



## **Annual post-market environmental monitoring (PMEM) report on the cultivation of genetically modified maize MON 810 in 2015 from Monsanto Europe S.A.**

Hanspeter Naegeli, Andrew Nicholas Birch, Josep Casacuberta, Adinda de Schrijver, Mikolaj Antoni Gralak, Philippe Guerche, Huw Jones, Barbara Manachini, Antoine Messéan, Elsa Ebbesen Nielsen, et al.

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## Annual post-market environmental monitoring (PMEM) report on the cultivation of genetically modified maize MON 810 in 2015 from Monsanto Europe S.A.

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### 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 2015 growing season of the Cry1Ab-expressing maize event MON 810 provided by Monsanto Europe S.A. The GMO Panel concludes that the insect resistance monitoring data submitted to EFSA do not indicate a decrease in susceptibility of field Iberian populations of corn borers to the Cry1Ab protein during the 2015 season. However, since the methodology for insect resistance monitoring remained unchanged compared to previous PMEM reports, the GMO Panel reiterates its previous recommendations on resistance monitoring to provide sufficient detection sensitivity. Although the farmer alert system to report complaints about product performance could complement the information obtained from the laboratory bioassays, the GMO Panel is currently not in a position to appraise its usefulness, and therefore encourages the consent holder to provide more information on this complementary resistance monitoring tool. The data on general surveillance (GS) do not indicate any unanticipated adverse effects on human and animal health or the environment arising from the cultivation of maize MON 810. The GMO Panel reiterates its previous recommendations on the analysis of farmer questionnaires, and advises the consent holder to provide more detailed information on the conducting and reporting of the literature search in future annual PMEM reports. Moreover, the GMO Panel encourages relevant parties to continue developing a methodological framework to use existing networks in the broader context of environmental monitoring. The GMO Panel concludes that the case-specific monitoring (CSM) and GS activities of maize MON 810 as carried out by the consent holder do not provide evidence that would invalidate previous GMO Panel evaluations on the safety of maize MON 810.

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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 the Cry1Ab-expressing maize event MON 810 during the 2015 growing season provided by Monsanto Europe S.A.

The 2015 case-specific monitoring (CSM) data set on maize MON 810 consists of a survey on compliance with non-*Bacillus thuringiensis* (non-Bt) refuges in Spain and Portugal, concentration–response and diagnostic concentration bioassays to monitor for changes in susceptibility to the Cry1Ab protein in target pests (European and Mediterranean corn borer) collected from Iberian populations, and the outcome of the farmer alert system. The 2015 PMEM report shows partial compliance with the implementation of non-Bt refuges 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 Cry1Ab in the tested target pests from the populations monitored in 2015.

The GMO Panel notes that the methodology for insect resistance monitoring remained unchanged compared to previous PMEM reports and that the monitoring protocol adopted does not provide the sufficient sensitivity to detect early cases of resistance. The GMO Panel reiterates its previous recommendations on resistance monitoring, in particular the recommendations to: (1) increase sampling efforts and ensure that as many field-collected larvae as possible are represented in the laboratory assays as F<sub>1</sub> larvae in order to provide sufficient detection sensitivity (i.e. 3% resistance allele frequency); and (2) implement annual monitoring of populations of both target pests exclusively from north-east Iberia (i.e. the Ebro valley) where adoption rate of maize MON 810 is the highest in the Iberian Peninsula and field resistance to Cry1Ab is more likely to evolve.

The consent holder has implemented a farmer alert system allowing farmers to report complaints about product performance (including unexpected field plant damage caused by target pests). Although this farmer alert system could complement the information received from the laboratory bioassays, the GMO Panel is currently not in a position to appraise its usefulness, and therefore encourages the consent holder to provide more information on this complementary resistance monitoring tool. More information is required to determine whether appropriate communication mechanisms and fit-for-purpose educational programs are implemented that ensure the timely and effective reporting of farmer complaints.

The 2015 general surveillance (GS) data set 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 2015 and May 2016). 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. Therefore, the GMO Panel considers that the GS activities of maize MON 810 as carried out by the consent holder do not provide evidence that would invalidate previous GMO Panel evaluations on the safety of maize MON 810.

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 develop these.

Similar methodological shortcomings to those observed in previous annual PMEM reports on maize MON 810 were identified in the analysis of farmer questionnaires, and the conducting and reporting of the literature search. The GMO Panel therefore strongly reiterates its recommendations to provide more detailed information on the sampling methodology and measures taken to reduce the possible selection bias in farmer questionnaires. To improve the sampling frame of the farmer survey, the GMO Panel reiterates the importance of operational 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.

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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 2015, maize MON 810 was grown in Spain (107,749 ha), Portugal (8,017 ha), the Czech Republic (997 ha), Slovakia (104 ha) and Romania (2.5 ha) over a total area of approximately 116,867 ha.<sup>1</sup>

According to Article 13 of Directive 2001/18/EC,<sup>2</sup> 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. Annex VII was supplemented by notes providing guidance on the objectives, general principles and design of the monitoring plan.<sup>3</sup>

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 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 the GMO Panel) assesses these annual PMEM reports on the cultivation of maize MON 810 (EFSA GMO Panel, 2011a, 2012a, 2013, 2014a, 2015a,b, 2016).

### 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.<sup>4</sup> 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. In July 2004, Monsanto notified MON 810 maize seeds for cultivation, as *existing products*, according to Article 23 of Regulation (EC) No 1829/2003.

Following the request by the consent holder for the renewed market authorisation of maize MON 810, the GMO Panel adopted a scientific opinion on the renewal applications submitted under Regulation (EC) No 1829/2003 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).

In the EU, a harmonised insect resistance management (IRM) plan (EuropaBio, 2012) for the single lepidopteran-active Bt-maize events MON 810, Bt11 and 1507 is being implemented for the cultivation of maize MON 810 since 2003. Implemented resistance management measures are based on the 'high-dose/refuge' strategy, and aim at delaying resistance evolution (Tabashnik et al., 2013). The 'high-dose/refuge' strategy prescribes planting Bt-crops that produce a very high concentration of the insecticidal Bt-protein (25 times the amount needed to kill susceptible individuals (Gould, 1998)), so that nearly all target insect pests that are heterozygous for resistance do not survive on it. In addition,

<sup>1</sup> At present, maize MON 810 is the only GM maize event cultivated in the EU. Maize Bt176, also producing the protein Cry1Ab, was cultivated in the EU between 1998 and 2005.

<sup>2</sup> 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, pp. 1–39.

<sup>3</sup> 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, pp. 27–36.

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



a nearby structured refuge of the non-*Bt*-crop is required where the target insect pest does not encounter the *Bt*-protein. In addition, resistance and compliance monitoring is conducted to allow the periodic evaluation of the adequacy and efficacy of the IRM strategy. Resistance monitoring is designed to detect early warning signs indicating increases in tolerance of target pests in the field; a timely detection of such signs enables actions to limit the survival of resistant insects, and slow or prevent their spread should resistance have evolved among field populations.

In the context of annual PMEM reports on the cultivation of maize MON 810, the consent holder follows a two-pronged approach for resistance monitoring: (1) monitoring for changes in susceptibility to the Cry1Ab protein in ECB/MCB in laboratory bioassays and (2) monitoring of unexpected field damage caused by ECB/MCB through a farmer alert system.

From 2005 onwards, the consent holder submitted to the European Commission 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), which focuses on resistance and compliance monitoring to allow the periodic evaluation of the adequacy and efficacy of the IRM strategy, and general surveillance (GS), which focuses on detecting unanticipated adverse effects caused by the cultivation of maize MON 810.

Since 2010, the European Commission requested the GMO Panel to assess the annual PMEM reports on the cultivation of maize MON 810. The GMO Panel therefore adopted scientific opinions on the 2009 to 2014 annual PMEM reports (EFSA GMO Panel, 2011a, 2012a, 2013, 2014a, 2015a,b, 2016). 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 several recommendations to improve future annual PMEM reports on maize MON 810.

On 22 May 2012, the European Commission requested the European Food Safety Authority (EFSA) to compile an inventory of existing environmental surveillance networks at European and national level, and develop a set of assessment criteria to support the selection of such networks for PMEM of genetically modified (GM) plants. Following this request, EFSA's Assessment and Methodological Support Unit (hereafter referred to as AMU Unit) commissioned an external report that: reviewed statistical methods used in the analysis of ecological and environmental data sets; provided an inventory of statistical approaches in ecological and environmental monitoring and identification of data requirements for the items in the inventory; delivered an inventory of European, National and Regional existing surveillance networks/programmes; and gave recommendations of the most appropriate analysis methodologies for PMEM of agro-ecosystems (Centre for Ecology and Hydrology, Perseus et al., 2014). In this external report and other publications (e.g. Smets et al., 2014), several existing networks were identified as potentially suitable for GS of GM plants, although their usefulness is limited due to issues pertaining to data accessibility, data reporting format, and data connectivity with GMO registers (EFSA GMO Panel, 2014b).

