reports (e.g. number and type of complaints, how complaints referring to potential lack of efficacy of maize MON 810 are followed up by the consent holder, how complaints to other consent holders marketing maize MON 810 are collated and analysed).

The consent holder states that *not a single MON 810 performance complaint allegedly caused by reduced target pest susceptibility was received from farmers in 2014*.

3.3. Farmer questionnaires

In its annual 2014 PMEM report, the consent holder submitted a survey completed between December 2014 and March 2015 based on 261 questionnaires received from farmers in two European countries: 213 in Spain and 48 in Portugal. No farmers from the Czech Republic, Romania and Slovakia, representing 2% of the maize MON 810 grown in the EU in 2014, were interviewed. The consent holder concluded that the analysis of the questionnaires *did not identify any potential adverse effects that might be related with to MON 810 plants and their cultivation*.

The GMO Panel, in close collaboration with the AMU Unit, assessed the methodology followed by the consent holder to analyse the farmer questionnaires. Alongside the methodological guidance for a systematic evaluation of farmer questionnaires, the evaluation of the overall 2014 farmer's survey (including, for example, sampling of farmers, types of questions, method of conduct interviews, data validation, method used for the design of the statistical analysis) is given in Annex A.

The methodology, consisting of the use of farmer questionnaires, followed by the consent holder to identify unanticipated adverse effects caused by the cultivation of maize MON 810 did not differ from previous annual PMEM reports (EFSA GMO Panel, 2011a, 2012a, 2013, 2014a, 2015b). Similar weaknesses in the methodology as in previous annual PMEM reports were observed, and recommendations to the consent holder for the improvement of the methodology are listed in Annex A.

The GMO Panel examined the results of the analysis of the 2014 farmer questionnaires on maize MON 810, and acknowledges that there is no indication that unanticipated adverse effects have been observed.

The GMO Panel notes that in the annual 2015 PMEM report of maize MON 810 approximately 2,500 farmers will have been surveyed over 10 years since the first questionnaires were completed in 2006. This is the total sample size determined by the consent holder (EFSA GMO Panel, 2011a) deemed necessary to achieve sufficient power to identify unanticipated adverse effects caused by maize MON 810. The GMO Panel strongly recommends that the consent holder performs statistical analyses pooling all the surveys obtained over the last 10 years and report the results of these analyses in the annual 2015 PMEM report.

3.4. Existing monitoring networks

Directive 2001/18/EC and Council Decision 2002/811/EC propose to make use of existing monitoring networks, as it complements farmer questionnaires and provides an additional tool for the GS of GM plants. The Member States have various networks in place – some of which have a long history of data collection – that may be helpful in the context of GS of GM plants. The networks involved in routine monitoring offer recognised expertise in a specific domain and have the tools to capture information on important environmental aspects over a large geographical area.

The consent holder referred to an ongoing EuropaBio project that aims to map the existing European monitoring networks, but that has not yet delivered information on possible networks that could be involved in the GS of maize MON 810. Therefore, as in the previous annual PMEM reports, the consent holder did not report information gathered by existing monitoring networks in the EU. However, the GMO Panel notes that efforts have been made to develop a methodological framework to facilitate the use of existing networks in the broader context of environmental monitoring (Centre for Ecology and Hydrology et al., 2014; EFSA GMO Panel, 2014b; Smets et al., 2014). The GMO Panel encourages that these efforts are continued by relevant parties (EFSA GMO Panel, 2011b).

3.5. Literature review

3.5.1. Relevant scientific publications reported by the consent holder

The consent holder performed a literature search to identify publications related to maize MON 810 and/or the Cry1Ab protein that were published in the peer-reviewed scientific literature between June 2014 and the beginning of June 2015.

The consent holder used Web of Science[SM] [26] as the only scientific literature database to identify relevant publications.

