Annual monitoring report on the cultivation of MON 810 in 2016

Czech Republic, Portugal, Slovakia, and Spain

Submitted by

MONSANTO EUROPE S.A.

Dept. Regulatory Affairs
Avenue de Tervuren 270-272
Tervurenlaan 270-272
B-1150 Brussels
BELGIUM

September 2017

Data protection.
This application contains scientific data and other information which are protected in accordance with Art. 31 of Regulation (EC) No 1829/2003.

TABLE OF CONTENTS

TABLE OF CONTENTS **2**
LIST OF APPENDICES **3**
1. GENERAL INFORMATION **4**
1.1 Crop/trait(s) **6**
1.2 Decision authorisation number pursuant to Directive 2001/18/EC, and number and date of consent pursuant to Directive 2001/18/EC **6**
1.3 Decision authorisation number and date of authorisation pursuant to Regulation (EC) No 1829/2003 ..... **6**
1.4 Unique identifier **6**
1.5 Reporting period **6**
1.6 Other monitoring reports **6**
2. EXECUTIVE SUMMARY **7**
3. MONITORING RESULTS **8**
3.1 General Surveillance **8**
3.1.1 Description of General Surveillance 11
3.1.2 Details of surveillance networks used to monitor environmental effects during General Surveillance and description of other methodologies 12
3.1.2.1 Farmer questionnaire 12
3.1.2.2 Company stewardship activities 14
3.1.2.3 Alerts on environmental issues 15
3.1.3 Details of information and/or training provided to operators and users, etc. 16
3.1.4 Results of General Surveillance 16
3.1.4.1 Farmer questionnaires 16
3.1.4.2 Company stewardship activities 16
3.1.4.3 Alerts on environmental issues 16
3.1.5 Additional information 16
3.1.6 Review of peer-reviewed publications 17
3.1.6.1 Peer-reviewed publications on the safety of MON 810 and/or the Cry1Ab protein published in 2016 – 2017 17
3.2 Case specific monitoring **28**
3.2.1 Description and results of case-specific monitoring (if applicable) 28
3.2.1.1 Refuge 29
3.2.1.2 Baseline studies and resistance monitoring in the target pests 30
3.2.1.3 Farmer complaint system 33
3.2.1.4 Communication and grower education 34
3.2.2 Monitoring and reporting of adverse effects resulting from accidental spillage (if applicable) 36
3.3 Concluding remarks **36**
4. SUMMARY OF RESULTS AND CONCLUSIONS **36**
5. ADAPTATIONS OF THE MONITORING PLAN AND ASSOCIATED METHODOLOGY FOR FUTURE YEARS **37**
REFERENCES **38**

LIST OF APPENDICES

APPENDIX 1. POST MARKET MONITORING OF INSECT PROTECTED BT MAIZE MON 810 IN EUROPE – BIOMETRICAL ANNUAL REPORT ON THE 2016 GROWING SEASON

APPENDIX 2. 2016 MON 810 FARMER QUESTIONNAIRE

APPENDIX 3. EXAMPLES OF TECHNICAL USER GUIDES

- **Appendix 3.1. CZECH REPUBLIC_TUG**
- **Appendix 3.2. PORTUGAL_TUG**
- **Appendix 3.3. SLOVAKIA_TUG**
- **Appendix 3.4. SPAIN_TUG**

APPENDIX 4. 2016 FARMER QUESTIONNAIRE – USER'S MANUAL

- **Appendix 4.1. Portugal User manual annexes**
- **Appendix 4.2. SPAIN USER MANUAL ANNEXES**

APPENDIX 5. MON 810 LITERATURE REVIEW (JUNE 2016 – MAY 2017)

- **Appendix 5.1. MON 810 LITERATURE REVIEW – FOOD/FEED**
- **Appendix 5.2. MON 810 LITERATURE REVIEW - ENVIRONMENT**
- **Appendix 5.3. MON 810 LITERATURE REVIEW – LIST OF ALL HITS (JUNE 2016 – MAY 2017) – WEB OF SCIENCE™ CORE COLLECTION DATABASE**
- **Appendix 5.4. MON 810 LITERATURE REVIEW – LIST OF ALL HITS (JUNE 2016 – MAY 2017) – CABI CAB ABSTRACTS® DATABASE**

APPENDIX 6. EUROPABIO HARMONISED INSECT RESISTANCE MANAGEMENT (IRM) PLAN FOR CULTIVATION OF BT MAIZE (SINGLE INSECTICIDAL TRAITS) IN THE EU, JULY 2017

APPENDIX 7. INSECT RESISTANCE MONITORING REPORT FOR SESAMIA NONAGRIOIDES ASSOCIATED WITH MON 810 MAIZE CULTIVATION IN THE EU: SEASON 2016

APPENDIX 8. INSECT RESISTANCE MONITORING REPORT FOR OSTRINIA NUBILALIS (ECB) ASSOCIATED WITH MON 810 MAIZE CULTIVATION IN THE EU: SEASON 2016

APPENDIX 9. IBERIAN REFUGE IMPLEMENTATION COMMUNICATION MATERIALS

- **Appendix 9.1. Spain_Yieldgard technical guide**
- **Appendix 9.2. IRM ADVERTISEMENTS**
  - **Appendix 9.2.1 Advert 1 - 12 Feb 2016**
  - **Appendix 9.2.2 Advert 2 - 26 Feb 2016**
- **Appendix 9.3. REFUGE POSTCARD**
- **Appendix 9.4. SLIDE DECK ON IRM REQUIREMENTS**
- **Appendix 9.5. IRM POSTER**
- **Appendix 9.6. Portugal_YieldGard Technical Guide**

1. GENERAL INFORMATION

Using modern biotechnology, Monsanto Company has developed insect-protected YieldGard® Corn Borer maize MON 810 (hereafter referred to as MON 810) that produces the naturally occurring *Bacillus thuringiensis (Bt)* protein, Cry1Ab. MON 810 is protected from foliage feeding and stalk tunneling damage by the European corn borer (*Ostrinia nubilalis*) and the pink stem borer (*Sesamia nonagrioides*).

In 1995, Monsanto submitted an application for import and use of MON 810 as any other maize (including cultivation) under Directive 90/220/EEC to France, the country acting as *rapporteur*. France subsequently forwarded the dossier to the European Commission with a favorable opinion. The other EU Member States raised objections. The European Commission sought the opinion of the Scientific Committee on Plants (SCP) that adopted a scientific opinion on 10 February 1998, concluding that "*there is no evidence that the seeds of insect-resistant maize (expressing the cry1Ab gene and protein) when grown, imported and processed in the manner indicated, are likely to cause adverse effects on human or animal health and the environment*"[1]. After receiving a qualified majority at the Regulatory Committee, composed of Member State experts, on 18 March 1998, MON 810 was approved for import and use (including cultivation) (Commission Decision, 1998). France, as *rapporteur*, ratified the Commission Decision on 3 August 1998. According to this Decision, Monsanto is required to inform the European Commission and the competent authorities of the European Union Member States about the results of monitoring for insect resistance.

On 4 May 2007, Monsanto submitted an application for renewal of authorisation of MON 810 maize products to the European Commission in accordance with Article 20(1)(a) (Commission Regulation, 2003)[2] of Regulation (EC) No 1829/2003 on genetically modified food and feed. In support of this renewal application, a monitoring plan (developed according to Annex VII of Directive 2001/18/EC) and previously submitted monitoring reports have been provided as part of the information required under Article 23(2) of Regulation (EC) No 1829/2003. A positive scientific opinion from the European Food Safety Authority (EFSA), confirming the conclusions of the original safety assessment, was adopted on 15 June 2009 (and published as part of an EFSA overall opinion on 30 June 2009 (EFSA, 2009)). According to the legal framework, these authorised products remain lawfully on the market until a decision on re-authorisation is taken. Due to continuing discussions at political level on nationalization of GMO cultivation to provide freedom to the Member States to decide on the cultivation of genetically modified crop, the renewal applications failed to progress since the positive EFSA opinion was published in 2009. Therefore, in order to provide certainty on the

® YieldGard is a registered trademark of Monsanto Technology LLC.

[1] Opinion of the Scientific Committee on Plants Regarding the Genetically Modified, Insect Resistant Maize Lines Notified by the Monsanto Company - http://ec.europa.eu/ (Accessed 27 September 2017)

[2] For products previously authorised under Directive 90/220/EEC. Other food and/or feed aspects previously authorised under Regulation (EC) No 258/97 or notified under Articles 8 and 20 of Regulation (EC) No 1829/2003 were covered in separate renewal applications according to Articles 8(1)(a), 8(1)(b) and 20(1)(b) of Regulation (EC) No 1829/2003.

international trade of MON 810 for food and feed uses, Monsanto requested the European Commission on 9 March 2016 to progress separately two complementary decisions for the renewal applications EFSA-GMO-RX-MON 810 (8-1a, 20-1a and 8-1b/20-1b), *i.e.*, the renewal of authorization for (1) existing food and food ingredients produced from MON 810; feed consisting and/or containing MON 810 and food and feed additives, and feed materials produced from MON 810; and (2) the use of seed for cultivation. Following Directive (EU) 2015/412 of 11 March 2015, the geographical scope of the authorization for cultivation of MON 810 was adapted on 3 March 2016 (European Commission, 2016). On 8 July 2016, the European Commission presented the Draft Commission Implementing Decision authorizing the renewal of existing food and food ingredients produced from MON 810; feed consisting and/or containing MON 810 and food and feed additives, and feed materials produced from MON 810 to the Standing Committee on Plants, Animals, Food and Feed (PAFF) for a vote, where no qualified majority was reached. On 4 July 2017, the European Commission adopted the renewal of the authorisation for the placing on the market of MON 810 for all uses, with the exception of pollen and cultivation (European Commission, 2017).

In 2016, MON 810 was planted in the EU on approximately 136 334 hectares across four countries: Czech Republic (75,26 ha[3]), Portugal (7 056 ha[4]), Slovakia (122,13 ha[5]) and Spain (129 081 ha[6]).

Results of Insect Resistance Management (IRM) are provided to the European Commission on an annual basis (*i.e.* this report) in line with the obligations under Commission Decision 98/294/EC of 22 April 1998. In addition, Monsanto has also always reported on a voluntary basis about its activities to identify the occurrence of adverse effects of MON 810 or its use on human health or the environment which were not anticipated in the environmental risk assessment (General Surveillance monitoring). In addition to any reporting obligation in terms of annual monitoring activities, in case an investigation establishes that MON 810 is the cause of an adverse effect, Monsanto will immediately inform the European Commission. Monsanto, in collaboration with the European Commission and based on a scientific evaluation of the potential consequences of the observed adverse effect, will then define and implement management measures to protect human health or the environment, as necessary.

MON 810 monitoring reports were submitted to the European Commission since 2005 (Monsanto Europe S.A., 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2015, 2016, 2017). Since 2010, the reports follow the format as laid out in Annex I to Commission Decision 2009/770/EC (Commission Decision, 2009).

---

[3] Ministry of Agriculture of the Czech Republic, 2017 - http://eagri.cz/public/web/mze/zemedelstvi/aktualni-temata/gm-plodiny-pestovani-geneticky/ (Accessed 27 September 2017)

[4] Anpromis, 2017 - http://www.anpromis.pt/dados-estatisticos/ (Accessed 27 September 2017)

[5] Ministry of Agriculture and rural development of the Slovak Republic, 2017 - http://www.mpsr.sk/index.php?navID=764&navID2=764&sID=40&id=11635 (Accessed 27 September 2017)

[6] Ministry of Agriculture, Food and Environment of Spain, 2015 - http://www.mapama.gob.es/es/calidad-y-evaluacion-ambiental/temas/biotecnologia/superficiecultivadaomg2016_tcm7-435282.pdf (Accessed 27 September 2017)

1.1 Crop/trait(s): Maize/insect resistance

1.2 Decision authorisation number pursuant to Directive 2001/18/EC, and number and date of consent pursuant to Directive 2001/18/EC: Not available

1.3 Decision authorisation number and date of authorisation pursuant to Regulation (EC) No 1829/2003: Not available

1.4 Unique identifier: MON-ØØ81Ø-6

1.5 Reporting period: July 2016 - July 2017

1.6 Other monitoring reports have been submitted in respect of:

- **Import and Processing** Yes voluntary (September 2017)
- **Food/Feed** Not applicable

2. EXECUTIVE SUMMARY

In 2016, MON 810 was planted in the EU on approximately 136 334 hectares across four countries. As part of stewardship of the technology, industry has implemented an Insect Resistance Management (IRM) plan to proactively delay the potential development of pest resistance to the Cry1Ab protein. The adherence to this stewardship measure in the context of the 2016 cultivation of MON 810 maize in Europe is detailed in this report.

The planting of MON 810 in the 2016 season was accompanied by a rigorous IRM plan involving five main elements: a farmer complaint system, farmer education, refuge implementation, susceptibility monitoring and good stewardship practices. The initiatives developed to educate farmers about the importance of the implementation of IRM measures were continued in 2016 and the success of these initiatives was reflected in the high levels of compliance with requirements for refuge implementation observed in the 2016 season. A comprehensive IRM program demonstrated that there were no changes in susceptibility of neither *O nubilalis* nor *S nonagrioides* to the Cry1Ab protein in the major MON 810 growing regions in Europe in 2016. No complaint allegedly caused by reduced target pest susceptibility to MON 810 was received from farmers in 2016.