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 IRM strategy for maize MON 810.<sup>5</sup> EFSA concluded that the previous conclusions and recommendations by the GMO Panel remain valid (EFSA, 2015).

On 18 December 2015, the National Committee of Biosafety of the Spanish Competent Authority supplied several considerations about EFSA's recommendations on the IRM plan for maize MON 810.<sup>6</sup>

In two 2016 Technical Reports (EFSA, 2016a,b), following the requests of the European Commission,<sup>7,8</sup> EFSA assessed the implications of new relevant scientific publications by Bøhn et al. (2016) and Hofmann et al. (2016) for the environmental risk assessment (ERA) of maize MON 810 for cultivation. EFSA considered that conclusions and risk management recommendations previously made by the GMO Panel remain valid and applicable.

In another 2016 Technical Report (EFSA, 2016c), EFSA assessed the available scientific information on teosinte for its relevance for the ERA of maize MON 810 for cultivation, as requested by the European Commission.<sup>9</sup> The presence of teosinte in the EU has been reported in maize fields in Spain (in the Ebro Valley (Aragón) and in the region of Cataluña in the summer of 2014) and, to a lesser

<sup>5</sup> <http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2015-00235>

<sup>6</sup> <http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2015-00650>

<sup>7</sup> <http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2016-00390>

<sup>8</sup> <http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2016-00389>

<sup>9</sup> <http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2016-00388>

extent, in France (in the region of Poitou-Charentes since 1990). Pathways to harm from the cultivation of maize MON 810 were hypothesised for situations where maize MON 810 and teosinte would grow sympatrically, focusing on specific areas of risk typically considered in ERAs of GM plants. For each of these pathways, it is unlikely that environmental harm will be realised. EFSA therefore concluded that there are no data that indicate the necessity to revise the previous ERA conclusions and risk management recommendations for maize MON 810 made by the GMO Panel.

On 1 September 2016, the European Commission received from the consent holder the annual PMEM report for the 2015 cultivation season of maize MON 810 (hereafter referred to as 2015 PMEM report).<sup>10</sup>

On 13 October 2016, the European Commission requested the GMO Panel to assess the 2015 PMEM report and, in particular, *to evaluate the findings of the monitoring activities, taking into consideration the comments received from Member States and to assess the appropriateness of the methodology if this is found to differ compared to the previous season.*

On 17 January 2017, the European Commission asked the consent holder to provide additional information to assess the 2015 PMEM report upon EFSA request. The requested information was received on 6 February 2017.

On 7 February 2017, EFSA held an applicant's hearing to which the consent holder participated as a hearing expert of the PMEM Working Group and provided clarifications on the methodology of the monitoring activities followed in the 2015 PMEM report.<sup>11</sup>

On 20 March 2017, the European Commission sent an updated version of the 2015 PMEM report and asked the GMO Panel to take it into consideration in the adoption of the Scientific Opinion.

## 2. Data and methodologies

### 2.1. Data

In delivering this scientific opinion, the GMO Panel took into account the data set derived from the CSM and GS activities performed by the consent holder:

- The IRM plan consisting of: (1) the 'high-dose/refuge' strategy, including surveys on farmers compliance with non-*Bt*-maize refuges; (2) field monitoring and laboratory assays to measure changes in baseline susceptibility to the Cry1Ab protein in target pest populations<sup>12</sup>; (3) a communication and education plan of farmers; (4) a remedial action plan in the event of any confirmed evolution of pest resistance<sup>13</sup>;
- the outcome of the farmer alert system;
- a survey based on 261 questionnaires received from farmers in the main two countries growing maize MON 810: 212 in Spain and 49 in Portugal<sup>14</sup>;
- company stewardship activities and technical user guides<sup>15</sup>;
- an assessment of 18 peer-reviewed scientific studies relevant to the risk assessment and/or management of maize MON 810, which were published between June 2015 and May 2016.<sup>16</sup>

The additional information provided by the consent holder on 6 February 2017 following a request from EFSA was also considered.

### 2.2. Methodologies

The GMO Panel conducted a scientific assessment on the data submitted in the context of the annual 2015 PMEM report, in accordance with Annex VII to Directive 2001/18/EC. Following the terms of reference of the European Commission mandate, the GMO Panel also considered whether the methodology applied in the monitoring activities during the 2015 growing season differed from that followed in the previous PMEM reports on maize MON 810.

<sup>10</sup> [http://ec.europa.eu/food/plant/gmo/reports\\_studies\\_en](http://ec.europa.eu/food/plant/gmo/reports_studies_en)

<sup>11</sup> <http://www.efsa.europa.eu/sites/default/files/gmopmemreports2017.pdf>

<sup>12</sup> 2015 PMEM report, Appendices 7 and 8.

<sup>13</sup> 2015 PMEM report, Appendix 6. More details on the key elements of the IRM plan are described in EFSA GMO Panel (2011a).

<sup>14</sup> 2015 PMEM report, Appendix 1.

<sup>15</sup> 2015 PMEM report, Appendices 3.1–3.5.

<sup>16</sup> 2015 PMEM report, Appendices 5.1–5.4.



The GMO Panel took into account the principles and requirements described in its guidance on the PMEM of GM plants (EFSA GMO Panel, 2011b), and EFSA's guidance on the application of systematic review methodology to food/feed safety assessments to support decision-making (EFSA, 2010).

The comments raised by Member States were taken into consideration during the assessment of the 2015 PMEM report and the development of this scientific opinion.<sup>17</sup>

### 3. Assessment

#### 3.1. Implementation of non-*Bt*-maize refuges

Compliance with refuge requirements and the implementation of the operational details of the IRM plan were assessed through the farmer questionnaires supplied as part of GS. The consent holder asked 212 farmers from Spain and 49 farmers from Portugal, the two main EU countries where maize MON 810 was cultivated in 2015 (i.e. approximately 99% of the maize MON 810 area in the EU), to complete a questionnaire which included a question on compliance with the refuge strategy.

In Spain, 200 farmers growing maize MON 810 complied with refuge requirements. Thirty-eight of those farmers (18% of the farmers surveyed) were not required to plant a refuge because the area of maize MON 810 was less than 5 ha (Appendix A). The 12 farmers that did not plant a refuge but cultivated an area of maize MON 810 of more than 5 ha provided the following two main reasons for their non-compliance (as indicated in the survey): (1) corn borers cause harvest losses in conventional maize (eight farmers); and (2) the implementation of refuges complicates the sowing (four farmers). The consent holder did not provide the exact location of the *Bt*-maize fields where no refuges were planted.

In Portugal, the four farmers that did not plant a refuge reported that they were part of a 'production area' which had made refuge arrangements to ensure refuge compliance, while the other 45 maize MON 810-growing farmers surveyed complied with the refuge requirements (none of them were exempted since the maize MON 810 area was more than 5 ha). In addition to the farmer questionnaires, the Portuguese authorities performed inspections at 66 farms (out of the 216 notifications received in 2015) where maize MON 810 was grown to check compliance with refuge and coexistence requirements outlined in Portuguese law. Based on these inspections, the Portuguese authorities concluded that there was full compliance with refuge requirements.

#### GMO Panel assessment

The 2015 PMEM report shows full compliance with refuge requirements in Portugal and partial compliance (93%) in Spain as observed in previous years (Appendix A). As pointed out by Tabashnik et al. (2013), Castañera et al. (2016) and others, refuge compliance is crucial to sustain the efficiency of the technology and delay resistance evolution, especially in regions of high adoption rate (i.e. > 60%).<sup>18</sup> The GMO Panel considers that there should be full compliance in high adoption areas. Therefore, the GMO Panel reiterates that the consent holder should strive to increase the level of compliance in those areas.

The GMO Panel reminds that the refuge requirements also apply to clusters of small maize MON 810 fields (i.e. a group of adjacent fields that can be from different farms) in which the aggregate area planted with maize MON 810 is greater than 5 ha.<sup>19</sup> The GMO Panel recommends the consent holder and Member States to develop appropriate information systems on GM crop cultivation.

<sup>17</sup> Comments were received from Austria, Germany, Hungary, Italy, the Netherlands, Romania and Spain.

<sup>18</sup> The adoption rate of maize MON 810 is expressed as a fraction of total maize cultivation in the same geographical area (i.e. geographical zone where maize is typically grown following similar agronomic practices isolated from other maize areas by barriers that might impair an easy exchange of target pests between those areas).

<sup>19</sup> When cultivating maize MON 810, the presence of refuge areas equivalent to at least 20% of the surface planted with maize MON 810, should be ensured when a single field cropped to maize MON 810 is larger than 5 ha, and when a cluster of adjacent fields cropped to maize MON 810 has an aggregated surface greater than 5 ha, irrespective of individual field and farm size (EFSA, 2009).