The search terms[27] used for the literature search were similar to those applied in the search reported in the previous annual PMEM report (EFSA GMO Panel, 2015b). The GMO Panel considered that the search terms used by the consent holder in its annual 2014 PMEM report are adequate to retrieve relevant scientific publications. The search terms are broad and include synonyms (*tolerant* and *resistant*), scientific and common names (*maize* and *Zea mays*), brand and generic names (*Yieldgard* or *Bt maize*), British and US variants (*maize* and *corn*), etc. Boolean operators (i.e. OR, AND) were appropriately used to combine terms, while wild card symbols allowed to retrieve variant spellings (e.g. *toleran**, *protec**).

The consent holder initially identified 26 scientific relevant scientific papers published between June 2014 and the beginning of June 2015 (Appendix A): 25 publications were reported initially by the consent holder in the 2014 annual PMEM report, and one additional publication (Zeljenkova et al., 2014) was reported in the updated 2014 annual PMEM report.

Two publications were relevant to the molecular characterisation (MC) of maize MON 810, six publications were relevant for the food and feed (FF) safety assessment (in terms of toxicity, allergenicity and nutrition) and 18 publications pertained to the environmental risk assessment (ERA) or risk management of maize MON 810 (mostly, studies assessing the interaction of maize MON 810 with target organisms (TOs) and non-target organisms (NTOs)). One of the MC-related publications, Trtikova et al. (2015) had already been assessed by EFSA (EFSA, 2015b) concluding that *the findings reported by Trtikova* et al. *(2015) present no new scientific information that would invalidate the EFSA GMO Panel's previous risk assessment conclusions and recommendations on risk management of maize MON 810*.

The GMO Panel notes that 13 relevant scientific publications related to Bt-maize/maize MON 810 and/or the Cry1Ab protein (published between June 2014 and beginning June 2015) were not reported by the consent holder (Appendix A) (see Section 3.5.2). Therefore, the GMO Panel makes the following recommendations:

- to perform a more comprehensive systematic search by considering the EFSA Guidance Document on systematic literature review methodology (EFSA, 2010) and to provide a protocol for the systematic search that includes information on the date of the search, the full list of retrieved scientific publications and clear criteria for exclusion/inclusion of relevant scientific publications;
- to use additional scientific literature databases such as CAB Abstracts®,[28] to increase the likelihood to retrieve all relevant scientific publications (EFSA, 2015c).

[26] http://apps.webofknowledge.com/ (Accessed: 4 March 2016).

[27] The list of keywords used to identify relevant scientific publications is given in Table 1 of the 2014 annual PMEM report on maize MON 810.

[28] http://www.cabi.org/publishing-products/online-information-resources/cab-abstracts/ (Accessed: 4 March 2016).

The GMO Panel encourages the consent holder to revise the protocol for the literature search accordingly, and to supply it to EFSA annually.

The GMO Panel assessed all the scientific publications selected by the consent holder, and acknowledges that these were adequately discussed and put into the context of the overall safety assessment of maize MON 810.

The GMO Panel considers that none of the publications reported adverse effects of maize MON 810 on human and animal health or the environment.[29]

3.5.2. Additional scientific publications assessed by the GMO Panel

EFSA conducted a literature search to identify additional relevant scientific publications. Several bibliographic databases were queried simultaneously to identify as many relevant peer-reviewed scientific publications as possible. These were BIOSIS Citation Index^SM,[30] CAB Abstracts®, Current Contents Connect®,[31] Medline®,[32] and Web of Science Core Collection^TM.[33] The databases were integrated into Web of Science^TM (Thomson Reuters, New York, NY, USA).

The literature search was performed using the same search terms and Boolean operators as the consent holder, targeting scientific publications in the peer-reviewed scientific literature between June 2013 and June 2014 (which was the time interval covered by the literature search conducted by the consent holder). The searches were subsequently refined by selecting only those publications (as *document type*) that were written in English.