The weight of evidence available to date confirms the initial conclusions of the safety assessment, namely that MON 810 is as safe as conventional maize with respect to human or animal health and the environment (*see* Section 3.1).

In 2016, Monsanto continued its General Surveillance monitoring program, implemented on a voluntary basis and aimed at identifying the occurrence of adverse effects of the GMO or its use on human or animal health or the environment, which were not anticipated in the environmental risk assessment. The analysis of 250 questionnaires from a survey of farmers cultivating MON 810 in two European countries in 2016 did not reveal any adverse effects associated with the genetic modification in MON 810. Furthermore, a detailed analysis of 37 publications related to MON 810 and/or Cry1Ab did not reveal any new scientific evidence that would invalidate the conclusions of the risk assessment concluding that MON 810 is as safe to human and animal health as its conventional counterpart, and confirms that there is negligible impact from the cultivation of MON 810 on biodiversity, abundance or survival of non-target species, and the environmental risk of MON 810 is considered to be negligible compared to conventional maize. Also, company stewardship activities did not reveal any adverse effects related to MON 810 cultivation in 2016. Taken together, these results demonstrate that there are no indications of adverse effects to be attributed to the cultivation of MON 810 in Europe in 2016.

3. MONITORING RESULTS

3.1 General Surveillance

Current EU legislation requires applicants to include in their monitoring plan strategies to identify the occurrence of adverse effects of the GMO on human or animal health or the environment which were not anticipated in the environmental risk assessment. This type of monitoring, termed General Surveillance (GS), is not a condition of the current authorization for MON 810 issued in 1998 or renewal in 2017. Nevertheless, Monsanto has been reporting on its activities for this non-hypothesis based monitoring on a voluntary basis since 2005. Over the years, several approaches to monitor unanticipated adverse effects were developed and their methodologies improved substantially. Several complementary approaches initially developed by Monsanto were taken up by EuropaBio in an effort to harmonize proportional and workable monitoring approaches across the technology providers. Monsanto has traditionally reported on four complementary GS activities: (1) analysis of farmer questionnaires, (2) literature searches on the safety of MON 810 in peer-reviewed journals, (3) alerts on the product through stewardship programs, and (4) the use of existing environmental networks (EENs).

The weight of evidence available to date confirms the initial conclusions of the EU safety assessment in 1998, namely that MON 810 is as safe as conventional maize with respect to human or animal health and the environment. MON 810 has been safely grown in multiple countries around the world since 1997 as a single event, and later as part of several stacks. Following its approval in 1998 in the EU, MON 810 was first grown in European countries in 2003. From 2005 to date, Monsanto submitted 12 post-market environmental monitoring (PMEM) reports covering 14 years of MON 810 cultivation in the EU and all reports confirm consistently its safety. These reports describe the activities undertaken by Monsanto to identify and analyse anticipated and allegedly unanticipated effects related to MON 810 cultivation (Monsanto Europe S.A., 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2015, 2016, 2017). The resulting weight of confirmatory safety evidence is summarized below. Furthermore, irrespective of any annual monitoring reporting obligations Monsanto will, in accordance with EU legislation, inform the European Commission and the appropriate national competent authorities of any confirmed adverse effect related to the MON 810 event should it occur.

Farmers growing MON 810 are the first to observe any effects related to the GM event (adverse as well as beneficial) should they occur. Therefore, two of the four GS approaches are focused on the farmer, *i.e.*, the farmer questionnaire and Monsanto's product stewardship efforts. For the farmer questionnaires, a sample size of 2 436 interviews was calculated to achieve the demands as specified in Appendix 1. These demands are very stringent in order to reduce false test decisions to a minimum. To achieve this sample size even in the case of questionnaires having to be excluded from the survey *e.g.* because of low quality, this number was rounded to 2 500 questionnaires. Since the first implementation of farmer interviews, more than 2 500 farmers have been questioned about their experience with MON 810 and in

particular about any observations or effects in the field that were different for MON 810 compared to conventional maize hybrids. As this years' PMEM report aims to describe the outcomes of the 2016 growing season, the results of the farmer questionnaires conducted in 2016 are provided. None of the reports, for which the results were statistically analysed, identified a statistically meaningful effect that indicated adverse effects to human or animal health, or the environment. The intended beneficial effects were observed in those reports as being evaluated in MON 810 fields compared to conventional maize fields.

The Council Decision 2002/811/EC and the EFSA guidance on PMEM of genetically modified plants (EFSA, 2011a), state that "*monitoring plans should not be viewed as static*" and "*it is fundamental that the monitoring plan and associated methodology are reviewed at appropriate intervals and* ***may need to be modified and adapted depending on the results of the monitoring information collected***". Following EFSA guidance, "*the monitoring results and experience may lead to adjustments of certain parts of the original monitoring plan*". Therefore, as the aimed sample size of 2 500 farmer questionnaires was obtained after the 2015 MON 810 growing season, an analysis with the pooled multiyear data is being conducted and the outcome will be reported in the form of a peer reviewed publication. It is at this stage a reasonable assumption (as the annual reports are providing consistent results) that the multiyear analyses will not identify adverse effect of MON 810 cultivation on human or animal health, or the environment. The monitoring plan and associated methodology for conducting farmer questionnaires will be modified and adapted based on the results of the monitoring information collected.

In addition to the results from the farmer questionnaires conducted in 2016, Monsanto's company-internal processes for managing product related incidents and complaints did not identify adverse effects caused by the MON 810 event. Furthermore, as a third pillar of the implemented GS, Monsanto reported on the peer reviewed articles that were published on the safety of MON 810. Across our regulatory submissions and monitoring reports, Monsanto has reported on more than 425 articles of which the vast majority is authored by independent academics and scientists. Allegations about the safety of the product were thoroughly reviewed, allowing Monsanto to confirm the validity of the initial conclusions on safety made in the food and feed risk assessment as well as the environmental risk assessment presented in our different applications for authorization of MON 810 in the EU. Finally, the value of using the reports of EENs to confirm the safety of GM crops in general and MON 810 in particular was assessed, but were considered of less additional value than the other approaches. EuropaBio identified and characterized potential relevant EENs for PMEM of GM crop cultivation, but concluded that EENs are not well suited as a primary tool for GS in GM crop monitoring (Smets *et al.*, 2014).

The aforementioned 12 PMEM, covering 14 years of MON 810 cultivation in the EU, all support the original conclusion reached in the initial application of authorization, *i.e.*, MON 810 is as safe as conventional maize in terms of human and animal health or the environment. Global regulators reached the same conclusions as MON 810 is authorized for cultivation in Argentina, Brazil, Canada, Colombia, Egypt, EU, Honduras, Paraguay, the

Philippines, South Africa, Uruguay and the USA[7]. More specifically in the EU, independent scientific panels, such as the EFSA have reviewed our regulatory submissions (EFSA, 2012c, 2012e), new scientific publications published from 2009 onwards (EFSA, 2012f, 2015b, 2015d, 2016a, 2016b, 2017), Monsanto's monitoring reports (Monsanto Europe S.A., 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2015, 2016, 2017) as well as challenges raised by various Member States related to human and animal health or the environment (EFSA, 2004, 2005, 2006, 2008a, 2008b, 2008c, 2008d, 2012a, 2012b, 2013a, 2013b, 2014c). EFSA's first opinion based on regulatory data presented in our three complementary regulatory renewal submissions (in 2009) concluded that "*maize MON 810 is as safe as its conventional counterpart with respect to potential effects on human and animal health. The EFSA GMO Panel also concludes that maize MON 810 is unlikely to have any adverse effect on the environment in the context of its intended uses*". All subsequent EFSA opinions consistently concluded that there is no specific scientific evidence, in terms of risk to human and animal health or the environment that would invalidate the previous EFSA GMO Panel risk assessments of maize MON 810.

In conclusion, the available weight-of-evidence continuing to support the safety of MON 810 and the absence of unintended adverse effects consists of:

- regulatory safety studies presented in the different EU applications,
- more than a dozen EFSA opinions concluding on the safety of MON 810,
- cultivation approvals for MON 810 in multiple countries around the world based on scientific risk assessment data and local safety opinions,
- hundreds of peer reviewed publications relevant to the safety assessment of MON 810 and the expressed Cry1Ab protein,
- more than 13 years of experience with MON 810 cultivation in the EU,
- more than 20 years of experience worldwide on millions of hectares,
- multiple PMEM reports for the EU reporting on the commercial experience confirming the initial safety conclusions (and endorsed by EFSA),
- absence (in the EU and on a global scale) of demonstrated field resistance for the targeted pests,
- absence of evidence indicating adverse effect related to the event.

The weight of evidence described above confirms that MON 810 is as safe as conventional maize with respect to human and animal health and the environment. Taking into consideration that GS is not a condition of the current authorization for MON 810 issued in 1998 (Commission Decision, 1998), reporting on GS activities of each growing season becomes disproportional to the available weight of evidence demonstrating the safety of MON 810.

[7] CropLife International: www.biotradestatus.com (Accessed on 17 August 2017).

However, the European Commission has stated on several occasions the necessity to report on GS activities for MON 810 on an annual basis. Even though Monsanto's position as explained above remains unchanged, the results of the 2016 GS activities are included in this report. Monsanto reiterates the need for adaptation of the monitoring plan and associated methodology based on the comprehensive experience and the information collected, and aligned with the spirit of the EFSA guidance on PMEM of genetically modified plants (EFSA, 2011a).

The types of GS monitoring that were implemented by Monsanto as well as the methodologies followed and the reporting conducted has not been an individual applicant's work. During the years, Monsanto always has communicated to different stakeholders and has informed and consulted, amongst others, the European Commission, EFSA GMO unit, Member States and biotech industry on its approach. Through feedback from a variety of workshops, meetings and reports, but also based on gained monitoring experience over time Monsanto has gradually improved the way it implemented GS monitoring. For these adjustments, Monsanto aims to secure the balance between information maximization at the one hand, and implementation practicalities and proportionality (to the perceived risk) at the other hand.

Monsanto acknowledges the fact that EFSA made several recommendations to improve the methodology on how to perform GS, *i.e.*, in their general guidance document for PMEM of GM crops in August 2011 (EFSA, 2011a) and seven specific opinions on MON 810 monitoring in the 2009, 2010, 2011, 2012, 2013, 2014 and 2015 growing seasons (EFSA, 2011b, 2012d, 2013c, 2014a, 2015c, 2016c, 2017). Monsanto has adapted its monitoring approaches where possible and feasible, taking into consideration the gained expertise on MON 810 monitoring and already established methodologies, in order to report on a voluntary basis on the results for the 2016 growing season. EFSA concluded that no adverse effects on human or animal health or the environment were identified due to MON 810 cultivation during the 2009, 2010, 2011, 2012, 2013, 2014 or 2015 growing seasons and that the outcomes of the monitoring reports did not invalidate the previous risk assessment conclusions (EFSA, 2011b, 2012d, 2013c, 2014a, 2016c, 2017). This confirms that Monsanto's methodologies are fit for the purpose of identifying adverse effects. In case an adverse effect is observed to the environment, human or animal health and confirmed to be caused by the MON 810 trait, it will immediately be reported to the European Commission and a mitigation plan will be developed in collaboration with the European Commission (*see* also Section 1).

3.1.1 Description of General Surveillance

In 2016, Monsanto continued the GS monitoring program initiated in 2005 on a voluntary basis. The objective of GS is to identify the alleged occurrence of adverse effects of the GMO or its use on human or animal health or the environment which were not anticipated in the environmental risk assessment. The main challenge of GS is determining whether *1)* an unusual effect has been observed (*i.e.*, an alteration that results in values that are outside the normal variation range given the constant change and flux of agriculture, agricultural

practices, the rural environment and the associated biota in the European Union), *2)* the effect is adverse, and *3)* the adverse effect is associated with the GM plant or its cultivation (EFSA, 2011a).

GS is focused on the geographical regions within the EU where the GM crop is grown, therefore takes place in representative environments, reflecting the range and distribution of farming practices and environments exposed to GM plants and their cultivation.

Where there is scientifically valid allegation of a adverse effect (whether direct or indirect), linked to the genetic modification, then further evaluation of the consequence of that effect should be science-based and compared with baseline information. Relevant baseline information will reflect prevalent agricultural practice and the associated impact of these practices on the environment. In many cases it may be complex to establish a causal link between a potential adverse effect and use of a particular GM crop.

The GS monitoring program performed by Monsanto in 2016 consisted of four elements:

- a farmer questionnaire designed to assess unusual observations in the areas where MON 810 has been cultivated,
- data collected from peer-reviewed scientific publications or reports relating to MON 810 and its comparative safety (to conventional counterparts) with respect to human, and animal health and the environment,
- company stewardship activities designed to ensure and maintain the benefits of the product,
- alerts on environmental issues by authorities, existing networks and the press that may reflect potential adverse effects associated with the product.