## 3.2. Resistance monitoring of target pests

### 3.2.1. Changes in baseline susceptibility to Cry1Ab in corn borers

#### 3.2.1.1. Field sampling of ECB/MCB populations

For the 2015 resistance monitoring activities, ECB and MCB larvae were collected at the end of the maize-growing season from corn borer populations<sup>20</sup> found in refuges and conventional maize fields adjacent to maize MON 810 fields (hereafter also referred to as sampling sites) occurring in two different geographical areas of the Iberian Peninsula: north-east and central for ECB, and north-east for MCB. Samples collected in 2015 included corn borer populations from north-east, which is the area with the highest adoption rate of maize MON 810 in the Iberian Peninsula (Appendix B), and therefore with the highest selection pressure (and thus where field resistance is more likely to evolve).

For ECB, 376 and 443 last-instars were collected from three fields in north-east and three fields in central Iberia, respectively (Table 1). Two and four additional sites were sampled in each area, but the minimum number of 100 larvae established in the harmonised IRM plan was not found. A total of 152 and 180 larvae reached the adult stage (40% and 41% of the field-collected larvae) and were placed in 10 and 9 oviposition cages. All cages were used for mating to obtain F<sub>1</sub> larvae for the bioassays.

For MCB, 529 last-instars were collected from three fields in north-east Iberia (Table 1). In the laboratory, 444 of the field-collected larvae reached the adult stage (16% pre-imaginal mortality) and were placed in 28 oviposition cages for mating. Of those, 12 oviposition cages, containing a total of 195 adults (37% of the number of field-collected larvae), were used to obtain F<sub>1</sub> larvae for the bioassays.

For both species, emerging adults from the different sampling sites of a given geographical area were pooled for mating.

**Table 1:** Field collection of *Ostrinia nubilalis* (ECB) and *Sesamia nonagrioides* (MCB) larvae in the 2015 growing season in different areas of the Iberian Peninsula

Target pest	Geographical area	Sampling site (Province)	No. larvae collected
ECB	North-east Iberia <sup>(a)</sup>	Alberuela de Tubo (Huesca)	132
		Candasnos (Huesca)	144
		Mélida (Navarra)	100
		<b>Total</b>	<b>376</b>
	Central Iberia <sup>(b)</sup>	La Herrera (Albacete)	106
		Motilleja (Albacete)	178
		Santa Ana (Albacete)	159
		<b>Total</b>	<b>443</b>
MCB	North-east Iberia	Alberuela de Tubo (Huesca)	107
		Candasnos (Huesca)	162
		Beire (Navarra)	260
		<b>Total</b>	<b>529</b>

Larvae were collected between 7 and 8 September 2015 from central Iberia and between 21 and 23 September 2015 from north-east Iberia. Geographical coordinates were not provided for the sampling sites. All ECB larvae collected were in diapause, while most of the MCB larvae collected were not.

(a): Two additional sites were inspected but did not yield sufficient larvae for further analysis.

(b): Four additional sites were inspected but did not yield sufficient larvae for further analysis.

#### 3.2.1.2. Insect bioassays

Concentration–response assays and diagnostic concentration assays<sup>21</sup> were conducted to assess the susceptibility of the ECB and MCB populations collected in 2015 to the Cry1Ab protein, and to monitor possible changes in baseline susceptibility. In both assays, neonate larvae of the subsequent

<sup>20</sup> The term *population* is referred in the present opinion to those corn borers sampled in a given geographical area (i.e. north-east and central Iberia).

<sup>21</sup> The terminology used to refer to the laboratory bioassays (i.e. concentration–response and diagnostic concentration) in the present opinion is different to that used by the consent holder (i.e. dose–response and diagnostic dose). Since the actual amount of Cry1Ab ingested by the tested larvae was not measured and, therefore, actual doses of Cry1Ab could not be estimated, the GMO Panel considers that the term *concentration* is more precise.

generation ( $F_1$  larvae) were used. The neonates were exposed to purified Cry1Ab protein in artificial-diet overlay assays.<sup>22</sup> In addition, a confirmatory experiment with leaves of maize MON 810 was used to check the absence of resistant individuals in the progenies obtained from field-collected larvae.

Raw data of the insect bioassays were not made available by the consent holder even following a specific request by EFSA on 17 January 2017.

## Concentration–response bioassays

### Methodology

Eight concentrations, ranging from 0.2 to 28.22 ng Cry1Ab/cm<sup>2</sup>, and seven to ten concentrations, ranging from 1 to 128 ng Cry1Ab/cm<sup>2</sup>, and a negative control (i.e. the same buffer solution in which the purified Cry1Ab protein was dissolved) were tested for the ECB and MCB populations, respectively. The concentration of 28.22 ng Cry1Ab/cm<sup>2</sup> was also used as the diagnostic concentration for ECB testing for resistance. In all bioassays, three replicates were used for each concentration and the control, each one consisting of 32 larvae (64 for the controls), giving a total of 96 larvae tested for each concentration (192 for the controls). Mortality and moult inhibition was assessed after seven days of exposure.<sup>23</sup> Moult inhibition concentration for 50% (MIC<sub>50</sub>) and 90% (MIC<sub>90</sub>) values, with a 95% confidence interval (CI), were estimated by probit analysis (Robertson et al., 2007).

The susceptibility of the ECB and MCB laboratory reference strains to Cry1Ab was assessed in the same manner as described above.<sup>24</sup>

Resistance ratios (RR) of field populations with 95% CI were calculated with respect to the MIC<sub>50</sub> and MIC<sub>90</sub> values obtained for the reference laboratory strains.<sup>25</sup> Moult inhibition concentrations of the field-collected populations were considered significantly different with respect to the respective laboratory reference strain if the 95% CI values for the RR MIC<sub>50,90</sub> did not include 1.

### Results

A concentration–response was not observed for any of the ECB populations tested and, consequently, 50% and 90% lethal concentration (LC<sub>50</sub> and LC<sub>90</sub>) values were not estimated. The consent holder indicated that *it is not uncommon to see Bt-susceptible insects survive the duration of the assay by feeding minimally or not at all (...) because Bt proteins have anti-feedant properties and the conditions under which such assays are run are relatively benign*. LC<sub>50</sub> and LC<sub>90</sub> values were not provided for the MCB population collected in 2015.

Moult inhibition (mean  $\pm$  standard deviation) in the control groups was  $2.1 \pm 3.6\%$  and  $3.1 \pm 1.6\%$  for the ECB populations of north-east and central Iberia, and  $7.4 \pm 2.7\%$  for the MCB population.

The MIC<sub>50</sub> and MIC<sub>90</sub> values estimated for the ECB and MCB populations tested in 2015 are given in Table 2 and Table 3, respectively. The MIC<sub>50</sub> and MIC<sub>90</sub> values are in the range of those reported in previous years.

Significant differences in MIC<sub>50</sub> were observed between the field-collected ECB and MCB populations and their respective susceptible laboratory reference strains due to a decrease in susceptibility of the latter compared to previous seasons (Appendix C). The consent holder indicated that *the decrease in susceptibility of the lab population could be due to variations that occur in laboratory-reared insects produced by a variety of reasons*. Regarding the susceptibility of the field-collected ECB and MCB populations, the consent holder concluded that *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*.

<sup>22</sup> For the bioassays with ECB larvae, batch 2a was used (1.6 mg Cry1Ab/mL in 50 mM sodium bicarbonate buffer, pH 10.25, 91% purity). For the bioassay with MCB larvae, batch B3 was used (1.8 mg Cry1Ab/mL in 50 mM sodium bicarbonate buffer, pH 10.25, 91% purity).

<sup>23</sup> Mortality is defined as larvae not showing any reaction when prodded whereas moult inhibition is defined as larvae that either have died or not moult to the second instar after 7 days.

<sup>24</sup> ECB strains were established from larvae collected from Niedernberg (Germany) in 2002 and from 145 larvae collected from Galicia (Spain) in 2015. The MCB strain was established from larvae collected from Andalucía (661 larvae), Madrid (793 larvae), Ebro (857 larvae) and Galicia (665 larvae) (Spain) in 1998 (González-Núñez et al., 2000). To preserve its vigour, the MCB strain was refreshed periodically with new individuals. To this end, the progenies of the populations collected for the monitoring bioassays are used, and between 10% and 15% of new individuals with respect to the laboratory strain are introduced. ECB strains have not been refreshed yet. The similarity in susceptibility is verified before the introduction of new individuals.

<sup>25</sup> In the 2015 PMEM report, RR for ECB and MCB were calculated differently: for ECB,  $RR = MIC \text{ laboratory reference strain} / MIC \text{ field-collected population}$ ; for MCB,  $RR = MIC \text{ field-collected population} / MIC \text{ laboratory reference strain}$ .