The search was conducted on 4 November 2015 and identified a total of 351 references. References were exported into an EndNote X5 database (Thomson Reuters) and duplicated references were deleted. The 339 remaining scientific publications were screened and assessed manually by title and abstract. Only peer-reviewed publications containing evidence specific to the risk assessment and/or management of maize MON 810 were considered for further assessment.

The GMO Panel identified a total number of 39 relevant scientific publications (Appendix A), of which 26 were reported by the consent holder in its annual 2014 PMEM report on maize MON 810 and one (Hofmann et al., 2014) which was previously assessed by the GMO Panel (EFSA GMO Panel, 2015c). For this scientific publication, the GMO Panel concluded that *the new information provided by Hofmann* et al. *(2014) does not impact greatly on the mortality estimates for NT Lepidoptera of conservation concern, occurring within protected habitats and potentially exposed to maize MON 810 pollen, and the previous recommendation for isolation distances around protected habitats, within which maize MON 810 should not be cultivated, remains valid*.

The remaining 12 scientific publications not previously assessed by EFSA or its GMO Panel are all relevant for the ERA and/or risk management of maize MON 810. No additional scientific publications relevant to the MC or FF safety assessment of maize MON 810 were identified.

The GMO Panel assessed the ERA-related scientific publications and concludes that no environmental safety concerns owing to maize MON 810 or Cry1Ab were identified.

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[29] In one of the scientific publications, Andreassen et al. (2015) investigated the immune responses (humoral and cellular) in mice after airway exposure to Cry1Ab (test material: maize MON 810 pollen suspension, maize MON 810 leaf extracts, purified Cry1Ab protoxin and trypsinised Cry1Ab). The authors reported no anti-Cry1Ab antibodies against the maize MON 810 materials tested. On the other hand, specific anti-Cry1Ab IgG1 and IgE were seen in the groups exposed to the purified Cry1Ab protein (Cry1Ab protoxin or trypsinised Cry1Ab). The immunogenic capacity of the Cry1Ab protein *per se* is well-known and it has been described in previous studies (Adel-Patient et al., 2011). This has been previously assessed by the GMO Panel (EFSA GMO Panel, 2012b). Specific IgE antibodies against the purified Cry1Ab protein were observed by Andreassen et al. (2015). The authors provided several hypotheses that in part might explain the difference in immune responses to Cry1Ab observed in the different groups. Among the rational provided, structural differences between the Cry1Ab proteins tested and differences in doses used were considered the main factors explaining the discrepancy in outcomes. The GMO Panel agrees with the authors that, in order to elucidate these aspects, additional studies on the immunogenicity of Cry1Ab proteins following an airway exposure would be useful. In the context of the risk assessment of maize MON 810, the study by Andreassen et al. (2015) does not put forward new elements that would invalidate the previous conclusions on maize MON 810 made by the GMO Panel. Specifically, no indications of safety concern due to the airway exposure of maize MON 810 pollen suspension and maize MON 810 leaf extract in mice were identified.

[30] http://wokinfo.com/products_tools/specialized/bci/ (Accessed: 4 March 2016).

[31] http://thomsonreuters.com/en/products-services/scholarly-scientific-research/scholarly-search-and-discovery/current-contents-connect.html (Accessed: 4 March 2016).

[32] https://www.nlm.nih.gov/bsd/pmresources.html (Accessed: 4 March 2016).

[33] http://thomsonreuters.com/en/products-services/scholarly-scientific-research/scholarly-search-and-discovery/web-of-science-core-collection.html (Accessed: 4 March 2016).

3.5.3. Conclusions of the literature review

Results reported in the relevant peer-reviewed scientific publications included by the consent holder in the annual 2014 PMEM report on maize MON 810 and additional ones identified by the GMO Panel do not provide new information that would invalidate the previous FF and ERA conclusions on maize MON 810 made by the Panel. Therefore, the GMO Panel considers that its previous conclusions on the safety of maize MON 810 remain valid and applicable (EFSA, 2009; EFSA GMO Panel, 2012b,c).