3.1.2 Details of surveillance networks used to monitor environmental effects during General Surveillance and description of other methodologies

3.1.2.1 Farmer questionnaire

Farmers are the closest observers of the cultivation of GM crops and routinely collect information on the cultivation and management of their crops at the farm level. Therefore, they can give details on GM plant-based parameters (referring to species/ecosystem biodiversity, soil functionality, sustainable agriculture, plant health and product performance) and on background and baseline environmental data (*e.g.*, soil parameters, climatic conditions and general crop management data such as fertilisers, crop protection, crop rotations and previous crop history). Additionally, farmers may give empirical assessments which can be useful within GS to reveal unexpected deviations from what is common for the crop and cultivation area in question, based on their historical knowledge and experience.

A questionnaire addressed to farmers cultivating GM crops is a monitoring tool that is specifically focused on the farm level. EFSA explicitly considers questionnaires a useful method to collect first hand data on the performance and impact of a GM plant and to

compare the GM plant with conventional plants (EFSA, 2011a). The questionnaire approach has also proven its applicability with other industries, *e.g.*, the pharmaceutical industry.

A farmer questionnaire has been developed as a key tool for monitoring of MON 810. It was inspired by the experimental questionnaire developed by the German Federal Biological Research Centre for Agriculture and Forestry (BBA), maize breeders and statisticians in Germany (Wilhelm *et al.*, 2004). It was first applied in 2005 and adapted based on experience to create a new version for 2006. The current version of the questionnaire has been used since 2009 (see Appendix 2). As appropriate, in each season adjustments were made to improve the statistical relevance of the collected data. Questions were designed to be unambiguous, easily understood and not to be too burdensome. Also, it is sufficiently pragmatic to take into account real commercial situations.

Farmers are asked for their observations and assessment in and around MON 810 cultivated fields in comparison to a baseline, this being their own historical local knowledge and experience. The 2016 GS for MON 810 focused on the Iberian geographical regions where the majority of MON 810 was grown in 2016 (Portugal and Spain, countries accounting for approximately 99.9% of the MON 810 plantings in the EU in 2016), reflecting the range and distribution of farming practices and environments exposed to MON 810 plants and their cultivation. This allows for cross-checking of information indicative of an unanticipated effect, and the possibility to establish correlations either by comparing questionnaires between regions, or associating answers to observations made by existing networks, such as meteorological services (weather conditions) or extension services (pest pressure).

In 2016, 13 farmers in Portugal and 237 farmers in Spain were asked to complete the questionnaire (250 in total). The farmers/fields were randomly selected depending on the market maturity and the size of the sample was considered large enough to give sufficient power to the test (*i.e.*, the probability to reject the null hypothesis while the value of the probability of the answer is small) (see Appendix 1 for details on methodology). The interviews have been completed between January and March 2017. In Spain, which represented the largest market, the survey was performed by Markin[8] while in Portugal, it was performed by Agro.Ges[9], two qualified, independent companies with a vast experience in the conduction of farmer surveys. All interviewers have been trained to understand the background of the questions. Here also experience gained during surveys of the previous years (uncertainties, misinterpretation of questions) could be shared. While questions have been carefully phrased to obtain accurate observations from farmers, previous experience with the questionnaire may increase awareness and thus result in slightly inconsistent observations from one year to the next. To assist the interviewers in filling in the questionnaires with the farmers, a 'user manual' was developed (*see* Appendix 4).

[8] Instituto Markin, Spain.
[9] Agro.Ges - Sociedade de Estudos e Projectos, Portugal.

The questionnaire was designed to collect data in four specific areas:

Part 1: Maize grown area

Responses to this section will enable records of general, basic data on maize cultivation, cultivation area and local pest and disease pressure (independent from GM or non-GM cultivation – background and possible influencing factors). It includes questions on 'fixed factors', *e.g.*, soil characteristics, and 'random factors', *e.g.*, diseases, pests and weeds.

Part 2: Typical agronomic practices to grow maize on the farm

Questions in this section aim to establish the agricultural practices to cultivate conventional maize. The data collected in this section constitutes a baseline against which insect protected maize cultivation can be compared. It includes questions on 'adjustable factors', *e.g.*, irrigation, soil tillage, planting technique, weed and pest control practices, and fertiliser.

Part 3: Observations of the insect protected maize event

Questions in this section collect information to assess the specific insect protected maize practices, observations and performance. It includes questions on 'monitoring parameters' for comparison with conventional maize, *e.g.*, germination, time to emergence, and yield.

Part 4: Implementation of insect protected maize event specific measures

Questions in this section are intended to survey the implementation of the recommendations for insect protected maize cultivation.

3.1.2.2 Company stewardship activities

Monsanto is committed to the management of its products in a responsible and ethical way throughout their entire life cycle, from the stages of discovery to their ultimate use. Stewardship activities include *1)* assessment of the safety of the products, *2)* management practices to endorse sustainability of the products, *3)* absolute respect of all the regulations in place, and *4)* explanation and promotion of the proper and responsible use of products and technologies. Details on growers' education in this context is given in Section 3.2.1.4.

As part of product stewardship and responsible use, Monsanto urges users to notify any unexpected potential adverse effects observed that might be linked to the use of its products. This can be done through the phone, fax or mail contact information given in the Technical User Guides (TUGs), (*see Appendix 3*.1 to Appendix 3.4). Alternatively, EuropaBio[10] and Monsanto[11] websites offer a contact point.

[10] EuropaBio contact webpage - http://www.europabio.org/contact (Accessed 27 September 2017)
[11] Monsanto product stewardship webpage - http://www.monsanto.com/products/pages/product-stewardship.aspx (Accessed 27 September 2017)

3.1.2.3 Alerts on environmental issues

Internal procedure on alerts on environmental issues

Since the commercial introduction of MON 810, attention to potential environmental issues has been raised through a number of sources. An issue management process has been put in place by Monsanto to deal with these 'issue alerts'. The process involves:

- identification of potential issues (by anticipation of potential or emerging issues through external relationships with regulators and academics or publication in media and scientific journals (*see* Section 3.1.6)),
- analysis of the potential issue and its relevance to the safety assessment of the product,
- sharing of expert commentary with regulators and other stakeholders (if warranted),
- communication of conclusions to internal and external stakeholders (if warranted)[12].

Alerts on environmental issues by existing networks

The EuropaBio Working Group on monitoring coordinated a harmonized effort to map EENs in Europe and to set up a unique reporting system (Smets *et al.*, 2014). The work done by EuropaBio resulted in the identification of numerous suitable EENs established in different individual EU Member States, as well as on a European level. The selection and identification was done in line with EFSA recommendations. The identified networks were divided into four groups, 1) governmental networks; 2) academic networks; 3) nature conservation networks and 4) professional networks. Whereas the monitoring expertise of these identified networks was recognized, it was concluded that it would not be possible for such a network to establish a relationship between a cause and an effect. More specifically, none of the identified EENs measured GM crop cultivation as an influencing factor, making it difficult to establish accurate correlations based on the collected data. Furthermore, additional limitations in the use of EENs as an early warning system part of GS efforts are 1) technical constraints (*e.g.* delayed publication of monitoring data); 2) lack of public availability of (raw) data; 3) harmonization between networks (*e.g.* data collection and processing). As also concluded in Smets *et al*. (2014), plant biotechnology companies have no authority to modify the practices used by EENs today, nor is there an interest to do so as this would influence their independence.

In addition, the EFSA has published a scientific opinion on the use of EENs for PMEM reports based on internal expertise and a report issued by a contracted consortium (Henrys *et al.*, 2014). EFSA's opinion concluded that "*In compliance with these assessment criteria, several existing ESNs have been identified as potentially suitable for GS of GMPs subject to further examination. However, the EFSA GMO Panel also identified several limitations pertaining to ESNs such as limited data accessibility, data reporting format and data connectivity with GMO registers*" (EFSA, 2014b).

[12] Channels of communication to external stakeholders include the Monsanto website - http://www.monsanto.com/newsviews/Pages/Issues-and-Answers.aspx (Accessed 27 September 2017)

3.1.3 Details of information and/or training provided to operators and users, etc.

Each purchaser of MON 810 receives a Technical User Guide (TUG) that provides a concise source of technical information about the product and sets forth use requirements and guidelines. Examples of the documents distributed in the 2016 season can be found in Appendix 3 (see *Appendix 3*.1 to Appendix 3.4). Additional details on growers' education in the context of refuge implementation is given in Section 3.2.1.4.

3.1.4 Results of General Surveillance

3.1.4.1 Farmer questionnaires

The methodology is described in Section 3.1.2.1. The analysis of 250 questionnaires from the survey of farmers cultivating MON 810 in Spain and Portugal during the 2016 growing season did not reveal any adverse effects that could be associated with the genetic modification in MON 810. The full report is presented in Appendix 1.

The farmer questionnaires are distributed, completed and collated each year. Reports are also prepared on an annual basis. If the findings of the surveys indicate adverse effects directly associated with MON 810 cultivation that require risk mitigation, these will be reported immediately to the Commission.

3.1.4.2 Company stewardship activities

The methodology is described in Section 3.1.2.2. To date, no unexpected potential adverse effects related to MON 810 have been reported or confirmed.

3.1.4.3 Alerts on environmental issues

The methodology is described in Section 3.1.2.3. No confirmed adverse effects related to MON 810 were reported in 2015.

3.1.5 Additional information

Not applicable as no adverse effects were observed.

3.1.6 Review of peer-reviewed publications

3.1.6.1 Peer-reviewed publications on the safety of MON 810 and/or the Cry1Ab protein published in 2016 – 2017

Monsanto maintained its strategy for the review of peer-reviewed publications by including 1) information on the search date; 2) the full list of retrieved scientific publications; 3) clear criteria for exclusion/inclusion of relevant scientific publications; and 4) a literature search in scientific literature databases (Web of Science™ Core Collection and CABI CAB Abstracts® and Global Health®).

An important source of information on MON 810 is the extensive independent research that is performed by scientists with a wide range of expertise such as insect and microbial ecology, animal toxicology, molecular biology or chemistry. During the period between the search conducted for the last MON 810 cultivation monitoring report, *i.e.*, June 2016, and beginning of June 2017, 37 publications related to MON 810 and/or Cry1Ab were published in high quality journals.

In order to be able to cite scientific work with the highest credibility, Monsanto uses publications from journals that are included in the Web of Science™ platform, a product of Clarivate Analytics (previously Thomson Reuters). This platform is one of the most commonly used ones for scientific literature reviews and is known for its comprehensive coverage of scientific journals and high quality standards. The platform covers over 33 000 of the impactful journals worldwide, including Open Access journals and over 7.4 million conference proceedings[13]. The web-based interface allows for a customized literature search using keywords in a certain combination and specific for each product.

The Web of Science™ platform offers one of the largest unified discovery platforms including more than 1 billion cited references and accesses the world's leading scholarly literature in the sciences, social sciences, arts, and humanities and examines proceedings of international conferences, symposia, seminars, colloquia, workshops, and conventions.

From the databases covered under the Web of Science™ platform; based on the coverage and relevance of the journals included, Monsanto selected the Web of Science™ Core Collection and the CABI CAB Abstracts® and Global Health® databases for performing the scientific literature searches. The Web of Science™ Core Collection database[14] includes literature captured under the following two catalogues: 1) the Science Citation Index Expanded (1995-present); and 2) the Conference Proceedings Citation Index- Science (1990-present) while the CABI CAB Abstracts® and Global Health® database[15] includes literature capture under the

[13] Web of Science™; http://clarivate.com/scientific-and-academic-research/research-discovery/web-of-science/ (Accessed 27 September 2017)

[14] Web of Science Core Collection; https://clarivate.com/products/web-of-science/web-science-form/web-science-core-collection/ - Accessed on 27 September 2017.

[15] CABI CAB Abstracts® and Global Health® database; http://www.cabi.org/cab-direct/ (Accessed on 27 September 2017)

CAB Abstracts (1973-present) catalogue. Further, these catalogues offer a complete view of item from a journal, including original research articles, reviews, editorials, chronologies, conference proceedings, bulletins, monographs, and technical reports.

Taking the above cited aspects into consideration, Monsanto's scientific literature search process can be summarized in five major steps:

Step 1. Set keywords and/or keyword combinations,
Step 2. Database search with keywords established in Step 1,
Step 3. Selection criteria. Reasons for including or excluding publications from further consideration,
Step 4. Screen retrieved articles and select relevant articles based on title and abstract,
Step 5. Assess the relevant articles using the full text and summary in prescribed format by consultation with experts.

Each of the above cited steps are detailed below.

Step 1 - Keywords and/or keyword combinations

The keywords used for a particular search and the Boolean operators used to combine them are provided when the results from a scientific literature search are communicated. These keywords take into account synonyms and abbreviations as well as spelling variants. The keywords and keyword combinations are established in English; hence, the search is expected to result in a list of articles written in English and/or articles written in other languages with at least a title, abstract or keywords in English. The keywords and keyword combinations are designed to give an excellent coverage and retrieve an as broad as possible number of articles related to the specific product. No restriction was applied when establishing the keywords.