**Table 2:** Historical data of susceptibility to the purified Cry1Ab protein of North East and Central Iberian field populations of *Ostrinia nubilalis* (ECB) collected in refuge areas and/or conventional maize fields adjacent to maize MON 810 fields

Population	Season	No. larvae collected (no. of sites)	Protein batch <sup>(a)</sup>	MIC <sub>50</sub> (95% CI) <sup>(b)</sup>	MIC <sub>90</sub> (95% CI) <sup>(b)</sup>	RR MIC <sub>50</sub> <sup>(c)</sup>	RR MIC <sub>90</sub> <sup>(c)</sup>
North-east Iberia	2008	401 (4)	1	7.03 (4.89–10.03)	23.91 (15.76–46.84)	3.11/3.18 <sup>*,(d)</sup>	2.93/5.35 <sup>*,(d)</sup>
	2009	509 (3)	1	6.40 (5.32–7.75)	13.68 (10.77–20.02)	1.75*	1.43
	2011	382 (6)	2	1.79 (1.54–2.07)	4.19 (3.45–5.48)	0.61*	0.67
	2013	452 (3)	2a	2.48 (2.03–3.02)	5.41 (4.27–7.61)	1.26	0.82
	2015	376 (3)	2a	2.12 (1.75–2.55)	5.43 (4.36–7.29)	0.53*	0.77
Central Iberia	2009	396 (2)	1	3.09 (2.03–4.33)	11.98 (8.12–22.31)	0.85	1.25
	2011	404 (3)	2	1.56 (1.27–1.91)	4.04 (3.12–5.91)	0.53*	0.64
	2013	432 (2)	2a	2.40 (2.04–2.83)	6.38 (5.18–8.34)	1.22	0.97
	2015	443 (3)	2a	1.88 (1.68–2.11)	3.38 (2.91–4.21)	0.47*	0.48*

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

(a): Data provided by the consent holder in previous monitoring reports showed that the Cry1Ab protein batches 1 and 2, and Protein batches 2 and 2a have similar insecticidal activity (see Appendix C).

(b): 50% and 90% moulting inhibition concentration (MIC<sub>50</sub> and MIC<sub>90</sub>) and their 95% confidence intervals (CI 95%) are expressed in ng Cry1Ab/cm<sup>2</sup>.

(c): Resistance ratio (RR) between MIC values of the field-collected populations and the reference laboratory strain for each cultivation season. RR were calculated by EFSA since the consent holder calculated them differently (i.e.  $RR = MIC_{\text{reference laboratory strain}} / MIC_{\text{field-collected population}}$ ).

(d): The laboratory reference strain was tested two times in 2008 (see Appendix C).

**Table 3:** Historical data of susceptibility to the purified Cry1Ab protein of North East Iberian field populations of *Sesamia nonagrioides* (MCB) collected in refuge areas and/or conventional maize fields adjacent to maize MON 810 fields

Population	Season	No. larvae collected (no. of sites)	Protein batch <sup>(a)</sup>	MIC <sub>50</sub> (95% CI) <sup>(b)</sup>	MIC <sub>90</sub> (95% CI) <sup>(b)</sup>	RR MIC <sub>50</sub> (95% CI) <sup>(c)</sup>	RR MIC <sub>90</sub> (95% CI) <sup>(c)</sup>
North-east Iberia	2005	400 (2)	B1	9 (3–15)	76 (54–117)	0.5 (NR) <sup>(d)</sup>	0.8 (NR) <sup>(d)</sup>
	2007	457 (3)	B1	14 (8–20)	99 (71–158)	0.9 (NR)	1.0 (NR)
	2009	489 (3)	B1	22 (16–28)	188 (138–277)	1.1 (0.8–1.7)	1.6 (NR)
	2011	564 (4)	B2	20 (14–27)	135 (91–232)	2.2 (1.6–3.0)*	2.0 (1.3–2.9)*
	2013	742 (5)	B2	19 (14–25)	163 (108–287)	2.6 (2.0–3.4)*	3.4 (2.2–5.2)*
	2015	529 (3)	B3	17 (13–21)	84 (63–124)	0.6 (0.5–0.8)*	1.3 (0.9–1.8)

NR: not reported.

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

(a): Data provided by the consent holder in previous monitoring reports showed that the Cry1Ab protein batches B1 and B2, and B2 and B3 have similar insecticidal activity (see Appendix C).

(b): 50% and 90% moulting inhibition concentration (MIC<sub>50</sub> and MIC<sub>90</sub>) and their 95% confidence intervals (CI 95%) are expressed in ng Cry1Ab/cm<sup>2</sup>.

(c): Resistance ratio (RR) between MIC values of the field-collected populations and of the susceptible laboratory strain for each cultivation season.

(d): MIC<sub>50</sub> and MIC<sub>90</sub> values of the laboratory reference strain used to calculate RR MIC<sub>50</sub> and RR MIC<sub>90</sub> correspond to those estimated in 2004.

## Diagnostic concentration assays

### Methodology

A diagnostic concentration assay was performed with the MCB population collected in 2015 using a concentration of 726 ng Cry1Ab/cm<sup>2</sup>.<sup>26</sup> The highest concentration of the concentration–response bioassay (28.22 ng Cry1Ab/cm<sup>2</sup>) was used as the diagnostic concentration for the ECB field

<sup>26</sup> The diagnostic concentration for ECB was established by pooling data from populations collected in the Czech Republic, France, Germany, Italy, Hungary, Poland, Portugal, Romania and Spain between 2005 and 2012. This resulted in a MIC<sub>99</sub> value of 48.2 (42.8–55.1) ng Cry1Ab/cm<sup>2</sup>. Due to a change in the protein batch in 2012, the diagnostic concentration was re-calibrated, resulting in a MIC<sub>99</sub> value of 28.22 ng Cry1Ab/cm<sup>2</sup>. The diagnostic concentration for MCB was established by pooling data from populations collected in north-east, central and south-west between 2008 and 2012. This resulted in a MIC<sub>99</sub> value of 726 (548–1013) ng Cry1Ab/cm<sup>2</sup>.

populations collected in 2015. For both species, three replicates were used, each one consisting of 32 larvae, giving a total of 96 larvae tested. The negative controls were the same as for the concentration–response bioassays. Moulting inhibition was assessed after seven days.

### Results

Table 4 shows the results of the diagnostic concentration assays with ECB and MCB populations. Moulting inhibition of ECB larvae collected in north-east and central Iberia, and MCB larvae collected in north-east Iberia in 2015, was 100% since no single larvae survived after 7 days of exposure.

**Table 4:** Moulting inhibition of north-east and central Iberian field populations of *Ostrinia nubilalis* (ECB) and *Sesamia nonagrioides* (MCB) tested with a diagnostic concentration of Cry1Ab

Species	Population	Season	Protein batch <sup>(a)</sup>	Moulting inhibition (% $\pm$ SE)
ECB	North-east Iberia	2013	2a	100
		2015	2a	100
	Central Iberia	2013	2a	100
		2015	2a	100
MCB	North-east Iberia	2013	B2	97 $\pm$ 2
		2015	B3	100

Diagnostic concentration is defined as the concentration causing 99% of moulting inhibition to first instars. The diagnostic concentration was 28.22 ng Cry1Ab/cm<sup>2</sup> for ECB populations and 726 ng Cry1Ab/cm<sup>2</sup> for MCB. For both species, three replicates were used, each one consisting of 32 larvae, giving a total of 96 larvae tested.

SE: standard error.

(a): Data provided by the consent holder in previous monitoring reports showed that the Cry1Ab protein batches B2 and B3 have similar insecticidal activity (see Appendix C).

### Confirmatory experiment with maize MON 810 leaves

#### Methodology

The survival of the four ECB larvae that survived the concentration–response bioassay at a concentration of 14.1 and 20 ng Cry1Ab/cm<sup>2</sup> (two larvae for each concentration), all surviving MCB larvae from the concentration–response assay (574 larvae), and the approximate 1,400 MCB F<sub>1</sub> larvae that were not used in the bioassays was tested in a confirmatory experiment with maize MON 810 leaves. Larvae were fed maize MON 810 leaves *ad libitum*. No negative control treatments (i.e. larvae fed near-isogenic maize leaves) were used.

### Results

None of the ECB and MCB larvae fed maize MON 810 leaves survived.

### GMO Panel assessment

Changes in the methodology compared with the 2014 PMEM report were not identified. Consistent with its previous recommendations, the GMO Panel acknowledges that the consent holder has provided further details on the laboratory rearing of corn borers, the reference laboratory strains and the methodology of the bioassays (EFSA GMO Panel, 2016). To evaluate and verify data quality, and to be in a position to fully assess the analysis of the laboratory assays performed with the progeny of the corn borers sampled, the consent holder should provide the raw data in future PMEM reports.