4. Conclusions

The data reported in the annual 2014 PMEM report on maize MON 810 do not indicate any adverse effects on human and animal health or the environment arising from the cultivation of maize MON 810 during the 2014 growing season. The GMO Panel therefore concludes that the CSM and GS of maize MON 810 as carried out by the consent holder did not provide evidence that would invalidate previous GMO Panel evaluations on the safety of maize MON 810 (EFSA, 2009; EFSA GMO Panel, 2012b,c). However, the GMO Panel identified limitations that need further action from the consent holder.

5. Recommendations

5.1. Case-specific monitoring

The GMO Panel considers that the detection levels of resistance allele frequency in target pest populations set by risk managers should consider sufficient time to implement appropriate mitigation measures to prevent field resistance and sample sizes (i.e. number of larvae to be screened). The GMO Panel is of the opinion that the sample size of target pest populations needs to be proportionate to the detection levels of resistance allele frequency set by risk managers.

The GMO Panel considers that a detection level of 1% would be an appropriate threshold because it would give more time to undertake mitigation measures and offers more options to reduce the potential for field resistance to evolve than a detection level of 3%. However, owing to practical sampling limitations, the GMO Panel recommends that a threshold of 3% should be achieved in practice, meaning that the minimum number of field larvae tested should be above *ca.* 1,000 in a given sampling area in those regions where the adoption rates of maize MON 810 are above 60%, such as the Ebro valley. If achievable, a lower detection limit should be aimed at by the consent holder.

The GMO Panel maintains its previous recommendation to perform annual monitoring of bi-/multivoltine target pests when adoption rate of maize MON 810 is higher than 60% in order to ensure early detection of change in susceptibility of the ECB and MCB field populations.

While adoption rates remain low in other regions growing maize MON 810, the GMO Panel recommends focusing the sampling effort exclusively in North East Iberia (i.e. the Ebro valley), where field resistance to Cry1Ab is more likely to develop. In that geographical area, samples should be collected in three zones of approximately 10 km × 10 km, where adoption rate of maize MON 810 is higher than 50% for at least three consecutive years. The consent holder should clearly identify cases where larger zones are required to ensure that sufficient numbers of larvae are collected. In order to acquire information on maize MON 810 cropping areas, the GMO Panel reiterates the importance for the Member States to implement national GMO cultivation registers, as referred to in Article 31.3 (b) of Directive 2001/18/EC.

For all sampling sites, the consent holder is encouraged to provide the geographical coordinates. Samples from each sampling zone should be analysed separately in order to detect potential interpopulation variation in the susceptibility of target pest populations.

The GMO Panel considers that the consent holder should provide more information on the farmer alert system (e.g. number and type of complaints, how complaints referring to potential lack of efficacy of maize MON 810 are followed up by the consent holder, how complaints to other consent holders marketing maize MON 810 are collated and analysed) in order to determine whether appropriate communication mechanisms and fit-for-purpose educational programmes are in place to ensure timely and effective reporting of farmer complaints regarding product performance.

The GMO Panel recommends that further details on the methodology (e.g. preimaginal mortality of field-collected larvae, number of adults mated to obtain F_1 larvae, number of individuals screened and number of replicates in the diagnostic dose assay) in the laboratory bioassays should be provided for an appropriate estimation of the detection limit of resistance allele frequency. Regarding the dose–response bioassays to assess changes in susceptibility to Cry1Ab, the GMO Panel reiterates its previous recommendations to provide both LC and MIC values in the future PMEM reports.

Considering the implementation of non-Bt refugia, the GMO Panel recommends that the consent holder consolidates its efforts to increase the level of compliance, especially in regions of high maize MON 810 adoption.