Step 2 - Database search with keywords established in Step 1

After the keywords are established and combined via Boolean operators, they are used in a search of the Web of Science™ database[16]. All journals included in the database go through a verification process and as a minimum requirement, journals that are written in other languages than English need to include English-language bibliographic information (title, abstract, keywords), and all need to be peer-reviewed. In general, English is considered the universal language of science[17], which is why Clarivate Analytics focuses on journals publishing in English. For this reason, the journals most important to the international research community will publish either full text or a minimum of bibliographic information in English, which is especially true in the scientific domain of natural sciences. Full text in English is highly desirable if the journal intends to serve an international community of researchers. Therefore, it is expected that even if there is a relevant article for the food and feed safety of GM plants in a language different than English, the article will include title/abstract/keywords in English and the guarantee for retrieving these articles is provided by

[16] Web of Knowledge; http://apps.webofknowledge.com/ (Accessed 27 September 2017) (*Note* access to the database requires a subscription).
[17] Web of Science™; http://wokinfo.com/essays/journal-selection-process/ (Accessed 27 September 2017)

the use of keywords and keyword combinations in English (Step 1). These English titles, abstracts and keywords are used to screen the articles for relevance and the full text is checked in case needed.

Steps 3 - Selection criteria. Reasons for including or excluding publications from further consideration

From the full reference list of retrieved hits (*see* example for MON 810 in Appendix 5), taking into account i) the objective(s) of the studies, *i.e.* assessment of potential effects on human and animal health or the environment of MON 810 and ii) the scope of the application, *i.e.* authorization for import, processing and all uses as any other maize, including the cultivation of MON 810 in the EU, an assessment is conducted in order to conclude whether a certain publication is considered a relevant hit or not. Selection criteria have been established to categorize the publications that are part of the full reference list as either relevant or not. When a publication belongs to a category related to the risk assessment of the product, that publication will be included for further consideration. The following is a non-exhaustive list of categories publications can belong to:

Food/Feed safety assessment

- Molecular characterization
- Protein expression
- Crop compositional studies
- Agronomic and phenotypic studies
- Toxicology / Animal feeding studies
- Toxicology / *In vitro* studies
- Allergenicity studies of the protein or the whole food/feed
- Nutritional studies
- Protein / DNA fate in digestive tract

Environmental safety assessment

- Agronomy
- Non Target Organisms (NTO)
- Gene flow
- Protein/DNA fate in soil or in stream water
- Insect Resistance Management (IRM) / Impact of Management Practices
- Ecology

It should be noted that the selection criteria are well defined and reassessed annually. In case of disagreements on eligibility for the inclusion of studies, the reviewers (external and internal experts), discuss together. If uncertainty remains, the study is included for further consideration.

Steps 4 and 5- Screening and assessment of relevant publications

All publications that resulted from the search as described in Step 2 are screened by three different reviewers (one internal and two external experts) with a solid experience in the risk assessment of GM plants. The articles are assessed based on their title and abstract and deemed relevant or not based on the selection criteria as described in Step 3.

Later, the selected relevant articles are further assessed using the full text and summarized following the format as laid down in Commission Decision 2009/770/EC (Commission Decision, 2009). They are further subject to an analysis by experts with a solid experience in the risk assessment of GM plants and by experts with technical experience in the specific area of the selected publication. This analysis is conducted to formally assess the included studies (methodological quality) and the result is then used to assess if the conclusions on the food/feed safety of the risk assessment based on the comprehensive weight of evidence are still valid.

Results of the scientific literature search for MON 810

The key words used for this search and the operators to combine them are provided in Table 1. The detailed analysis of these peer reviewed publications is presented in Appendix 5. Publications were classified into the categories of food/feed (Toxicology/Animal feeding study; Allergenicity studies of the protein or the whole food/feed and Crop compositional studies; – *see* Appendix 5.1) and environment (Protein fate; Non-Target Organisms (NTO); Insect Resistance Management (IRM) and Agronomy – *see* Appendix 5.2).

Table 1. List of key words and operators used to obtain relevant publications related to MON 810 in Clarivate Analytics Web of Science™ database

Set	Search criteria
#7	((#4 OR #5 OR #6)) *DocType=All document types; Language=All languages;*
#6	(TS=(MON810 OR "MON 810")) *DocType=All document types; Language=All languages;*
#5	(TS=(Cry1Ab OR "Cry1 Ab" OR "Cry 1 Ab" OR "Cry 1Ab" OR CryIAb OR "CryI Ab" OR "Cry I Ab" OR "Cry IAb")) *DocType=All document types; Language=All languages;*
#4	((#1 and #2) OR (#1 and #3)) *DocType=All document types; Language=All languages;*
#3	(TS=(Yield Gard OR Yieldg* OR "Bt maize" OR "Bt corn")) *DocType=All document types; Language=All languages;*
#2	(TS=((TOLERAN* OR RESISTANT* OR PROTEC*) near/3 (Corn near Borer* OR CornBorer OR Lepidoptera OR Ostrinia OR Sesamia))) *DocType=All document types; Language=All languages;*
#1	(TS=(maize* OR corn* OR "zea mays" OR "z mays")) *DocType=All document types; Language=All languages;*

As a result, articles were retrieved from the search conducted using the Web of Science™ Core Collection database and the CABI CAB Abstracts® and Global Health® database (s*ee* Appendix 5.3 and Appendix 5.4 for the full reference lists of all hits). Retrieved articles were

assessed according to the procedures described in Steps 3 and 4, leading to the conclusion that 37 of them were relevant for the assessment of the potential effects of MON 810 on human and animal health or the environment. The scientific literature search in both the Web of Science™ Core Collection and CABI CAB Abstracts® and Global Health® databases was conducted every month covering in total the period of June 2016 to May 2017 (see Appendix 5.3 and Appendix 5.4).

Six publications were evaluated in terms of **food/feed safety** of genetically modified (GM) MON 810 maize, expressing the *Bacillus thuringiensis* (Bt) insecticidal protein Cry1Ab. Three were dealing with animal feeding studies, one was addressing allergenicity and one was related to crop composition (Andreassen *et al.*, 2016; Chrenkova *et al.*, 2016; Ibrahim and Okasha, 2016; Korwin-Kossakowska *et al.*, 2016; Osborne *et al.*, 2016; Zeljenková *et al.*, 2016).

Ibrahim and Okasha (2016) studied the changes in the jejunal mucosa of adult male albino rats fed MON 810 maize at 30% in the diet for 90 days. There was no mortality and no clinical signs were recorded throughout the experiment. Furthermore, there were no effects on behaviour, feed consumption or average weight gain. However, the histological structure of the jejunal mucosa was altered in the MON 810 group compared to controls, with observations of proliferative and eroded hemorrhagic lesions in addition to several ultrastructural alterations. Possible mechanisms proposed by the authors included inflammation associated with goblet cell overexpression and PCNA upregulation. However, the jejunum varies greatly along its length and the observations reported are likely to reflect regional differences across this variable tissue on account of sampling bias rather than an effect of treatment. It should also be noted that the study used poorly described and insufficiently characterized diets/test materials and non-validated techniques to characterize potential hazards, used missing tabulated histological data to assess effects and did not show any evidence of adverse effects in the animal (*see* Appendix 5.1). **Zeljenková *et al.* (2016)** performed a one year oral toxicity study in Wistar Han RCC rats fed with MON 810 maize at 11 and 33% in the diet. Results on hematology, clinical biochemistry parameters, absolute and relative organ weights and histopathological analysis showed that MON 810 maize at a level of up to 33% in the diet did not lead to toxicologically relevant effects in male and female rats. The authors noted that more sensitivity would be required to distinctly identify specific potential effects. The group of **Korwin-Kossakowska *et al.* (2016)** studied the health status and uptake of transgenic DNA in breast muscle, eggs and organs of Japanese quail (*Coturnix cot. japonica*) fed MON 810 maize over 10 generations. The results suggested that there was no negative impact with regard to bird health status or to the presence of transgenic DNA in the final consumable product. **Chrenkova *et al.* (2016)** fed MON 810 maize to Holstein bulls for 258 days and found no effect on pH value, chemical composition or colour of the meat. **Andreassen *et al.* (2016)** investigated the immunogenic, allergenic and adjuvant properties of trypsinised Cry1Ab protein after intragastric exposure in a food allergy model in mice. No detectable immunogenic, allergenic or adjuvant capacity occurred after intragastric exposure, within the limitations of the model, the doses and the applied protein context. Finally, **Osborne *et al.* (2016)** conducted a three year field study in the northern USA maize belt to

evaluate the impact of genetic modification on maize plant residue, grain yield, grain nutrient composition and stalk nutrient composition independent of glyphosate use. A total of 18 maize hybrids from three different genetic platforms were used, including MON 810 maize. The side by side comparison of these hybrids grown under the same soil, environment and agronomic management did not result in any significant difference in above-ground biomass or grain yield.

Twenty one publications were reviewed in terms of **environmental safety**, more specifically on the subject of non-target organisms, agronomy, fate of Cry protein in the environment and insect resistance management (Andow and Zwalhen, 2016; Buuk *et al.*, 2016; Castañera *et al.*, 2016; Di Grumo and Lovei, 2016; Dos Santos *et al.*, 2016; Erasmus *et al.*, 2016; Griffiths *et al.*, 2017; Kotey *et al.*, 2017; Lee and Albajes, 2016; Mashiane *et al.*, 2017; Niu *et al.*, 2016; Omoto *et al.*, 2016; Peterson *et al.*, 2016; Shu *et al.*, 2017; Sousa *et al.*, 2016; Stenekamp *et al.*, 2016; Tefera *et al.*, 2016; Waquil *et al.*, 2016; Yang *et al.*, 2016; Yao *et al.*, 2017; Yinghua *et al.*, 2017)

In the field of non-target organisms, **Andow and Zwalhen (2016)** determined the direct and indirect exposure routes through which non-target organisms such as carabid beetles might acquire Cry1Ab protein in fields of MON 810 maize. Based on the results, the authors concluded that there was transfer of Cry1Ab proteins from living maize plant, residue or prey towards beetles and that significant differentiation among carabid species in their associations with live-plant and residue based food webs in agricultural fields exists. In this respect, it is important to note that a key principle of environmental risk assessment (ERA) is risk-based testing (CLI, 2016; Wolt *et al.*, 2010) following a tiered approach, limited to scenarios for which there is plausible scientific rationale for an adverse effect. Overall, although Andow and Zwalhen (2016) report interesting and important results, the current body of knowledge does not indicate that Cry1Ab expressed in *Bt* maize negatively impacts the predation ecosystem services provided by ground beetles (*see* Appendix 5.2). **Di Grumo and Lovei (2016)** determined the body size distribution of ground beetle (*Coleoptera: Carabidae*) assemblages in order to monitor the environmental impact of MON 810 maize. The authors did not find significant differences in inequality or mean body size between the assemblages in MON 810 *vs.* isogenic maize plots. **Lee and Albajes (2016)** assessed carabid beetle indicators that could reveal environmental impacts of GM maize. Their results showed that the best indicator species was *Pseudoophonus rufipes*. The best sampling location was field margins, where carabids were exposed to GM maize and were abundant enough to require smaller sample sizes to detect population changes. In addition, carabid abundance was shown to be the highest around pollen-shed. The group of **Peterson *et al.* (2016)** evaluated the potential risk associated with the uptake of *Bt* proteins from GM maize, including MON 810, by spiders. The researchers concluded that diverse members of a spider community took up *Bt* protein via pollen and prey consumption and that this should be factored into future risk assessment of *Araneae* in North America. As discussed above in the context of the research by Andow and Zwalhen (2016), knowledge on the mode of action of Cry 1Ab and results of studies on numerous species does not indicate that this protein expressed in *Bt* maize negatively impacts the predation ecosystem services provided by spiders (*see* Appendix 5.2).

In China, **Shu *et al.* (2017)** looked at the effects of MON 810 maize straw return on the bacterial community of earthworms. They found that *Bt* maize straw return caused changes in the living environment of the earthworm, mainly including soil nutrient levels and bacterial community, which might ultimately affect the growth, metabolism and functions of earthworms. The study design is flawed because it did not include maize reference varieties for comparison. Therefore, the results from this study do not impact the conclusion of the environmental risk assessment conducted on MON 810 and do not trigger adverse effect reporting for the reasons described in Appendix 5.2. In **Mashiane *et al.* (2017)**, bacterial communities associated with the roots of MON 810 and non-*Bt* maize roots from field-grown plants in South Africa differed more across developmental stages than between maize genotypes. These differences were more pronounced between the diversity and abundance of particular species, rather than in the species richness of the maize bacterial community. Finally, **Griffiths *et al.* (2017)** examined the occurrence, leaching and degradation of Cry1Ab protein from MON 810 maize into streams. The protein was commonly detected in streams sampled across an agricultural landscape. Combined with laboratory studies showing rapid leaching and degradation, this suggested to the authors that Cry1Ab may be pseudo-persistent at the watershed scale due to the multiple input pathways from the surrounding terrestrial environment.

With regard to maize pests, **Yinghua *et al.* (2017)** examined the response of the secondary lepidopteran pest *Spodoptera litura* (leafworm moth) to two *Bt* maize hybrids, 5422Bt1 (Bt11) and 5422CBCL (MON 810). Catalase, superoxide dismutase and glutathione-S-transferase activities in larvae fed Bt11 leaves were significantly higher than those in the MON 810 treatments. *S. litura* showed low susceptibility to Bt11 and MON 810 when larvae were fed kernels instead of leaves. **Stenekamp *et al.* (2016)** evaluated the effect of diet containing MON 810 maize on *Cydia pomonella* (codling moth) larval mortality, development and dispersal. *Bt* maize meal diet adversely impacted the number of moths produced. Delayed larval development appeared to be the most important parameter affected. **Tefera *et al.* (2016)** assessed the performance of MON 810 maize against *Busseola fusca* (stem borer) and *Chilo partellus* (spotted stem borer) in a biosafety greenhouse and confined field trial in Kenya. MON 810 was effective in controlling both species.