To ensure the timely implementation of remedial measures, the GMO Panel previously recommended that a detection level of 3% resistance allele frequency in target pest populations is the maximum level that should be achieved in practice (for further details, see EFSA, 2015 and EFSA GMO Panel, 2016). Between 152 and 195 emerging adults were placed in oviposition cages to obtain F<sub>1</sub> larvae for the susceptibility bioassays (representing between 32% and 38% of the field-collected larvae), and only 96 F<sub>1</sub> larvae were finally used in the diagnostic concentration assay (for instance, see Marçon et al., 2000 and Alcantara et al., 2011). This implies that a maximum of 192 alleles were tested for each population (i.e. two alleles per larvae, assuming that no siblings were tested), and therefore, the actual limit of resistance allele frequency that could be detected with the methodology used in the diagnostic concentration assay in 2015 was above the targeted threshold (for further details, see Roush and Miller, 1986 and Andow and Ives, 2002). The GMO Panel acknowledges that



several limitations exist for sampling adequate numbers of target pests in the fields to reach the targeted threshold, but encourages the consent holder to increase sampling efforts, and ensure that as many field-collected larvae as possible are represented in the laboratory assays as F<sub>1</sub> larvae in order to provide sufficient detection sensitivity.

The GMO Panel has previously recommended focusing the monitoring activities in north-east Iberia (i.e. the Ebro valley), where adoption rates of maize MON 810 are the highest in the Iberian Peninsula (Appendix B), and where field resistance to Cry1Ab is more likely to evolve. Within the Ebro valley, ECB and MCB larvae should be collected from at least three sampling zones of approximately 10 km × 10 km, where adoption rate of maize MON 810 is higher than 60% for at least three consecutive years (EFSA, 2015). Since information on the adoption rate of maize MON 810 is only available at the province level, the GMO Panel is not in a position to assess what has been the actual selection pressure in the vicinity of sampled fields over the last years. The GMO Panel therefore recommends the consent holder and Member States to develop appropriate information systems on GM crop cultivation and invites the consent holder to report estimates of historical adoption rates in the vicinity (e.g. *comarcas*, municipalities) of fields that were sampled.

In the 2015 PMEM report, only MIC values were provided for MCB and ECB. For ECB, LC values could not be estimated because a concentration–response was not observed (see Section 3.2.2). The consent holder claimed that it might be due to antifeeding properties of Bt-proteins but did not provide any evidence supporting this assumption. 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 Bt-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 report both LC and MIC values for ECB and MCB in future PMEM reports (EFSA GMO Panel, 2012a).

The MCB laboratory reference strain has been periodically refreshed with larvae collected from the same sampling zones as for the monitoring activities. It is likely that some of the individuals of the strain were subjected to selection pressure, therefore affecting the value of resistance ratios to measure changes in susceptibility to Cry1Ab. If refreshment of laboratory reference strains is needed, individuals should be collected from areas where Cry1-expressing maize is not grown.

The confirmatory experiment with maize MON 810 leaves was used to further test for 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. However, since there were no negative controls (i.e. larvae fed with near-isogenic maize leaves), these confirmatory tests cannot be used to reinforce the bioassays with purified Cry1Ab as uncertainty remains on the suitability of the test system and the reliability of the obtained results (Romeis et al., 2011). Moreover, further information (e.g. 50% lethal time (LT<sub>50</sub>), developmental stage at death, Cry1Ab protein expression in detached leaves, would be needed to correctly interpret the results.

### 3.2.1.3. Farmer alert system

The consent holder has implemented a farmer alert system allowing farmers to report complaints about product performance (including unexpected crop damage caused by target pests).

The consent holder stated that, during the 2015 cultivation season, more than 300 complaints were received and assessed. None of these complaints were related to infestation of maize MON 810 by corn borers.

Although this farmer alert system could be a useful complement to the information received from the laboratory bioassays, the GMO Panel is currently not in a position to appraise its usefulness, and therefore encourages the consent holder to provide more information on this complementary resistance monitoring tool. More information is required to determine whether appropriate communication mechanisms and fit-for-purpose educational programs (e.g. characterisation of the damage caused by corn borers) are implemented ensuring the timely and effective reporting of farmer complaints.

### 3.2.2. Conclusions on resistance monitoring of target pests

The analyses of the data set provided by the consent holder do not indicate a decrease in susceptibility to the Cry1Ab protein of the corn borer samples tested in the 2015 cultivation season. However, the GMO Panel is of the opinion that, due to the low number of field ECB and MCB genotypes tested, there is not enough power to assess whether the frequency of resistance alleles is actually below the targeted threshold of 3%.

### 3.3. Farmer questionnaires

In its annual 2015 PMEM report, the consent holder submitted a survey completed between December 2015 and February 2016 based on 261 questionnaires received from farmers in two European countries: 212 in Spain and 49 in Portugal (Table 5). No farmers from the Czech Republic, Romania and Slovakia, representing approximately 1% of the maize MON 810 grown in the EU in 2015, were interviewed. The consent holder concluded that the analysis of the questionnaires *did not identify any potential adverse effects that might be related to MON 810 plants and their cultivation*.

**Table 5:** Farmers surveyed and maize MON 810 areas monitored in 2015 through questionnaires

Country	No. of farmers surveyed	Mean maize MON 810 area monitored per farmer (ha)	Monitored maize MON 810 area (ha)	Total planted MON 810 area (ha)	Monitored maize MON 810 (% of total area)
Spain	212 <sup>(a)</sup>	26	5,466	107,749	5.1
Portugal	49 <sup>(b)</sup>	66	3,251	8,017	40.5

Farmers from the Czech Republic, Slovakia and Romania, representing approximately 1% of the cultivated area of maize MON 810 in the EU, were not surveyed.

(a): Eighty-six farmers were from Aragón, 61 from Cataluña, 23 from Andalucía, 18 from Extremadura, 13 from Navarra and 11 from Castilla la Mancha.

(b): Twenty-three farmers were from Alentejo, 15 from Lisbon, 8 from Central and 3 from North.

The methodology of the farmer questionnaires to identify unanticipated adverse effects caused by the cultivation of maize MON 810 was similar to that in previous annual PMEM reports (EFSA GMO Panel, 2011a, 2012a, 2013, 2014a, 2015b, 2016). In the assessment that follows, some important observations and comments on the farmer questionnaire survey are offered. Additional ones can be found in Annex A of EFSA GMO Panel (2016).

#### GMO Panel assessment

The GMO Panel makes the following observations and comments on the methods and results of the farmer questionnaire survey:

- The farmer questionnaires survey should ideally aim to collect responses relating to areas of actual maize MON 810 cultivation. However, it is stated that *the sampling frame for this survey cannot be based on the total population of fields with MON 810 cultivation in Europe*, and so farmers are sampled instead of fields. As the farmer questionnaires survey currently targets the population of European farmers growing maize MON 810, it should be conducted on a representative sample of that population. Survey design methodology requires the use of a sampling frame which is representative for the sampled target populations and that the random selection process is applied to the sample units in the sampling frame prior to proceeding with the interviews. In the annual 2015 PMEM report, it is not clear whether such sampling frames were used since it is indicated that *GMO cultivation register information – where publicly available – is used to identify the regions of cultivation. It cannot not be used to identify the cultivating farmers since in most countries the personal data of farmers are not freely available and Farmers therefore are selected from customer lists of the seed selling companies or interviewer companies, plus experience from previous surveys or search in the region*. Therefore, it cannot be ascertained that the selected farmers (from Portugal and Spain) are representative of the target farmer population. Additionally, it is unclear whether the current sampling strategy does take into account farmers growing maize MON 810 varieties bought from other companies. The GMO panel advises that the sampling frame used should take account of all maize MON 810 varieties cultivated in the EU.
- For the reasons stated above, the claim *The whole sampling procedure ensures that the monitoring area will be proportional to and representative of the total regional area under GM cultivation* cannot be substantiated, based on the information provided in the report. The selection procedure, reportedly gives emphasis on areas of high adoption of MON 810; however, it is not completely clear how this is done. The consent holder should clearly describe how the number of farmers sampled by country/area per year is calculated and how the results of these calculations ensure that areas of intensive maize MON 810 cultivation are appropriately represented in the survey. A description of the method to ensure that units are randomly

selected from the sampling frame should be included in the report, including, where relevant, the statistical software and/or the program code used for this procedure. The proportion of farmers cultivating maize MON 810 for the first time and of farmers with previous experience of maize MON 810 selected from the sampling frame in each region should be presented in the monitoring report in order to provide evidence that the sampling method ensures that areas of intensive maize MON 810 cultivation are appropriately covered by the survey.