5.2. General surveillance

The GMO Panel identified shortcomings in the methodology followed by the consent holder to analyse the farmer questionnaires similar to those found in previous reports. Therefore, the Panel reiterates its recommendations on the survey design and reporting to provide more detailed information on the sampling methodology and to reduce the possibility of selection bias (for further details on the recommendations, see Annex A), as this would give more confidence in the conclusion on the absence of adverse effects. In order to improve the sampling frame of the farmer survey, the GMO Panel reiterates the importance of national GMO cultivation registers and its recommendations to consent holders to consider how they may make best use of the information recorded in national registers and foster dialogue with those responsible for the administration of the registers of maize MON 810 cultivation.

The outcome of the literature review confirms the previous conclusions on the safety of maize MON 810 made by the GMO Panel. In addition, considering the relevant publications identified as missing, the GMO Panel advises the consent holder to improve the methodology followed in the literature review to ensure that all relevant publications are identified and assessed (e.g. following the EFSA guidance on systematic reviews (EFSA, 2010), use of additional databases, providing inclusion/exclusion criteria). Moreover, the consent holder is requested to continue screening, reviewing and discussing relevant scientific publications on possible adverse effects of maize MON 810 on rove beetles as previously recommended by the GMO Panel (EFSA GMO Panel, 2014a).

No information collected from existing monitoring networks in the EU was provided by the consent holder. However, the GMO Panel notes that initiatives have been taken to develop a methodological framework to use existing networks in the broader context of environmental monitoring and encourages the relevant parties to continue to develop these.

Documentation provided to EFSA

1) Letter from the European Commission, dated 26 October 2015, to the EFSA Executive Director requesting the assessment of the annual 2014 PMEM report on maize MON 810 cultivation during the 2014 growing season report provided by Monsanto; the PMEM report was annexed to the letter.
2) Comments from the Member States on the annual 2014 PMEM report on the cultivation of maize MON 810 during the 2014 growing season.
3) Acknowledgement letter, dated 13 November 2015, from the EFSA Executive Director to the European Commission.
4) Considerations from the National Committee of Biosafety of the Spanish Competent Authority expressed EFSA recommendations on the IRM plan for maize MON 810.
5) Email from the European Commission, dated 3 February 2016, to the GMO Unit requesting the assessment of an additional scientific publication.

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Abbreviations

Bt	*Bacillus thuringiensis*
CSM	case-specific monitoring
ECB	European corn borer
AMU Unit	EFSA Unit for Assessment and Methodological Support
GMO Panel	EFSA Panel on Genetically Modified Organisms
ERA	environmental risk assessment
FF	food and feed
GM	genetically modified
GMO	genetically modified organism
GS	general surveillance
IRM	insect resistance management
LC	lethal concentration
LT_{50}	median lethal time
MC	molecular characterisation
MCB	Mediterranean corn borer
MIC	moulting inhibitory concentration

NT	non-target
NTO	non-target organism
PMEM	post-market environmental monitoring
SE	standard error
TO	target organism

Appendix A – Peer-reviewed scientific publications relevant to the risk assessment and/or management of maize MON 810 assessed by the EFSA GMO Panel as part of the annual 2014 PMEM report on the cultivation of maize MON 810