In the field of insect resistance management, **Buuk *et al.* (2016)** assessed shifts relative to baseline susceptibility of the maize pest *Ostinia nubilalis* (European corn borer) to Cry1Ab protein from MON 810 maize. From 2004 to 2015, larvae were collected from 15 areas in Europe, then cultured in the laboratory and exposed to Cry1Ab protein. Results indicated that the *O. nubilalis* populations remained susceptible to Cry1Ab over the entire test period. The group of **Yao *et al.* (2017)** in the USA compared gut gene transcriptional responses using microarrays, between Cry1Ab-resistant and susceptible strains of *O. nubilalis* when larvae were fed Cry1Ab maize leaves. The study suggests enhanced adaptation of Cry1Ab-resistant larvae on Cry1Ab maize as revealed by the lower number and ratios of differentially expressed genes in the R-strain than in the S-strain of *O. nubilalis*. **Castañera *et al.* (2016)** investigated whether *Sesamia nonagroides* (Mediterranean corn borer) developed resistance to MON 810 maize cultivated in Spain and looked into possible reasons of the resistance

management success using evolutionary models. There was no shift in susceptibility to Cry1Ab protein in field populations after 16 years of cultivation. The low initial adoption rates and the EU policy decision to replace Event 176 with MON 810 *Bt* maize were key to delaying resistance evolution. Model results suggested that, if refuge compliance continues at the present 90%, *Bt* maize might be used sustainably in northeast Spain for at least 20 more years before resistance might occur. **Dos Santos *et al.* (2016)** evaluated the biological response of *Helicoverpa zea* (corn earworm) F1 and F2 generations and *Helicoverpa armigera* (cotton/corn earworm) F2 and F3 generations in *Bt* maize expressing various Cry proteins, including MON 810 and their isogenic conventional counterparts. In the isogenic plants, the adaptation index of *H. armigera* was higher than that of *H. zea*, which indicated greater ease of adaptation to the environment of that species. **Erasmus *et al.* (2016)** determined the movement and survival of *B. fusca* larvae within maize plantings in South Africa with different ratios of *Bt* seed (Cry1Ab and Cry1A.105 + Cry2Ab2) and their near-isogenic non-*Bt* isolines. Larval movement continued for five weeks and resulted in a significant incidence of *Bt* and non-*Bt* damaged plants, indicating that the movement behaviour of *B. fusca* is of such a nature that seed mixtures as an IRM strategy may not be effective to delay resistance evolution. Work conducted by **Kotey *et al.* (2017)** showed that *B. fusca* collected from fields in two *Bt* maize cultivating areas and a non-*Bt* maize cultivating area of the Eastern Cape region in South Africa were susceptible to *Bt* maize. **Niu *et al.* (2016)** evaluated the survival and plant injury of two strains of *Spodoptera frugiperda* (fall armyworm) resistant to Cry1A.105 protein. Their results evidenced cross resistance to Cry1F and Cry1Ab maize, but not to the *Bt* maize products containing Cry2Ab2 or Vip3A. In Brazil, from 2010-2015, **Omoto *et al.* (2016)** followed the susceptibility of field-collected *S. frugiperda* to Cry1Ab protein and the performance of MON 810 maize hybrids. The decrease in susceptibility of *S. frugiperda* to Cry1Ab was expected, but the specific contributions to this resistance by MON 810 maize could not be distinguished from cross-resistance to Cry1Ab caused by exposure to Cry1F maize. **Sousa *et al.* (2016)** determined whether exposure to *Bt* toxins in low- and moderate-dose transgenic crops induced sub-lethal effects and increased the rate of *Bt* resistance evolution in *S. frugiperda*. In Brazil, offspring of *S. frugiperda* that developed on single-gene Cry1Ab maize grown in field had reduced performance on Cry1Ab maize foliage in two populations studied. However, in the other three populations, these offspring had better overall performance on the *Bt* maize foliage than that of the *S. frugiperda* from non-*Bt* maize fields. This was possibly linked to the presence of Cry1Ab resistance alleles in these populations. **Waquil *et al.* (2016)** field-collected *S. frugiperda* in Brasil to evaluate their fitness index and lethal time of the population on *Bt*-maize expressing the Cry1Ab, Cry1F, Cry1A.105/Cry2Ab2 and Vip3Aa20 proteins. The Cry1Ab *Bt* maize showed a limited but durable efficiency. The authors concluded that the lethal time can be a variable that indicates evolution of resistance, since it is greater for populations with greater fitness. **Yang *et al.* (2016)** compared the performance of Cry1Ab-susceptible and Cry1Ab-heterozygous resistant populations of *Diatraea saccharalis* (Sugarcane borer) in sequential feedings on *Bt* (MON 810) and non-*Bt* maize plant tissue, in the greenhouse. The larval dispersal behaviour of *D. saccharalis* could result in survival and

successful reproduction of heterozygous resistant populations in seed mix plantings, even with high dose expression.

For the 2016-2017 period, a total of ten **review papers** on *Bt* maize were identified in the search output. Three publications were reviewed in terms of food and feed safety (Domingo, 2016; Joshi *et al.*, 2016; Schmidt *et al.*, 2016) seven in terms of environmental safety (Blanco *et al.*, 2016; Coates, 2016; Diaz-Gomez *et al.*, 2016; Han *et al.*, 2016a; Han *et al.*, 2016b; Van den Berg, 2016; Venter and Bøhn, 2016).

In the field of **food and feed safety**, **Domingo (2016)** reviewed literature from January 2011 to May 2016, retrieved from PubMed and Scopus, regarding the potential adverse health effects of GM plants but excluding the studies regarding allergenicity. An oral toxicity study showed no adverse effects when rats had up to 33% of MON 810 in the diet. Another study reported alterations in organ weights, hematology and serum biochemical analyses in rats fed *Bt* maize (including MON 810) after 1.5 months. However, the author highlighted the very low number of animals used in the study. Finally, a study where *Daphnia magna* were exposed to leaves of MON 810 maize showed adverse effects on body size and fecundity later in life However, this study from Holderbaum *et al.* (2015) as previously indicated in the 2015 monitoring report should be interpreted with caution. The study methods did not follow accepted testing guidelines. The duration of the tests was substantially longer (42 days vs. 21 days) than recommended OECD (2012). Control mortality must not exceed 20%; however, the control mortality exceeded 20% at day 21, long before the termination of the 42 day study. The authors noted that *Daphnia* fed maize-leaf diets displayed higher rates of mortality and reduced reproduction, indicating sub-optimal feed conditions, yet they drew conclusions based on these data. **Schmidt *et al.* (2016)** analysed the data of four 90 day and a 1 year feeding trials with MON 810 maize in Wistar Han RCC rats performed in the frame of EU–funded GRACE project. The comparison of the five feeding trials showed a clear study effect in the control data. It also showed inconsistency both in the frequency of statistically significant differences and in the difference values between control and test groups. **Joshi *et al.* (2016)** reviewed published literature to assess the potential adjuvanticity of Cry proteins, including Cry1Ab. Although the presumption exists that Cry proteins may have immune-stimulatory activity and therefore an adjuvanticity risk, the evidence shows that Cry proteins, including Cry1Ab, are expressed at very low levels in GM crops and are unlikely to function as adjuvants. The authors furthermore stress that the study design is a critical aspect in discerning the potential use of animal models to identify adjuvant effects from Cry protein exposure. The main concern appears to be the co-exposure to unwanted toxicants or known immune-modulating molecules such as LPS or mycotoxins when preparing purified Cry protein or test diets.

With respect to **environmental safety**, **Diaz-Gomez *et al.* (2016)** reviewed the relationship between the cultivation of GM *Bt* maize, and the contamination levels with different types of mycotoxins. The authors reported numerous studies on a significant reduction in pest damage, disease symptoms and fumonisin levels in *Bt* maize hybrids (including MON 810 maize), particularly in areas where the European corn borer was prevalent. When other pests were also

present, the *cry1Ab* gene alone offered insufficient protection, and combinations of insecticidal genes was required to reduce damage to plants caused by insects and, indirectly, mycotoxin levels. **Venter and Bøhn (2016)** reviewed the exposure, spread, breakdown rates and effects of *Bt* crops on non-target organisms and aquatic communities. Results on the effects of *Bt* crops in aquatic organisms are contradictory. However, in the case of MON 810 maize, *Daphnia magna* fed with *Bt* material (kernels and leaves) resulted in negative effects regarding survival and fecundity. However, this study from (Bøhn *et al.*, 2008) as previously indicated in the 2009 monitoring report should be interpreted with caution. There appear to be methodological problems with the paper which do not allow for any substantive conclusions to be drawn from the study and it did not adhere to any of the internationally accepted testing guidelines for Daphnia reproduction. The group of **Blanco *et al.* (2016)** reviewed the current situation of pests targeted by *Bt* crops in Latin America by asking growers, crop consultants and academics about *Bt*-resistance problems in agricultural fields. In six Latin American countries where *Bt* maize (including MON 810) is planted, no synthetic insecticide applications are used to control maize pests because they are controlled by *Bt* maize, with the exception of *Spodoptera frugiperda*. This insect, in some countries, is still effectively controlled by *Bt* maize but, in others resistance has evolved and necessitates supplemental insecticide applications and/or the use of *Bt* maize cultivars that express multiple *Bt* proteins. **Coates (2016)** reviewed the molecular genetic basis of *Bt* toxin (Cry1Ab) resistance with emphasis on resistance described for *Ostrinia nubilalis*. The author summarized the current knowledge on receptor-mediated resistance and genetic control of receptor dysregulation, concluding that effective IRM plans requires additional investigations into the genomic architecture of resistance and the complex biological and ecological interactions of the insect. Another review by **Han *et al.* (2016b)** assessed the current information related to the effects of insect-resistant (IR) GM crops on arthropod behavior. Almost all studies were conducted on *Bt* crops, including Cry1Ab maize. Effects were found in 54, 22 and 33% of the case studies on phytophagous arthropods, arthropod natural enemies and pollinators, respectively. The authors concluded that, while attention needs to be paid to several behavioral effects that may undermine the efficacy of IRGM crops in sustainable pest management, these generally do not disrupt their role in achieving the goal of integrated pest management and crop production. In the insect resistance management area, **Han *et al.* (2016a)** summarized the development of GM maize and control efficacy against target pest complexes, reporting that the combination of different genes (including *cry1Ab*) in GM maize was suggested to confer full protection against *O. furnacalis* and *H. armigera*. The susceptibility *O. furnacalis* to Cry1Ab maize across China showed small variations and laboratory data supported the potential for developing resistance in *O. furnacalis.* Further, Cry1Ab-resistant *O. furnacalis* showed cross-resistance to some other Cry-proteins. The mechanisms of resistance and the mode of action in *O. furnacalis* includes transcriptional regulation of different genes. The authors suggest that because there is no cross-resistance or sharing of receptors between Cry1Ie and Cry1Ab or Cry1Ac proteins in *O. furnacalis,* their pyramided use in GM maize is supported. For South Africa, a review by **Van den Berg (2016)** discussed data of ecological studies and stem borer surveys conducted over the past decade and showed that wild host plants are unsuitable for development and survival of sufficient numbers of stem borer

individuals. These grasses rather act as dead-end-trap plants and do not comply with refuge requirements of producing 500 susceptible individuals for every one resistant individual that survives on *Bt* maize.

The publications identified by this literature search confirm the conclusions of the risk assessment. The peer-reviewed literature demonstrates that MON 810 is as safe to human and animal health as its conventional counterpart and confirms that there is negligible impact from the cultivation of MON 810 on biodiversity, abundance, or survival of non-target species. The environmental risk of MON 810 is negligible compared to conventional maize. This assessment concurs with the previous scientific opinions from EFSA on MON 810.

On 3 June 2016, the European Commission requested EFSA to provide scientific assistance on new scientific information **(Bøhn *et al.*, 2016; Hofmann *et al.*, 2016)** in relation to the risk assessment of genetically modified *Bt* crops[18]. As indicated in Appendix 5.3 and 5.4, both publications were retrieved in the literature of this years´ search period. However, they were considered non-relevant because the studies were not conducted either on MON 810 or Cry1Ab protein derived from the plant. The lack of relevance of these papers for the risk assessment of MON 810 is further described in the EFSA assessments (EFSA, 2016a, 2016b).

[18] EFSA Register of Questions; http://registerofquestions.efsa.europa.eu/roqFrontend/login?0 (Accessed 27 September 2017)

3.2 Case specific monitoring

3.2.1 Description and results of case-specific monitoring (if applicable)

Decades of experience have taught entomologists that insect populations have the potential to adapt, sometimes quickly, when exposed to insecticides via a selection process of existing resistant individuals in natural populations. For this reason, as early as 1992 in the US, Monsanto established an expert advisory panel composed of leading pest and resistance management researchers from academia, USDA-ARS, and university extension services to develop efficient Insect Resistance Management (IRM) strategies for insect-protected maize.