- The questionnaire relies on a comparison between a representative GM maize field and a representative conventional field in order to detect unanticipated adverse effects. Consequently, the choice of representative fields and the recollection of similarities and differences are crucial to the success of the survey. The questionnaire provides a list of the GM and non-GM varieties grown by each farmer, but it is unclear which conventional and GM fields have been actually compared. The farmer questionnaire should be more specific on the comparison that has been made. If no comparators are being grown spatially or temporally close to the GM crop, then the rationale for selecting another comparator (e.g. maize grown in previous years) should be fully described. The specific comparators selected by the farmers for the survey should also be summarised in the monitoring report.
- Farmer questionnaires should focus only on changes that would be recognised by the farmer during the daily management of the farm. However, additional questions could be included to gain a better understanding of the intensity of GM maize cultivation on the farm (number of years of maize MON 810 cultivation and frequency of maize MON 810 in crop rotations), and further information on plant protection product usage (in particular, in the comparator field) should be obtained to facilitate a full understanding of any observed changes. Moreover, qualitative responses may sometimes relate to a subjective assessment on the part of the farmer. An effort should be made to use objective measurable outcomes, whenever this is possible. In addition, the possible answers to the question concerning the insect pest control (for the two corn borers) in the MON 810 maize compared to the conventional maize are 'very good', 'good', 'weak' and 'don't know'. Given that the question requests again a comparison with the conventional maize, this set of possible responses does not appear to be appropriate. It should be ensured that the interpretation of each of the offered response options is clear to the farmers and some relevant information should also be offered in the survey report. Based on the information provided in the report, it is not clear how exactly these response options were interpreted by the farmers, especially the option 'good' which has been indicated by 4.2% of the surveyed farmers for each of the two borers in the 2015 farmer questionnaire survey. No statistical tests have been presented for these variables.
- Focusing on the analysis of the data from the questionnaires included in the 2015 PMEM report, a similar comment as last year concerning the overall type I error in the statistical comparisons is warranted. The choice of statistical test should be based on the number of possible outcomes, since the use of a series of binomial tests for multinomial distributions would increase the experiment-wise Type I error rate (i.e. failure to detect a true adverse effect). In the current analysis, a closed principle test procedure is proposed to be used in order to address the issue of the overall type I error. This approach is acceptable, and can be effective for this purpose, if applied correctly, with the relevant not 'as usual' effects being assessed only when the null hypothesis  $p_{As\ usual} \leq 0.9$  is not rejected. However, in practice, in the monitoring characteristics comparisons presented in the 2015 PMEM report (table 5 of Appendix 1), the results of all possible tests are presented, when in the first hypothesis test the null hypothesis  $p_{As\ usual} \leq 0.9$  is not rejected. Therefore, in the current report whenever the  $p_{As\ usual} \leq 0.9$  hypothesis is rejected, the decision is based only on this outcome. When this hypothesis is not rejected then, in the case of questions with three possible responses, two additional tests (for the probabilities of *Plus*- and *Minus*-answers) are being conducted, and therefore, some correction for the overall type-I error rate is still necessary.
- The GMO Panel considers that the sample size calculation provided in Annex I of the 2015 PMEM report indicates that the pre-set requirements (including the achievement of the pre-specified power) would be satisfied with a sample size of 2,500 questionnaires. Moreover, the statistical analysis should be planned to allow an analysis of the monitoring characteristics according to the length of GM crop cultivation in order to assess residual effects and possible trends. Certain effects may reach a sufficient magnitude for detection only with repeated cultivation of a GM crop, and so amendments to study design and the analysis plan should be considered in order to assess the effect of multiple years of GM crop cultivation. For all these

reasons, and also in order to achieve the statistical power described in the sample size calculations, the analysis needs to be pooled after 10 years. The 2015 PMEM report represents the 10th year report, but a pooled analysis of all the data of the 10 years was not provided. Therefore, the GMO Panel recommends that the analysis should be pooled at this 10-year period and analysis of the combined data sets is carried out. In such an analysis, consideration should be given to the consistency of questions to assess monitoring characteristics and the comparability of the obtained data from year to year, the possible inclusion of the same farmers in more than one year in the survey (and the enumeration of these farmers in the report) and the interim analyses performed for the annual reports.

### 3.3.1. Conclusions on farmer questionnaires

The consent holder reported that the analysis of the 2015 farmer questionnaires on maize MON 810 did not show any unanticipated adverse effects *related to MON 810 plants and their cultivation*.

The GMO Panel notes that in the 2015 PMEM report of maize MON 810 it is stated that 2,627 questionnaires have been completed over 10 years since the first questionnaires were conducted in 2006. A sample size of 2,500 has been 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. In its scientific opinion on the 2014 PMEM report of maize MON 810 (EFSA GMO Panel, 2016), the GMO Panel strongly recommended the consent holder to perform statistical analyses pooling all the data from the surveys obtained over the last 10 years and report the results of these analyses in the annual 2015 PMEM report. On 17 January 2017, the GMO panel invited the consent holder to submit such analysis for assessment by risk managers and EFSA. The consent holder informed on 6 February 2017 that *the analysis with the pooled data has not been included in the 2015 PMEM report as Monsanto's intention is to publish the results in a peer-reviewed journal*. The GMO Panel is of the opinion that the assessment of the results of the 10-year analysis is needed in order to evaluate the farmer questionnaire methodology for the detection of unintended effects caused by the cultivation of maize MON 810.

## 3.4. Existing monitoring networks

Directive 2001/18/EC and Council Decision 2002/811/EC propose to make use of existing monitoring networks, as such networks can complement farmer questionnaires and provide an additional tool for the GS of GM plants. 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.

As in 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, Perseus 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 searching

### 3.5.1. Relevant scientific studies reported by the consent holder

The consent holder performed a literature search to identify studies on maize MON 810 and Cry1Ab that were published in the peer-reviewed scientific literature between June 2015 and May 2016.

The consent holder used the databases Web of Science Core Collection<sup>27</sup> and CABI CAB Abstracts<sup>28</sup> to identify relevant studies. The scientific literature search conducted in the Web of Science Core Collection database was conducted every month, covering the period of June 2015 to May 2016. The single search performed in the CABI CAB Abstracts database was performed on 27 May 2016.

<sup>27</sup> <http://thomsonreuters.com/en/products-services/scholarly-scientific-research/scholarly-search-and-discovery/web-of-science-core-collection.html> (Accessed 19 April 2017).

<sup>28</sup> <http://www.cabi.org/publishing-products/online-information-resources/cab-abstracts/> (Accessed 19 April 2017).



The search strategy<sup>29</sup> used for the literature search was similar to that applied in the search reported in the previous annual PMEM report (for further details see EFSA GMO Panel, 2016).

The consent holder defined *a priori* eligibility/inclusion criteria to categorise the relevance of retrieved studies. Studies pertaining to a category related to the risk assessment of maize MON 810 were considered relevant.

After applying the eligibility/inclusion criteria, the consent holder identified 18 relevant primary research studies (hereafter referred to as publications) published between June 2015 and May 2016 (Appendix D). Five publications were relevant for the food and feed (FF) safety assessment (in terms of toxicity and allergenicity), 13 publications pertained to the ERA or the IRM of maize MON 810 (most studies assessed the interaction of maize MON 810 with target organisms and non-target organisms). Three of the 18 publications, Böttger et al. (2015), Holderbaum et al. (2015) and Xu et al. (2015), have already been assessed by EFSA (EFSA, 2016b) or the GMO Panel (EFSA GMO Panel, 2016), and have concluded that no environmental safety concerns owing to maize MON 810 and Cry1Ab were identified.

### GMO Panel assessment

The GMO Panel acknowledges that the consent holder has revised the protocol for the literature search accounting for the EFSA guidance on systematic review methodology (EFSA, 2010) and previous GMO Panel recommendations on literature searching performed in the context of PMEM reports (i.e. report the dates of the search, use of a second database, report the eligibility/inclusion criteria for relevance, and provide the full list of retrieved publications) (EFSA GMO Panel, 2016). However, the GMO Panel still considers that the consent holder should: (i) better define the review question, including its key elements, and its purpose; (ii) better report the results of the literature search; in particular, the number of scientific publications retrieved from each database, the number and list of publications remaining after removing duplicates, and the number of publications that were considered not relevant based on title and abstract on each of the steps of the searching process; and (iii) detail how the selection of relevant publications was conducted, provide the reason(s) for categorising each publication as not relevant, and report which of the relevant publications were previously risk assessed by EFSA.

The GMO Panel assessed all the scientific publications selected by the consent holder, and considers that these were adequately discussed and put into the context of the overall safety assessment of maize MON 810. The GMO Panel did not identify food/feed or environmental safety concerns pertaining to maize MON 810 or Cry1Ab from any the publications.

The findings of one publication confirms that, in addition to the target species, some regionally important lepidopteran pests, such as *Mythimna unipuncta* (Lepidoptera: Noctuidae), exposed to Lepidoptera-active Bt-maize events may also have the potential to evolve resistance to Cry1 proteins (García et al., 2015), and therefore, they should be considered within the PMEM of maize MON 810. For some of these regionally important lepidopteran pests, the Cry1Ab protein might not be expressed in relevant plant tissues at high toxic concentrations, meaning that one of the underlying assumptions contributing to the success of the 'high-dose/refuge' strategy in delaying resistance evolution is not fulfilled (EFSA, 2009). However, the likelihood of regionally occurring non-target lepidopteran pests to evolve resistance is lower than that of target pests due to the lower exposure and, therefore, routine insect resistance monitoring would not be proportionate at this time. Instead, GS through farmer questionnaires and/or literature searching is currently used to report information on the occurrence of regionally important lepidopteran pests other than ECB and MCB, and the occurrence of damaged maize MON 810 plants. Outbreaks of regionally important lepidopteran pests should trigger subsequent investigations, including CSM if necessary.