Authors	Title	Journal	Year	Identified by	Relevant field[(a)]
Achon MA, Serrano L, Sabate J and Porta C	Understanding the epidemiological factors that intensify the incidence of maize rough dwarf disease in Spain	Annals of Applied Biology	2015	EFSA	ERA
Andreassen M, Bohn T, Wikmark OG, Van den Berg J, Lovik M, Traavik T and Nygaard UC	Cry1Ab protein from *Bacillus thuringiensis* and MON 810 cry1Ab-transgenic maize exerts no adjuvant effect after airway exposure	Scandinavian Journal of Immunology	2015	Consent holder & EFSA	FF – Allergenicity
Andreassen M, Rocca E, Bohn T, Wikmark O-G, van den Berg J, Lovik M, Traavik T and Nygaard UC	Humoral and cellular immune responses in mice after airway administration of *Bacillus thuringiensis* Cry1Ab and MON 810 cry1Ab-transgenic maize	Food and Agricultural Immunology	2015	Consent holder & EFSA	FF – Allergenicity
Babu A, Cook DR, Caprio MA, Allen KC and Musser FR	Prevalence of *Helicoverpa zea* (Lepidoptera: Noctuidae) on late season volunteer corn in Mississippi: implications on Bt resistance management	Crop Protection	2014	EFSA	ERA – TO
Boettger R, Schaller J, Lintow S and Dudel EG	Aquatic degradation of Cry1Ab protein and decomposition dynamics of transgenic corn leaves under controlled conditions	Ecotoxicology and Environmental Safety	2015	EFSA	ERA
Bowen KL, Flanders KL, Hagan AK and Ortiz B	Insect damage, aflatoxin content, and yield of Bt corn in Alabama	Journal of Economic Entomology	2014	Consent holder & EFSA	ERA
Bowers E, Hellmich R and Munkvold G	Comparison of fumonisin contamination using HPLC and ELISA methods in Bt and near-isogenic maize hybrids infested with European corn borer or western bean cutworm	Journal of Agricultural and Food Chemistry	2014	Consent holder & EFSA	ERA
Camastra F, Ciaramella A and Staiano A	A note on some mathematical models on the effects of Bt-maize exposure	Environmental and Ecological Statistics	2014	EFSA	ERA – NTO
Campos RC and Hernandez MIM	Changes in the dynamics of functional groups in communities of dung beetles in Atlantic forest fragments adjacent to transgenic maize crops	Ecological Indicators	2015	Consent holder & EFSA	ERA – NTO
Chavez C, Recio-Totoro B, Flores-Escobar B, Lanz-Mendoza H, Sanchez J, Sobero M and Bravo A	Nitric oxide participates in the toxicity of *Bacillus thuringiensis* Cry1Ab toxin to kill *Manduca sexta* larvae	Peptides	2015	EFSA	ERA – TO

Authors	Title	Journal	Year	Identified by	Relevant field[a]
Čerevková A and Cagan L	Effect of transgenic insect-resistant maize to the community structure of soil nematodes in two field trials	Helminthologia	2015	Consent holder & EFSA	ERA – NTO
Cotta SR, Franco Dias AC, Marriel IE, Andreote FD, Seldin L and van Elsas JD	Different effects of transgenic maize and non-transgenic maize on nitrogen-transforming *Archaea* and *Bacteria* in tropical soils	Applied and Environmental Microbiology	2014	Consent holder & EFSA	ERA – Soil microorganisms
Cruz D and Eizaguirre M	Do *Sesamia nonagrioides* (Lepidoptera; Noctuidae) gravid females discriminate between Bt or multivitamin corn varieties? role of olfactory and visual cues	Journal of Insect Science	2015	Consent holder & EFSA	ERA – TO
da Silva DAF, Cotta SR, Vollu RE, Jurelevicius DD, Marques JM, Marriel IE and Seldin L	Endophytic microbial community in two transgenic maize genotypes and in their near-isogenic non-transgenic maize genotype	BMC Microbiology	2014	Consent holder & EFSA	ERA – Soil microorganisms
Erasmus A and Van den Berg J	Effect of Bt-maize expressing Cry1Ab toxin on non-target Coleoptera and Lepidoptera pests of maize in South Africa	African Entomology	2014	Consent holder & EFSA	ERA – NTO
Furgal-Dieriuk I, Strzetelski J, Twardowske M, Kwiatek K and Mazur M	The effect of genetically modified feeds on productivity, milk composition, serum metabolite profiles and transfer of tDNA into milk of cows	Journal of Animal and Feed Sciences	2015	Consent holder & EFSA	FF – Nutritional assessment
Giron-Perez K, Oliveira AL, Teixeira AF, Guedes RNC and Pereira EJG	Susceptibility of Brazilian populations of *Diatraea saccharalis* to Cry1Ab and response to selection for resistance	Crop Protection	2014	Consent holder & EFSA	ERA – TO
Grabowski M, Lipska A, Zmijewska E, Kozak M and Dabrowski ZT	Transfer of the Cry1Ab toxin in tritrophic bioassays involving transgenic maize MON 810, the herbivore *Tetranychus urticae* Koch and the predatory ladybird beetle *Adalia bipunctata* L. (Coleoptera: Coccinellidae)	Egyptian Journal of Biological Pest Control	2014	Consent holder & EFSA	ERA – NTO
Gu J, Bakke AM, Valen EC, Lein I and Krogdahl A	Bt-maize (MON 810) and non-GM soybean meal in diets for Atlantic Salmon (*Salmo salar* L.) juveniles – impact on survival, growth performance, development, digestive function, and transcriptional expression of intestinal immune and stress responses	PLoS ONE	2014	Consent holder & EFSA	FF – Toxicology
Gulli M, Salvatori E, Fusaro L and Pallacani C	Comparison of drought stress response and gene expression between a GM maize variety and a near-isogenic non-GM variety	PLoS ONE	2015	Consent holder & EFSA	ERA
Habustova OS, Svobodova Z, Spitzer L, Dolezal P, Hussein HM and Sehnal F	Communities of ground-dwelling arthropods in conventional and transgenic maize: background data for the post-market environmental monitoring	Journal of Applied Entomology	2015	Consent holder & EFSA	ERA – NTO