Following this example, Monsanto along with three other companies[19] established the European Union Working Group on Insect Resistance Management and developed together a harmonized IRM plan specific for the EU which was implemented until the 2011 growing season (reported on in 2012, see Monsanto Europe S.A. (2012)). This plan enabled the implementation of the management strategy described in Appendix II of the notification submitted to the French Commission du Génie Biomoléculaire (Monsanto Company, 1995), and has been based on published research, current EU legislation, the European Commission's Scientific Committee on Plants (SCP) opinion on IRM[20] and practical experience gained during the implementation of IRM plans in other parts of the world.

Meanwhile, EFSA published an updated guidance document on PMEM of GM crops as well as seven specific opinions on the monitoring conducted by Monsanto on MON 810 in the 2009, 2010, 2011, 2012, 2013, 2014 and 2015 growing seasons (EFSA, 2011b, 2012d, 2013c, 2014a, 2015c, 2016c, 2017). One of the elements described in the original plan was to update it in view of the findings and new scientific information. Taking into account the related EFSA´s opinions, the historical data on *Bt*-maize cultivation, data in the scientific literature, and the experience gained from IRM plans established in other regions, the EuropaBio Monitoring working group has updated the IRM plan in 2017 (*see* Appendix 6). The purpose of the IRM plan is to proactively monitor the potential development of target pests resistance to the Cry protein(s) expressed in these single *Bt* maize events in the EU. This harmonized IRM plan contains guidance on the following key elements: (1) Refuge; (2) Resistance monitoring in the target pests; (3) Farmers compliant system; (4) Remedial plan in case of *Bt* maize failure to protect against target pests; and (5) Communication and Grower education.

[19] Syngenta Seeds, Pioneer Hi-Bred International Incorporated and Dow AgroSciences.

[20] SCP (1999), Opinion of the Scientific Committee on Plants on *Bt* resistance monitoring (Opinion expressed on March 04, 1999), *Document SCP/GMO/094-Rev.5* - http://iatp.org/files/Opinion_of_the_Scientific_Committee_on_Plants_.htm (Accessed 27 September 2017)

3.2.1.1 Refuge

According to the *Harmonised insect resistance management (IRM) plan for cultivation of Bt maize (single insecticidal trait) in the EU* (*see* Appendix 6), farmers planting more than five hectares of MON 810 must have a refuge area planted with maize that does not express Cry1Ab and that corresponds to at least 20% of the surface planted with MON 810.

Many initiatives have been taken to educate the farmers on the importance of implementing IRM measures (*see* Section 3.2.1.4). For cultural reasons, certain farming communities are reluctant to accept 'signed agreements' requiring them to adhere to particular agricultural practices. Moreover, seeds are usually sold through distributors and farmer cooperatives, which adds another 'step' in the commercial chain. The absence of direct sales between end-users and seed companies makes signed agreements very difficult. Consequently, the seed industry has put emphasis on the development of communication tools.

In the context of Monsanto's 2016 GS, 250 farmers across Spain and Portugal where MON 810 was commercially cultivated were surveyed for their implementation of a refuge (*see* Appendix 1). This GS took place in representative environments, reflecting the range and distribution of farming practices and environments exposed to MON 810 plants and their cultivation.

92.0% of the farmers indicated that they followed the technical guidelines regarding the implementation of a refuge (70.8 % planted a refuge and 21.2 % had less than 5 ha planted with MON 810 on their farm[21]). Both countries reported a very high level of compliance with refuge requirements. The farmers in Portugal were all in compliance with refuge requirements. Responses of the Monsanto 2016 Farmer Questionnaire Survey show that 91.6% of the farmers in Spain were compliant with refuge planting while 20 farmers out of 237 (*i.e.*, 8.4%) indicated they did not plant a refuge. The farmers gave three main reasons for not being compliant with the refuge requirements: (1) lack or not enough information about the technical guidelines and fear of yield losses in conventional maize (10/20, 50.0%); (2) their neighbours' refuge was taken to be sufficient or the refuge was smaller than 20% of MON 810 area (7/20, 35.0%) and (3) the refuge implementation complicates the sowing (3/20, 15.0%).

In Portugal, an independent Monitoring Report on the planting of MON 810 varieties (including IRM communication and refuge implementation) during the 2016 growing season was prepared by the Portuguese authorities (DGAV, 2016). In addition to the farmers trained in previous seasons, and in compliance with the Portuguese law, 74 new farmers[22] were trained in 2016 on national and EU legislations that regulate the cultivation of GM varieties and to learn about the main characteristics of MON 810 maize. Furthermore, 79 inspections were performed on farmers planting MON 810 maize out of the total 242 cultivation notifications registered in 2016. These inspections showed high compliance in general terms,

[21] The IRM plan states that no refuge is required if there is less than 5 ha of MON 810 planted on the farm.

[22] So far, 1773 farmers have been trained on national and EU legislations since 2005.

with minor changes compared to the information declared in the notification, and no sanctions were needed. Full compliance with refuge and labelling requirements was found. In addition, 61 farmer questionnaires were completed by farmers growing MON 810 maize in Portugal. None of them declared that an adverse effect related to the GM crop was observed. All the interviewed farmers stated that the technical information on the seed bags was sufficient and clear.

In conclusion, the results from the presented surveys (Portuguese authorities and Monsanto) during the 2016 season are consistent and do show a high level of compliance, probably due to the high effectiveness of the growers' education. Regardless of these results, the message on the importance of refuge implementation is being repeated in countries growing MON 810 in the 2017 cultivation season with special focus in new growing areas. It is important to continue educating the farmers on the necessity to implement refuges and align them with a responsible use of the technology.

It would be also recommended that refuge planting would be integrated as requirement for direct payments under the Common Agricultural Policy or other national rules, then compliant farmers would be encouraged to continue implementing refuges, whereas those farmers reluctant to be compliance could be subjected to reductions or exclusions from direct support schemes.

3.2.1.2 Baseline studies and resistance monitoring in the target pests

Baseline studies

Baseline studies with Cry1Ab were performed in Spain with *S. nonagrioides* and *O. nubilalis* populations collected in the three major regions where insect pressure justifies the use of MON 810 (Ebro Valley, centre of Spain and Extremadura-Andalusia) prior to the introduction of *Bt* maize in Spain (Gonzalez-Nunez *et al.*, 2000). These results were reported in the 2003-2004 Monitoring Report (Monsanto Europe S.A., 2005). The baseline susceptibility to Cry1Ab was also established for the French and Portuguese field populations of *S. nonagrioides* and for the Portuguese populations of *O. nubilalis* (Monsanto Europe S.A., 2006, 2007). Overall, the susceptibility to Cry1Ab of these species was within the range obtained in baseline studies and subsequent monitoring performed after *Bt*176 maize cultivation (Farinós *et al.*, 2004; Gonzalez-Nunez *et al.*, 2000), prior to MON 810 introduction. In addition, the baseline susceptibility of *O. nubilalis* to Cry1Ab was explored from 2005 to 2007 in other major European maize growing regions based on the potential MON 810 adoption. During this period, levels of susceptibility to Cry1Ab have been determined for one laboratory colony and several field collected *O. nubilalis* populations in maize fields in the Czech Republic, France, Germany, Italy, Hungary, Slovakia, Poland, Portugal and Romania (Monsanto Europe S.A., 2006, 2007, 2008).

Resistance monitoring in the target pests

Monitoring for changes in susceptibility to Cry1Ab in *O. nubilalis* and *S. nonagrioides* across the Ebro Valley, central Spain and Extremadura-Andalucia since 1999 was in place following the commercialisation of *Bt*176 maize varieties from Syngenta, that also expressed the Cry1Ab protein (Farinós *et al.*, 2004). During 2004-2011, monitoring for *O. nubilalis* and *S. nonagroides* susceptibility to Cry1Ab expressed in MON 810 was performed following the IRM plan developed by a European Union Working Group on Insect Resistance Management in those geographical areas with considerable commercial plantings of MON 810. During 2012-2015, monitoring for *O. nubilalis* and *S. nonagroides* susceptibility to Cry1Ab expressed in MON 810 was performed following the 2012 EuropaBio harmonised IRM plan updated in the view of the related EFSA's opinions, historical data on *Bt*-maize cultivation, scientific literature and worldwide experiences on IRM plans.

One of the elements described in the harmonized IRM plan is to keep it updated based on new learnings and scientific information. Accordingly, EuropaBio updated the IRM plan in 2017 taking into account recent EFSA opinions, the large amount of additional data generated in the scientific literature, and the experience gained from IRM plans established in Europe and in other world areas (*see* Appendix 6). In the 2017 harmonized IRM plan, the sampling approach and monitoring protocol were revised. The sampling approach as defined in Table 3 of the EuropaBio harmonised IRM plan was implemented to connect sampling frequency to the MON 810 adoption rate and the ecology of the target pests (*i.e.*, multivoltine versus univoltine life cycles). The monitoring protocol as described in the IRM plan was implemented to consider as many larval samplings as possible, ultimately targeting a detection level of 3% resistance allele frequency in the target pest population, and the recurring practical limitations to meet this requirement.

Monsanto acknowledges that EFSA made several recommendations to improve the methodology for resistance monitoring in the target pests (EFSA, 2011b, 2012d, 2013c, 2014a, 2015a, 2015c, 2016c, 2017). EFSA provided recommendations for the sampling of the target pests, suggesting to "*increase sampling efforts and ensure that as many field-collected larvae as possible are represented in the laboratory assays as F_1 larvae in order to provide sufficient detection sensitivity (i.e. 3% resistance allele frequency)*"(EFSA, 2015c, 2016c, 2017). Monsanto followed previously fit-for-purpose methodologies gained through experience and in line with harmonized IRM plans allowing EFSA to conclude that no adverse effects related to the target pests have been identified due to MON 810 cultivation and that the findings do not invalidate the previous risk assessment conclusions (EFSA, 2011b, 2012d, 2013c, 2014a, 2015c, 2016c, 2017). The monitoring studies performed with *O. nubilalis* and *S. nonagrioides* from 2004 to 2016 showed that the susceptibility of the collected insect samples to Cry1Ab were within what is considered the normal historical range, demonstrating no change in susceptibility. The findings were further affirmed by scientific literature which demonstrated the absence of resistance development in the target pests (*O. nubilalis* and *S. nonagroides*) to the Cry1Ab protein after years of MON 810 cultivation in the EU (Castañera *et al.*, 2016; Farinós *et al.*, 2017; Thieme *et al.*, 2017).

Nevertheless, in light of all the continued EFSA recommendations (EFSA, 2015c, 2016c, 2017), Monsanto has extensively increased the efforts to sample as many larvae as possible, although EFSA now acknowledges also the difficulties and uncertainties of being able to meet the above recommendation (EFSA, 2017).

MON 810 adoption in the Czech Republic, Slovakia and some regions in Spain and Portugal (Central Iberia, the Southwest of Spain and Portugal)) was well below 60%. The area identified in the entire EU region where adoption of MON 810 in the 2016 maize growing season was expected to be greater than 60% was the Ebro valley (Northeast Iberia) in Spain. According to EFSA's opinion, the Ebro valley is also where adoption rate of MON 810 is the highest, field resistance to Cry1Ab is most likely to evolve and where annual monitoring of the both target pest populations should be exclusively implemented (EFSA, 2016c, 2017). Therefore, larvae sampling of *O. nubilalis* and *S. nonagrioides* for the monitoring activities in the 2016 maize growing season concentrated in the Ebro valley as described in the revised IRM plan (Appendix 6) and as recommended by EFSA. No larval samples for *O. nubilalis* and *S. nonagrioides* were collected from the other growing areas for the reasons explained above.

Aligned with the newly revised EuropaBio harmonised IRM plan, the objective of the sampling efforts in the 2016 growing season was to collect approximately 1 000 larvae per population in the Ebro valley, which ultimately target the detection of 3% (recessive) resistance allele frequency, as suggested by EFSA (EFSA, 2016c). From the experience gained in 14 years of MON 810 PMEM and information from independent resources[23], it was demonstrated that such collections may not be always feasible because the target pests' pressure and the number of larvae in the region have decreased drastically since MON 810 introduction and as a result of MON 810 performance. Consequently, despite intensified efforts of larvae collection, the significant reduction of the pest population over the years as well as occurrence of further drops in the pest population due to various reasons in certain growing seasons may make collecting 1 000 larvae impossible. Therefore, as indicated in the 2017 EFSA opinion (EFSA, 2017), flexibility on the number of larvae samples should be granted provided that the responsible parties can demonstrate to have undertaken the necessary steps to ensure the collection of as many larvae as possible.

During the 2016 growing season, Monsanto increased its effort to collect for both target pests as many larvae as possible for the laboratory assays. The details of the sampling efforts and laboratory assay are presented in Appendix 7 (Insect resistance monitoring report for *S. nonagrioides* associated with MON 810 maize cultivation in the EU) and Appendix 8 (Cry1Ab susceptibility in European origins of *O. nubilalis*). In 2016, a bioassay based on a single diagnostic concentration (DC) estimated from MIC_{99} values was used to evaluate changes in susceptibility of the target pests to the Cry1Ab protein. The use of a diagnostic

[23] Catalunya Research Institute, IRTA, 2014; https://www.ruralcat.net/c/document_library/get_file?uuid=52ce0d40-0c2f-42c8-ac9f-3609cc656237&groupId=10136 (Accessed 27 September 2017)

concentration assay is found appropriate based on the experience gained as well as scientific literature (Roush and Miller, 1986). This method increases the effectiveness and sensitivity of the assay for detecting changes in susceptibility to the Cry protein. In addition, comprehensive details of the larvae used and the data generated in the bioassays are clearly elaborated based on appropriate statistical analysis in both reports.