### 3.5.2. Conclusions on literature searching

The results reported in the relevant peer-reviewed scientific studies identified and considered by the consent holder in its 2015 PMEM report do not provide new information that would invalidate the previous FF and ERA conclusions on maize MON 810 made by the GMO Panel.

For future literature searches performed in the context of the 2016 PMEM report, the GMO Panel recommends the consent holder to follow the recommendations given in the EFSA's explanatory note to the guidance on literature searching (EFSA, 2017).

<sup>29</sup> The search strategy used to identify relevant scientific publications is given in Table 1 of the 2015 PMEM report.



## 4. Conclusions

The data reported in the 2015 PMEM report do not indicate any adverse effects on human and animal health or the environment arising from the cultivation of maize MON 810 during the 2015 growing season. The GMO Panel therefore concludes that the CSM and GS activities of maize MON 810 as carried out by the consent holder do 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 methodological limitations pertaining to insect resistance monitoring and farmer questionnaires that need further consideration by the consent holder, because the resistance monitoring activities do not provide sufficient sensitivity for an early detection of potential resistance of target pests in the field, and the sampling frame for the farmer questionnaires does not allow the assessment of the representativeness of the results.

## 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 provide sufficient time to implement appropriate mitigation measures to prevent field resistance. Therefore, the GMO Panel reiterates its previous recommendation that a threshold of 3% should be achieved in practice. The GMO Panel notes that the monitoring protocol adopted does not provide the sufficient sensitivity to detect early cases of resistance, and urges the consent holder to increase sampling efforts and ensure that as many field-collected larvae as possible are represented in the laboratory assays as  $F_1$  larvae.

The GMO Panel has previously recommended focusing the monitoring activities in north-east Iberia (i.e. the Ebro valley), where field resistance to Cry1Ab is more likely to develop. In that geographical area, insects should be collected annually from three sampling zones of approximately 10 km × 10 km where adoption rate of maize MON 810 has been higher than 60% for at least three consecutive years. In order to acquire this information, non-GM and maize MON 810 cropping areas at an appropriate scale should be made available by Member States.

For future PMEM reports, the GMO Panel recommends the consent holder:

- to provide both LC and MIC values and their 95% CI, for ECB and MCB field populations and their reference laboratory strains;
- to calculate RR for ECB by dividing the  $MIC_{50,90}$  of the field-collected population by the  $MIC_{50,90}$  of the respective laboratory reference strain;
- to include a negative control (i.e. larvae fed with near-isogenic maize leaves) and provide further information (e.g.  $LT_{50}$ , developmental stage at death, Cry1Ab protein expression in detached leaves) in the confirmatory experiment with maize MON 810 leaves so that these confirmatory tests can be appropriately assessed, and thus used to reinforce the bioassays with purified Cry1Ab;
- to collect individuals from areas where Cry1-expressing maize is not grown when refreshing the reference laboratory strains so that newly individuals have not been subjected to selection pressure;
- to disclose the raw data of insect bioassays to (1) evaluate and verify the quality of the data; and (2) to assess the analysis of such assays.

The GMO Panel considers that the consent holder should provide more information on the farmer alert system in order to appraise its usefulness as complementary resistance monitoring tool, and determine whether appropriate communication mechanisms and fit-for-purpose educational programs are implemented that ensure the timely and effective reporting of farmer complaints. Considering the implementation of non-*Bt*-refuges, the GMO Panel reiterates that the consent holder should pursue its efforts to further 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 GMO

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, 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 GMO Panel recommends the applicant to provide the pooled analysis from the surveys obtained over the last ten years in order to (1) confirm that no unintended effects caused by the cultivation of maize MON 810 have been observed; and (2) to evaluate the farmer questionnaire methodology for the detection of unintended effects caused by the cultivation of maize MON 810.

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.

Regarding the protocol for the literature search, the GMO Panel makes recommends the consent holder:

- to better define the review question, including its key elements, and its purpose;
- to better report the results of the literature search; in particular, the number of scientific publications retrieved from each database, the number and list of publications remaining after removing duplicates, and the number of publications that were considered not relevant based on title and abstract on each of the steps of the searching process;
- to detail how the selection of relevant publications was conducted, provide the reason(s) for categorising each publication as not relevant, and report which of the relevant publications were previously risk assessed by EFSA.

For future literature searches, the GMO Panel recommends the consent holder to follow the recommendations given in the EFSA's explanatory note to the guidance on literature searching (EFSA et al., 2017).

## Documentation provided to EFSA

- 1) Letter from the European Commission, dated 12 October 2016, to EFSA requesting the assessment of the annual PMEM report on the cultivation of maize MON 810 during the 2015 season (2015 PMEM report) provided by Monsanto; the PMEM report was annexed to the letter.
- 2) Comments from the Member States on the 2015 PMEM report.
- 3) Acknowledgment letter dated 30 November 2016 from EFSA to the European Commission.
- 4) Letter from the European Commission, dated 17 January 2016, to the consent holder requesting additional information.
- 5) Letter from the European Commission, dated 20 March 2017, to EFSA requesting to take into account the updated version of the 2015 PMEM report.

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## Abbreviations

AMU	Assessment and Methodological Support Unit
Bt	<i>Bacillus thuringiensis</i>
CI	confidence interval
CSM	case-specific monitoring
ECB	European corn borer
ERA	environmental risk assessment
FF	food and feed
GM	genetically modified
GMO	genetically modified organism
GMO	Panel EFSA Panel on Genetically Modified Organisms
GS	general surveillance
IRM	insect resistance management

LC	lethal concentration
LT	lethal time
MCB	Mediterranean corn borer
MIC	moulting inhibition concentration
PMEM	post-market environmental monitoring
RR	resistance ratio



## Appendix A – Compliance with refuge requirements by Spanish farmers between 2009 and 2015 from two sources

Growing season	No. farmers surveyed	No. farmers planting refuges	No. farmers not planting refuges		Compliance (%) <sup>(a)</sup>	Source <sup>(b)</sup>
			Field < 5 ha <sup>(a)</sup>	Field > 5 ha		
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

NR: not reported.

(a): Farmers planting < 5 ha of maize MON 810 in the farm are not required to plant a refuge. For the FQ, only farmers who were required to plant a refuge were considered for the calculation of non-compliance with refuge requirements.

(b): FQ: farmer questionnaires; Antama: Study sponsored by Spanish foundation supporting the use of new technologies in agriculture. In the surveys conducted by Antama all farmers were from the Ebro valley (Spain).

## Appendix B – Area and adoption rate of maize MON 810 in the north-east Iberia (Aragón and Cataluña), central Iberia (Albacete) and south-west Iberia (Extremadura and Andalucía) between 2011 and 2015 recorded by two sources

Season	Area maize MON 810 (ha) <sup>(a)</sup>	Source			
		Avances <sup>(b)</sup>		ESYRCE <sup>(c)</sup>	
		Total maize (ha)	Adoption rate (%)	Total maize (ha)	Adoption rate (%)
North-east Iberia					
2011	71,000	113,299	62.7	125,297	56.7
2012	75,200	108,621	69.2	107,564 <sup>(d)</sup>	69.9
2013	88,447	125,293	70.6	119,859 <sup>(d)</sup>	73.8
2014	90,422	128,959	70.1	141,218	64.0
2015	73,402	121,758 <sup>(e)</sup>	60.3	113,380 <sup>(d)</sup>	64.7
2011–2015	–	–	66.6	–	65.8
Central Iberia					
2011	5,041	15,718	32.1	15,967 <sup>(d)</sup>	31.6
2012	6,453	17,701	36.5	19,297 <sup>(d)</sup>	33.4
2013	6,564	16,950	38.7	20,698 <sup>(d)</sup>	31.7
2014	5,696	14,700	38.8	16,585 <sup>(d)</sup>	34.3
2015	4,027	9,300 <sup>(e)</sup>	43.3	14,895 <sup>(d)</sup>	27.0
2011–2015	–	–	37.9	–	31.6
South-west Iberia					
2011	15,811	85,295	18.5	94,621	16.7
2012	26,313	101,649	25.9	118,039 <sup>(d)</sup>	22.3
2013	31,058	113,437	27.4	123,097 <sup>(d)</sup>	25.2
2014	24,507	96,999	25.3	108,574	22.6
2015	21,298	87,212 <sup>(e)</sup>	24.4	100,210 <sup>(d)</sup>	21.3
2011–2015	–	–	24.3	–	21.6

NA: not available.

(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 19 April 2017).

(b): Avances de superficies y producciones de cultivos: <http://www.magrama.gob.es/es/estadistica/temas/estadisticas-agrarias/agricultura/avances-superficies-producciones-agricolas/> (Accessed 19 April 2017).

(c): Encuesta sobre superficies y rendimiento de cultivos (ESYRCE): <http://www.magrama.gob.es/es/estadistica/temas/estadisticas-agrarias/agricultura/esyrce/> (Accessed 19 April 2017).