Authors	Title	Journal	Year	Identified by	Relevant field[a]
Hofmann F, Otto M and Wosniok W	Maize pollen deposition in relation to the distance from the nearest pollen source under common cultivation – results of 10 years of monitoring (2001–2010)	Environmental Sciences Europe	2014	EFSA[b]	ERA – NTO
Hurej M, Mietkiewski R and Twardowski JP	The effect of Cry1AB insecticidal protein on the incidence of entomopathogenic fungi infecting aphids on *Bt* maize	Zemdirbyste-Agriculture	2014	Consent holder & EFSA	ERA – NTO
Kapazoglou A, Andreadis SS, Drossou V, Madesis P, Savopoulou-Soultani M and Tsaftaris AS	Cadherin characterization and cytochrome oxidase (COI) HRM analysis in different geographical populations of the Mediterranean corn borer, *Sesamia nonagrioides*	Journal of Agricultural Science	2014	EFSA	ERA – TO
La Paz JL, Pla M, Centeno E, Vicient CM and Puigdomenech P	The use of massive sequencing to detect differences between immature embryos of MON 810 and a comparable non-GM maize variety	PLoS ONE	2014	Consent holder & EFSA	MC
Leite AN, Mendes MS, Dos Santos AC and Pereira EJ	Does Cry1 Ab maize interfere in the biology and behavioural traits of *Podisus nigrispinus*?	Bulletin of Insectology	2014	Consent holder & EFSA	ERA – NTO
Meissle M, Zund J, Waldburger M and Romeis J	Development of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) on pollen from Bt-transgenic and conventional maize	Scientific Reports	2014	Consent holder & EFSA	ERA – NTO
Munoz P, Lopez C, Moralejo M, Perez-Hedo M, Eizaguirre M	Response of last instar *Helicoverpa armigera* larvae to Bt toxin ingestion: changes in the development and in the CYP6AE14, CYP6B2 and CYP9A12 gene expression	PLoS ONE	2014	EFSA	ERA – TO
Rausch MA, Kroemer JA, Gassmann AJ and Hellmich RL	On-plant selection and genetic analysis of European corn borer (Lepidoptera: Crambidae) behavioral traits: plant abandonment versus plant establishment	Environmental Entomology	2014	EFSA	ERA – TO
Reiner D, Lee RY, Dekan G and Epstein MM	No adjuvant effect of *Bacillus thuringiensis*-maize on allergic responses in mice	PLoS ONE	2014	Consent holder & EFSA	FF – Allergenicity
Reisig DD, Akin DS, All JN, Bessin RT, Brewer MJ, Buntin DG, Catchot AL, Cook D, Flanders KL, Huang FN, Johnson DW, Leonard BR, McLeod PJ, Porter RP, Reay-Jones FPF, Tindall KV, Stewart SD, Troxclair NN, Youngman RR and Rice ME	Lepidoptera (Crambidae, Noctuidae, and Pyralidae) injury to corn containing single and pyramided Bt traits, and blended or block refuge, in the Southern United States	Journal of Economic Entomology	2015	Consent holder & EFSA	ERA – TO
Romeis J, Meissle M, Naranjo SE, Li Y and Bigler F	The end of a myth-Bt (Cry1Ab) maize does not harm green lacewings	Frontiers in Plant Science	2014	EFSA	ERA – NTO
Swiatek M, Kielkiewicz M, Zagdanska B	Insect-resistant Bt-maize response to the short-term non-target mite-pest infestation and soil drought	Acta Physiologiae Plantarum	2014	EFSA	ERA