As reported in Appendix 7, from the 1364 larvae of *S. nonagrioides* collected in the Ebro valley Spain, 960 adults (70%) emerged, and the offspring of 95% of these adults (911) were used in the bioassays and treated with the DC of 1091 ng Cry1Ab/cm^2. The treatment with the DC caused moulting inhibition of 97.96% (S.E. 0.71%) to the F1 neonates, which was not significantly lower than the expected value of 99 %. As shown in Appendix 8, from the 1111 larvae of *O. nubilalis* collected in the Ebro valley Spain, 554 adults survived the diapause period, reached the adult stage and mated. Of the 1562 *O. nubilalis* larvae exposed to the discriminating concentration 3 larvae died, 1547 survived but did not reach the 2nd larval stage, and 12 reached the second larval stage. The resulting effect of Cry1Ab on moulting inhibition (this criterion used accounts for both death and complete moulting (growth inhibition) was 99.23 %. In addition, all of the *S. nonagrioides* and *O. nubilalis* larvae that survived in the bioassays died after feeding on leaves of *Bt* maize. In conclusion, no evidence was detected of a decrease in Cry1Ab susceptibility of *O. nubilalis* and *S. nonagroides* during the monitoring duration.

3.2.1.3 Farmer complaint system

Monsanto and the seed companies offering MON 810 varieties have a robust farmer complaint systems which provides a means for farmers to report any complaint related to maize seeds performance, including failure in protection against corn borers in MON 810 varieties. Farmers are first in line to detect a change in product performance, including reduced target pest insect control. Farmer complaint systems are available without any limitations for the entire farming community and for every field where MON810 is commercially cultivated. Therefore, the farmer complaint system serves as the primary tool to detect insect resistance development (Sumerford *et al.*, 2015). The farmer complaint system is a primary venue for the farmer to record any unexpected effect when cultivating *Bt* maize in their field. As a result, Monsanto believes based on gained experiences that incidence of reduced susceptibility to Cry1Ab protein in the target pest populations is most likely to be detected and reported rather via the farmer complaint system then the laboratory bioassays.

Farmers can complain to the seed suppliers about product related issue via the local sales representatives or customer service routes. The specific procedure can slightly differ between seed suppliers, but in all of them, once a validated product-specific complaint is received, an internal procedure for verification, potential analysis, and follow up is triggered. In the case of Spain, all companies offering MON 810 varieties have committed to monitor insect protection during the cultivation, as part of the Monitoring Plan requested by the registration in the Spanish variety catalogue. In case the analysis of the complaint indicates potential insect resistance development, a procedure will be followed that includes on-site follow-up by company representatives and additional testing of the larvae susceptibility to the protein

Cry1Ab and plants expressing MON 810. If this assessment would confirm insect-resistance development, a remedial plan as described in the EuropaBio harmonized IRM plan will be implemented without prejudice to specific actions that may be required by country or local authorities. In Spain the mitigation plan would be compulsory and established at the Monitoring Plan associated to MON 810 varieties registration.

During the 2016 growing season, Monsanto representatives did not receive any complaint related to MON 810 target pest efficacy. A survey has been performed in Spain among Asociación Nacional de Obtentores Vegetales (ANOVE, the National Breeder Association in Spain) member companies commercializing MON 810 maize to have an overview of the farmer complaint schemes. The effectiveness of the system was demonstrated because a total of 1.556 complaints were received related to any issue with maize seeds, by the companies which are marketing MON 810. Only 6 complaints were related to efficacy and after investigation, all of them were related to errors or misunderstanding by the farmers. The high number of complaints indicate that this communication route is well established within the farming community.

Both Monsanto's survey as well as the independent survey in Portugal by the local authorities further demonstrate the effectiveness of the education program to raise awareness on refuge implementation (Section 3.2.1.1 of this report). Users have received information through the Technical User Guides (TUG) attached to the seed bags and went through training sessions. It demonstrates a high level of commitment with these requirements from both seed companies and farmers.

3.2.1.4 Communication and grower education

An extensive annual repeated grower education program is essential for the successful implementation of the IRM plan. Each purchaser of MON 810 receives a Technical User Guide (*see* in Appendix 3 the Technical User Guides used in the countries growing MON 810). It contains the latest information on the growers' IRM obligations. The user guide requires farmers to implement IRM measures, including refuge planting. In addition to the widespread dissemination of information pertaining to refuge requirements to users of the technology, a grower education programme is also conducted with sales and agronomic advisory teams to ensure that farmer awareness of refuge compliance is reinforced.

In addition to the above and as in previous seasons, for the 2016 planting season in Spain (the main county growing MON 810), a number of initiatives were taken to emphasise the importance of refuge implementation. A comprehensive program to raise awareness of refuge requirements and educate personnel, distributors, cooperatives and individual farmers was continued. Activities included:

1) Ensuring continuous communication about IRM implementation in all sales tools (leaflets, brochures, catalogues, *etc.*). The TUG (Appendix 3), was included in seed bags and has been extensively distributed. Other, more detailed communication materials like the Guía Técnica YieldGard® (YieldGard Technical Guide) (*see* Appendix 9.1) were available electronically.

2) Stewardship requirements and IRM compliance for MON 810 cultivation are reviewed and extensively communicated with licensee companies and Monsanto sales teams every season. The working group of *Bt* maize within the ANOVE annually reviews and prepares an updated set of communication materials to be used by individual companies and through the jointly industry activities. This ensures common messages across the market and to the farmers regardless of the seed provider (European-Seed, 2016). In 2016, the following actions were taken:

    a. Advertisement about refuge compliance, articles and references to the TUG were published in key agricultural magazines and copies of the IRM materials sent to regional and national authorities (*see* Appendix 9.2.1 and 9.2.2).

    b. A postcard (by mail or electronic means) reminding refuge obligations (on behalf of ANOVE) was sent from each company to farmers in their database located in MON 810 growing areas (*see* Appendix 9.3)

    c. Sales and marketing teams of ANOVE members were encouraged to include IRM requirements in farmer meetings/farmer talks. In addition to the materials distributed in the previous season, summary slide decks on farmers obligations were distributed and each company committed to widely use it. (*see* Appendix 9.4)

    d. Posters reminding the obligation to plant a refuge distributed among seed distributors and point of sales (*see* Appendix 9.5)

    e. Communication plan for cooperatives, small points of sales and farmers: Trained ANOVE inspectors completed 103 interviews to cooperatives and point of sales at planting time in all the in MON 810 growing areas. The objectives were to check the degree of information and availability of materials, training or complement the information available by seed distributors, as needed offer materials and in the end, ensure that farmers are well informed on refuge implementation when buying MON 810 seeds. 97% of the interviewed entities considered their customers well informed. In general, all the entities expressed their willingness to support the dissemination of communication materials about refuges and contribute to a sustainable use of the technology.

3) IRM information has been exhibited at different national and regional agricultural fairs.

3.2.2 Monitoring and reporting of adverse effects resulting from accidental spillage (if applicable)

Not applicable.

3.3 Concluding remarks

Monitoring results obtained via questionnaires (*see* Section 3.1.4.1 and Appendix 1), the scientific literature (*see* Section 3.1.6 and Appendix 5.1 and Appendix 5.2), company stewardship activities (*see* Section 3.1.4.2) and alerts on environmental issues (*see* Section 3.1.4.3) demonstrated that there are no adverse effects attributed to the cultivation of MON 810 in Europe.

4. SUMMARY OF RESULTS AND CONCLUSIONS

Monsanto and the seed companies marketing maize expressing the Cry1Ab protein have been operating together to establish and implement an IRM programme that is adapted to the EU agricultural landscape, and will continue to work closely together to assess its implementation and subsequently build on this learning. The commercial planting of MON 810 in Europe has been accompanied by a rigorous proactive Insect Resistance Management (IRM) plan, involving these key elements: a farmer complaint system, refuge implementation, susceptibility monitoring, farmer education and company stewardship activities.

Following the establishment and reinforcement of an effective education and communication program in countries where MON 810 was grown in 2016, the percentage of farmers implementing refuges in their fields was very high.

The results of the analysis of 2016 farmer questionnaires did not identify potential adverse effects that might be related to MON 810 plants and their cultivation. Company stewardship activities, systems and issue alerts did not reveal adverse effect related to MON 810 cultivation. A review of high quality publications confirmed the negligible potential of MON 810 and/or the Cry1Ab protein to cause adverse effects. Also, no issues related to insect resistance were experienced for the 2016 cultivation season as confirmed by the absence of farmer complaints related to allegedly reduced MON 810 target pest product performance.

A comprehensive insect resistance monitoring program demonstrated that there were no changes in susceptibility of either targeted pest *O. nubilalis* or *S. nonagrioides* to the Cry1Ab protein in the MON 810 growing regions in Europe in 2016. This is consistent with the observation that also on a global level no resistance is found for *O. nubilalis* and *S. nonagrioides* (Tabashnik *et al.*, 2013) and demonstrates the appropriateness of the implemented IRM plan.

The weight of evidence available to date confirms the initial conclusions of the EU safety assessment in 1998, namely that MON 810 is as safe as conventional maize with respect to human or animal health and the environment. Indeed, MON 810 has been safely grown in multiple countries around the world since 1997. Following its approval in 1998 in the EU,

MON 810 was first grown in European countries in 2003. From 2005 to date, Monsanto submitted 12 PMEM reports covering 14 years of MON 810 cultivation in the EU and all confirming its safety. These reports describe the activities undertaken by Monsanto to identify and analyse anticipated and unanticipated effects related to MON 810 cultivation. In summary, the weight of evidence continues to support the safety conclusions and consists of regulatory safety studies presented in the different EU applications, more than a dozen EFSA opinions concluding on the safety of MON 810, cultivation approvals for MON 810 in multiple countries around the world based on scientific risk assessment data and local safety opinions, hundreds of peer reviewed publications relevant to the safety assessment of MON 810 and the expressed Cry1Ab protein, more than 12 years of experience with MON 810 cultivation in the EU, more than 20 years of experience worldwide on millions of hectares, multiple PMEM reports for the EU reporting on the commercial experience confirming the initial safety conclusions (and endorsed by EFSA), and absence of confirmed adverse effect related to the event. All together, these results demonstrate that there are currently no adverse effects attributed to the cultivation of MON 810 in Europe. The result of the 2016 monitoring efforts are consistent with the results observed since monitoring was started in 2003.

5. ADAPTATIONS OF THE MONITORING PLAN AND ASSOCIATED METHODOLOGY FOR FUTURE YEARS

The current monitoring plan and associated methodologies were considered to be adapted to the purpose of monitoring for adverse effects. As indicated in the monitoring plan submitted as part of the renewal application EFSA-GMO-RX-MON810 (20.1a), the validity of the methodology for the different aspects to environmental monitoring are continuously evaluated. The improvements that were implemented over the years are the result of experience gained while conducting environmental monitoring of MON 810 cultivation for fourteen years in Europe, and result from discussions with different stakeholders such as the European Commission, EFSA GMO unit, Member States, independent experts and other biotech industries. Based on these discussions, this report includes adaptations to the current monitoring plan and has been implemented as from the 2016 maize cultivation season. In anticipation of the approval of other *Bt* maize events conferring protection against Lepidoptera, Monsanto has collaborated with the other applicants towards a harmonized approach for environmental monitoring of these GM maize varieties. The PMEM plan includes this harmonized approach towards case-specific monitoring (IRM), which is currently a condition of the MON 810 authorization in the EU.

Signed: [redacted]

Date:

REFERENCES

Andow DA and Zwalhen C, 2016. Ground beetle acquisition of Cry1Ab from plant- and residue-based food webs. Biological Control, 103, 204-209.

Andreassen M, Bohn T, Wikmark O-G, Bodin J, Traavik T, Lovik M and Nygaard UC, 2016. Investigations of immunogenic, allergenic and adjuvant properties of Cry1Ab protein after intragastric exposure in a food allergy model in mice. BMC Immunology, 17, 1-12.

Blanco CA, W Chiaravalle W, Dalla-Rizza M, Farias JR, Garcia-Degano MF, Gastaminza G, Mota-Sanchez D, Murua MG, Omoto C, Pieralisi BK, Rodriguez JC, Teran-Santofimio H, Teran-Vargas AP, Valencia SJ and Willink E, 2016. Current situation of pests targeted by Bt crops in Latin America. Elsevier, 15, 131-138.

Bøhn T, Macagnan Rover C and Semenchuk PR, 2016. *Daphnia magna* negatively affected by chronix exposure to purified Cry-toxins. Food and Chemical Toxicology, 91, 130-140.

Bøhn T, Primicerio R, Hessen DO and Traavik T, 2008. Reduced fitness of *Daphnia magna* fed a *Bt*-transgenic maize variety. Archives of Environmental Contamination and Toxicology, 55, 584-592.