(d): Data for maize as a second crop are not included.

(e): Provisional data.

## Appendix C – Susceptibility to purified Cry1Ab protein of the laboratory reference strains of *Ostrinia nubilalis* (ECB) and *Sesamia nonagrioides* (MCB)

Target pest (strain)	Season	Protein batch	MIC <sub>50</sub> (95% CI) <sup>(a)</sup>	MIC <sub>90</sub> (95% CI) <sup>(a)</sup>
ECB (G.04) <sup>(b)</sup>	2006	1	1.20 (0.50–2.21)	4.78 (2.57–14.38)
	2007	1	1.44 (0.86–2.06)	3.94 (2.68–8.28)
	2008	1	2.21 (1.89–2.55)	4.47 (3.70–6.00)
	2008	1	2.26 (1.49–3.01)	8.16 (5.95–13.50)
	2009	1	3.65 (2.77–4.90)	9.56 (6.72–17.75)
	2010	1	2.77 (2.22–3.27)	6.03 (4.93–8.41)
	2011	1	4.01 (2.58–6.12)	10.07 (6.50–28.96)
	2011	2	2.94 (2.33–3.60)	6.27 (4.97–8.91)
	2012	2	0.37 (0.14–0.62)	1.13 (0.67–6.39)
	2013	2	1.97 (0.78–5.59)	5.66 (2.67–95.34)
	2013	2a	1.96 (0.84–4.60)	6.57 (3.13–50.53)
	2014	2a	0.28 (0.24–0.33)	0.46 (0.38–0.62)
	2015	2a	4.03 (2.85–4.86)	7.03 (5.83–9.91)
ECB (ES.ref) <sup>(c)</sup>	2015	2a	1.82 (1.53–2.16)	2.95 (2.43–4.54)
MCB <sup>(d)</sup>	2004	B1	18 (11–25)	99 (66–208)
	2007	B1	16 (11–22)	94 (69–147)
	2008/2009	B1	19 (10–30)	120 (76–255)
	2010	B1	8 (5–11)	74 (51–117)
	2011	B2	9 (6–13)	68 (45–127)
	2012	B2	7 (5–10)	62 (41–107)
	2013	B2	7 (5–10)	48 (31–88)
	2013	B3	5 (3–9)	42 (26–87)
	2014	B3	17 (11–25)	91 (57–209)
	2015	B3	28 (21–36)	67 (50–110)

(a): 50% and 90% moulting inhibition concentration (MIC<sub>50</sub> and MIC<sub>90</sub>) and their 95% confidence intervals (CI 95%) are expressed in ng Cry1Ab/cm<sup>2</sup>.

(b): The G.04 strain was established from ECB larvae collected from Niedernberg (Germany) in 2002. This strain has never been refreshed with field-collected individuals.

(c): The ES.ref strain was established from 145 ECB diapausing larvae collected from three sampling sites in Galicia (Spain) in 2015, of which 75 survived the diapause, reached the adult stage and mated.

(d): The MCB strain was established from larvae collected from Andalucía (661 larvae), Madrid (793 larvae), Ebro (857 larvae), and Galicia (665 larvae) (Spain) in 1998 (González-Núñez et al., 2000). To preserve its vigour, the MCB strain is refreshed periodically with new individuals. To this end, the progenies of the populations collected for the monitoring bioassays are used, and between 10 and 15% of new individuals with respect to the laboratory strain are introduced.

## Appendix D – Scientific publications relevant to the risk assessment and/or management of maize MON 810 assessed by the GMO Panel as part of the annual 2015 PMEM report on the cultivation of maize MON 810

Authors	Title	Journal	Year	Relevant field <sup>(a)</sup>
Arias-Martin M, García M, Lucíañez MJ, Ortego F, Castañera P and Farinós GP	Effects of three-year cultivation of Cry1Ab-expressing Bt maize on soil microarthropod communities	Agriculture, Ecosystems and Environment, 220, 125–134	2016	ERA
Böttger 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, 113, 454–459	2015	ERA <sup>(b)</sup>
Crespo ALB, Alves AP, Wang Y, Hong B, Flexner JL, Catchot A, Buntin D and Cook D	Survival of corn earworm (Lepidoptera: Noctuidae) on Bt maize and cross-pollinated refuge ears from seed blends	Journal of Economic Entomology, 109, 288–298	2015	ERA
Czerwiński J, Bogacki M, Jalali BM and Konieczka PS	The use of genetically modified Roundup Ready soybean meal and Genetically modified MON 810 maize in broiler chicken diets. Part 1. Effects on performance and blood lymphocyte subpopulations	Journal of Animal and Feed Sciences, 24, 134–143	2015	FF
Czerwiński J, Skupecka-Ziemilska M, Woliński J, Barszcz M, Konieczka P and Smulikowska S	The use of genetically modified Roundup Ready soybean meal and Genetically modified MON 810 maize in broiler chicken diets. Part 2. Functional status of the small intestine	Journal of Animal and Feed Sciences, 24, 144–152	2015	FF
García M, Ortego F, Crespo PH, Farinós GP and Castañera P	Inheritance, fitness costs, incomplete resistance and feeding preferences in a laboratory-selected MON 810-resistant strain of the true armyworm <i>Mythimna unipuncta</i>	Pest Management Science, 71, 1631–1639	2015	IRM
Holderbaum DF, Cuhra M, Wickson F, Orth AI, Nodari RO and Bohn T	Chronic responses of <i>Daphnia magna</i> under dietary exposure to leaves of a transgenic (Event MON810) Bt–maize hybrid and its conventional near-isoline	Journal of Toxicology and Environmental Health, Part A, ISSN 1087-2620 (Online), 1–16	2015	ERA <sup>(c)</sup>
Mahur C, Kathuria PC, Dahiya P and Singh AB	Lack of detectable allergenicity in genetically modified maize containing “Cry” proteins as compared to native maize based on in silico & in vitro analysis	PLoS ONE, 10, e0117340	2015	FF
Resende DC, Mendes SM, Marucci RC, Silva AD, Campanha MM and Waquil JM	Does Bt maize cultivation affect the non-target insect community in the agro ecosystem?	Revista Brasileira de Entomologia, 60, 1–12	2016	ERA
Rocha LO, Barroso VM, Andrade LJ, Pereira GHA, Ferreira-Castro FL, Duarte AP, Michelotto MD and Correa B	Gene expression profile and fumonisin production by <i>Fusarium verticillioides</i> inoculated in Bt and non-Bt maize	Frontiers in Microbiology, 6, 1503	2016	ERA

Authors	Title	Journal	Year	Relevant field <sup>(a)</sup>
Sartowska KE, Korwin-Kossakowska A and Sender G	Genetically modified crops in a 10-generation feeding trial on Japanese quails - Evaluation of its influence on birds' performance and body composition	Poultry Science, 94, 2909–2916	2015	FF
Shu Y, Zhang Y, Cheng M, Zeng H and Wang J	Multilevel assessment of Cry1Ab Bt-maize straw return affecting the earthworm <i>Eisenia fetida</i>	Chemosphere, 137, 59–69	2015	ERA
Szénási A and Markó V	Flea beetles (Coleoptera: Chrysomelidae, Alticinae) in Bt-(MON810) and near isogenic maize stands: Species composition and activity densities in Hungarian fields	Crop Protection, 77, 38–44	2015	ERA
Taverniers I, De Meyers L, Van Droogenbroeck B, Messens K and De Loose M	Influence of plant developmental stage on DNA yield and extractability in MON 810 maize	Agricultural and Food Science, 24, 128–138	2015	ERA
Valldor P, Miethling-Graff R, Martens R and Tebbe CC	Fate of the insecticidal Cry1Ab protein of GM crops in two agricultural soils as revealed by <sup>14</sup> C-tracer studies	Environmental Biotechnology, 99, 7333–7341	2015	ERA
Vidal N, Barbosa H, Jacob S and Arruda M	Comparative study of transgenic and non-transgenic maize ( <i>Zea mays</i> ) flours commercialized in Brazil, focussing on proteomic analyses	Food Chemistry, 180, 288–294	2015	FF
Xu LN, Wang YQ, Wang ZY, Ling YH and He KL	Transcriptome differences between Cry1Ab resistant and susceptible strains of Asian corn borer	BMC Genomics, 16, 173	2015	ERA <sup>(b)</sup>
Zeng H, Tan F, Shu Y, Zhang Y, Feng Y and Wang J	The Cry1Ab protein has minor effects on the arbuscular mycorrhizal fungal communities after five seasons of continuous Bt maize cultivation	PLOS ONE, 10, e0146041	2015	ERA

(a): ERA, environmental risk assessment; FF, food and feed; IRM, insect resistance management; MC, molecular characterisation.

(b): Already assessed by the GMO Panel (EFSA GMO Panel, 2016).

(c): Already assessed by EFSA (EFSA, 2016b).