Authors	Title	Journal	Year	Identified by	Relevant field[(a)]
Trtikova M, Wikmark OG, Zemp N, Widmer A and Hilbeck A	Transgene expression and Bt protein content in transgenic Bt maize (MON 810) under optimal and stressful environmental conditions	PLoS ONE	2015	Consent holder & EFSA[(c)]	MC
Truter J, Van Hamburg H and Van Den Berg J	Comparative diversity of arthropods on Bt maize and non-Bt maize in two different cropping systems in South Africa	Environmental Entomology	2014	Consent holder & EFSA	ERA – NTO
Xu L-N, Wang Y-Q, Wang Z-Y, Hu B-J, Ling Y-H and He K-L	Transcriptome differences between Cry1Ab resistant and susceptible strains of Asian corn borer	BMC Genomics	2015	EFSA	ERA – TO
Zeljenkova D, Ambrusova K, Bartuova M, Kebis A, Kovriznych J, Krivosikova Z, Kuricova M, Liskova A, Rollerova E, Spustova V, Szabova E, Tulinska J, Wimmerova S, Levkut M, Revajova V, Sevcikova Z, Schmidt K, Schmidtke J, La Paz JL, Corujo M, Pla M, Kleter GA, Kok EJ, Sharbati J, Hanisch C, Einspanier R, Adel-Patient K, Wal JM, Spoek A, Poeting A, Kohl C, Wilhelm R, Schiemann J and Steinberg P	Ninety-day oral toxicity studies on two genetically modified maize MON 810 varieties in Wistar Han RCC rats (EU 7th Framework Programme project GRACE)	Archives of Toxicology	2014	Consent holder & EFSA	FF – Toxicology
Zeng HL, Tan FX, Zhang YY, Feng YJ, Shu YH and Wang JW	Effects of cultivation and return of *Bacillus thuringiensis* (Bt) maize on the diversity of the arbuscular mycorrhizal community in soils and roots of subsequently cultivated conventional maize	Soil Biology and Biochemistry	2014	Consent holder & EFSA	ERA – Soil microorganisms
Zhang T, He M, Gatehouse AMR, Wang Z, Edwards MG, Li Q and He K	Inheritance patterns, dominance and cross-resistance of Cry1Ab-and Cry1Ac-selected *Ostrinia furnacalis* (Guenee)	Toxins	2014	EFSA	ERA – TO

(a): ERA: environmental risk assessment; FF: food and feed; MC: molecular characterisation; NT: non-target organism; TO: target organism.
(b): This publication was already assessed by the EFSA GMO Panel (2015c).
(c): This publication was already assessed by EFSA (2015b).

Annex A – EFSA AMU Unit technical report on the evaluation of farmer questionnaires

Annex A can be found in the online version of this output: http://dx.doi.org/10.2903/j.efsa.2016.4446