Buuk C, Gloyna K and Thieme T, 2016. Is there any change in susceptibility of European corn borer (*Ostrinia nubilalis*) to Cry1Ab protein? IOBC-WPRS Bulletin, 114, 1-6.

Castañera P, Farinós G, Ortego F and Andow D, 2016. Sixteen Years of Bt Maize in the EU Hotspot: Why Has Resistance Not Evolved? Plos One, 1-13.

Chrenkova M, Pomikalova S, Chrastinova L, Polacikova M, Formelova Z, Rajsky M and Mlynekova Z, 2016. Effect of Crimped Maize Grain Ensiled with High Moisture Grains of Transgenic Bt Maize in Fattening Bulls. 159-162.

CLI, 2016. Performance criteria for evaluating regulatory systems that oversee approvals of biotechnology-derived or genetically modified crops. CropLife International,

Coates BS, 2016. *Bacillus thuringiensis* toxin resistance mechanisms among Lepidoptera: progress on genomic approaches to uncover causal mutations in the European corn borer, *Ostrinia nubilalis*. Elsevier, 15, 70-77.

Commission Decision, 1998. Commission Decision 98/294/EC 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. Official Journal, L 131/32, 1-2.

Commission Decision, 2009. Commission Decision of 13 October 2009 establishing standard reporting formats for presenting the monitoring results of the deliberate release into the environment of genetically modified organisms, as or in products, for the purpose of placing on the market, pursuant to Directive 2. Official Journal, L 275/9, 1-19.

Commission Regulation, 2003. Regulation (EC) No 1829/2003 of the European Parliament and of teh Council of 22 September 2003 on genetically modified food and feed. Official Journal of the European Communities, 268/1, 1-23.

DGAV, 2016. Coexistência Entre Culturas Geneticamente Modificadas E Outros Modos De Produção Agrícola Relatório De Acompanhamento - 2016. Relatório de acompanhamento-2016, 1-27.

Di Grumo D and Lovei GL, 2016. Body size inequality in ground beetle (Coleoptera: Carabidae) assemblages as a potential method to monitor environmental impacts of transgenic crops. Periodicum Biologorum, 118, 223-230.

Diaz-Gomez J, Marin S, Capell T, Sanchis V and Ramos AJ, 2016. The impact of Bacillus thuringiensis technology on the occurrence of fumonisins and other mycotoxins in maize. World Mycotoxin Journal, 9, 475-486.

Domingo JL, 2016. Safety assessment of GM plants: An updated review of the scientific literature. Elsevier, 95, 12-18.

Dos Santos CA, Marucci RC, Barbosa TAN, Araujo OG, Waquil JM, Dias AS, Hebach FC and Mendes SM, 2016. Desenvolvimento de Helicoverpa spp. em milho Bt corn expressao de diferentes proteinas. Pesquisa Agropecuaria Brasileira, 51, 537-544.

EFSA, 2004. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the Austrian invoke of Article 23 of Directive 2001/18/EC1 (Question No EFSA-Q-2004-062). The EFSA Journal, 78, 1-13.

EFSA, 2005. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the safeguard clause invoked by Hungary according to Article 23 of Directive 2001/18/EC. The EFSA Journal, 228, 1-14.
EFSA, 2006. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the safeguard clause invoked by Greece according to Article 23 of Directive 2001/18/EC and to Article 18 of Directive 2002/53/EC (Question No EFSA-Q-2006-048). The EFSA Journal, 411, 1-26.
EFSA, 2008a. Request from the European Commission related to the safeguard clause invoked by Austria on maize MON 810 and T25 according to Article 23 of Directive 2001/18/EC. The EFSA Journal, 891, 1-64.
EFSA, 2008b. Request from the European Commission related to the safeguard clause invoked by France on maize MON 810 according to Article 23 of Directive 2001/18/EC and the emergency measure according to Article 34 of Regulation (EC) No 1829/2003. The EFSA Journal, 850, 1-45.
EFSA, 2008c. Request from the European Commission related to the safeguard clause invoked by Greece on maize MON 810 according to Article 23 of Directive 2001/18/EC. The EFSA Journal, 757, 1-12.
EFSA, 2008d. Request from the European Commission related to the safeguard clause invoked by Hungary on maize MON 810 according to Article 23 of Directive 2001/18/EC. The EFSA Journal, 756, 1-18.
EFSA, 2009. Applications (EFSA-GMO-RX-MON 810) for renewal of authorisation for the continued marketing of (1) existing food and food ingredients produced from genetically modified insect resistant maize MON 810; (2) feed consisting of and/or containing maize MON 810, including the use of seed for cultivation; and of (3) food and feed additives, and feed aterials produced from maize MON 810, all under Regulation (EC) No 1829/2003 from Monsanto. The EFSA Journal, 1149, 1-84.
EFSA, 2011a. Guidance on the post-market environmental monitoring (PMEM) of genetically modified plants. The EFSA Journal, 9 (8):2316, 1-40.
EFSA, 2011b. Scientific Opinion on the annual post-market environmental monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2009. The EFSA Journal, 9 (10), 1-66.
EFSA, 2012a. Scientific Opinion on a request from the European Commission related to the emergency measure notified by France on genetically modified maize MON 810 according to Article 34 of Regulation (EC) No 1829/2003. The EFSA Journal, 10 (5), 1-21.
EFSA, 2012b. Scientific Opinion on a request from the European Commission related to the safeguard clause notified by Greece on genetically modified maize MON 810 according to Article 23 of Directive 2001/18/EC. The EFSA Journal, 10 (9), 1-20.
EFSA, 2012c. Scientific Opinion on an application (EFSA-GMO-NL-2012-107) for the placing on the market of maize MON 810 pollen under Regulation (EC) No 1829/2003 from Monsanto. The EFSA Journal, 10 (12), 1-9.
EFSA, 2012d. Scientific Opinion on the annual post-market environmental monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2010. The EFSA Journal, 10 (4), 1-35.
EFSA, 2012e. Scientific Opinion supplementing the conclusions of the environmental risk assessment and risk management recommendations for the cultivation of the genetically modified insect resistant maize *Bt*11 and MON 810. The EFSA Journal, 10(12), 1-32.
EFSA, 2012f. Scientific Opinion updating the risk assessment conclusions and risk management recommendations on the genetically modified insect resistant maize MON 810. The EFSA Journal, 10 (12), 1-98.
EFSA, 2013a. Scientific Opinion on a request from the European Commission related to the emergency measure notified by Italy on genetically modified maize MON 810 according to Article 34 of Regulation (EC) No 1829/2003. The EFSA Journal, 11 (9), 1-7.

EFSA, 2013b. Scientific Opinion on a request from the European Commission related to the emergency measure notified by Luxembourg on genetically modified maize MON 810 according to Article 34 of Regulation (EC) No 1829/2003. The EFSA Journal, 11 (9), 1-7.
EFSA, 2013c. Scientific Opinion on the annual post-market environmental monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2011. The EFSA Journal, 11 (12), 1-38.
EFSA, 2014a. Scientific Opinion on the annual post-market environmental monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2012. The EFSA Journal, 12 (6), 1-29.
EFSA, 2014b. Scientific Opinion on the use of existing environmental surveillance networks to support the post-market environmental monitoring of genetically modified plants. The EFSA Journal, 12 (11), 1-24.
EFSA, 2014c. Statement on a request from the European Commission related to the emergency measure notified by Greece on genetically modified maize MON 810 according to Article 18 of Directive 2002/53/EC. The EFSA Journal, 12 (6), 1-7.
EFSA, 2015a. Clarifications on EFSA GMO Panel recommendations on the Insect Resistance Management plan for genetically modified maize MON 810. The EFSA Journal, 1-14.
EFSA, 2015b. Relevance of a new scientific publication (Trtikova et al., 2015) on previous EFSA GMO Panel conclusions on the risk assessment of maize MON 810 and other Cry1Ab-expressing Bt-maize events. The EFSA Journal, 12, 1-11.
EFSA, 2015c. Scientific Opinion on the annual post-market environmental monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2013. The EFSA Journal, 13(3), 1-11.
EFSA, 2015d. Updating risk management recommendations to limit exposure of non-target Lepidoptera of conservation concern in protected habitats to *Bt*-maize pollen. The EFSA Journal, 1-31.
EFSA, 2016a. Relevance of a new scientific publication (Bøhn et al., 2016) for previous environmental risk assessment conclusions on the cultivation of Bt-maize events MON 810 and Bt11. The EFSA Journal, 13, 1-16.
EFSA, 2016b. Relevance of a new scientific publication (Hofmann et al., 2016) for previous environmental risk assessment conclusions and risk management recommendations on the cultivation of Bt-maize events MON 810, Bt11 and 1507. The EFSA Journal, 13, 1-13.
EFSA, 2016c. Scientific opinion on the annual post-market environmental monitoring (PMEM) report on the cultivation of genetically modified maize MON 810 in 2014 from Monsanto Europe S.A. The EFSA Journal, 14 (4), 1-26.
EFSA, 2017. Scientific opinion on the annual post-market environmental monitoring (PMEM) report on the cultivation of genetically modified maize MON 810 in 2015 from Monsanto Europe S.A. The EFSA Journal, 15(5):4805, 1-27.
Erasmus A, Marais J and Van den Berg J, 2016. Movement and survival of *Busseola fusca* (Lepidoptera: Noctuidae) larvae within maize plantings with different ratios of non-Bt and Bt seed. Society of Chemical Industry, 72, 2287-2294.
European-Seed, 2016. The future of the Biotech in Europe: what does it hold? Spain's GM maize production. European-Seed, 3, 1-3.
European Commission, 2016. Commission implementing decision (EU) 2016/321 of 3 March 2016 adjusting the geographical scope of the authorisation for cultivation of genetically modified maize (*Zea mays* L.) MON 810 (MON-ØØ81Ø-6). Official Journal, L 60/90, 1-3.
European Commission, 2017. Commission implemeting decision (EU) 2017/1207 of 4 July 2017 renewing the authorisation for the placing on the market of genetically modified maize MON 810 (MON-ØØ81Ø-6) products pursuant to Regulation (EC) No 1829/2003 of the European Parliament and of the Council. Official Journal of the European Union, L 173/18, 1-5.
Farinós GP, de la Poza M, Hernandez-Crespo P, Ortego F and Castanera P, 2004. Resistance monitoring of field populations of the corn borers *Sesamia nonagrioides* and *Ostrinia nubilalis* after 5 years of Bt maize cultivation in Spain. Entomologia Experimentalis Et Applicata, 110, 23-30.

Farinós GP, Hernández-Crespo P, Ortego F and Castañera P, 2017. Monitoring of Sesamia nonagrioides resistance to MON 810 maize in the European Union: lessons from a long-term harmonized plan. Pest Management Science, doi: 10.1002/ps.4735,

Gonzalez-Nunez M, Ortego F and Castanera P, 2000. Susceptibility of Spanish populations of the corn borers *Sesamia nonagrioides* (Lepidoptera: *Noctuidae*) and *Ostrinia nubilalis* (Lepidoptera: *Crambidae*) to a *Bacillus thuringiensis* endotoxin. J. Economic Entomology, 93, 459-463.

Griffiths NA, Tank JL, Royer TV, Rosi EJ, Shogren AJ, Frauendorf TC and Whiles MR, 2017. Occurrence, leaching, and degradation of Cry1Ab protein fromtransgenic maize detritus in agricultural streams. Elsevier, 592, 97-105.

Han L, Jiang XX and Peng Y, 2016a. Potential resistance management for the sustainable use of insect-resistant genetically modified corn and rice in China. Elsevier, 15, 139-143.

Han P, Velasco-Hernandez MC, Ramirez-Romero R and Desneux N, 2016b. Behavioral effects of insect-resistant genetically modified crops on phytophagous and beneficial arthropods: a review. Springer, 89, 859-883.

Henrys P, Thompson J, Smets G, Rudelshiem P and Hails R, 2014. Review of statistical methods and data requirements to support post market environmental monitoring of agro ecosystems. Centre for Ecology and Hydrology, 1-151.

Hofmann F, Kruse-Plass M, Kuhn U, Otto M, Schlechtriemen U, Schröder B, Vögel R and Wosniok W, 2016. Accumulation and variability of maize pollen deposition on leaves of European Lepidoptera host plants and relation to release rates and deposition determined by standardised technical sampling. Environmental Sciences Europe, 28, 1-19.

Holderbaum DF, Cuhra M, Wickson F, Orth AI, Nodari RO and Bøhn T, 2015. Chronic Responses of Daphnia magna 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.

Ibrahim MAA and Okasha EF, 2016. Effect of genetically modified corn on the jejunal mucosa of adult male albino rat. Elsevier, 68, 579-588.

Joshi S, Barnett B, Doerrer NG, Glenn K, Herman RA, Herouet-Guicheney C, Hunst P, Kough J, Ladics GS, McClain S, Papineni S, Poulsen LK, Rascle J-B, Tao AL, Van Ree R, Ward J and Bowman CC, 2016. Assessment of potential adjuvanticity of Cry proteins. Elsevier, 79, 149-155.

Korwin-Kossakowska A, Sartowska K, Tomczyk G, Prusak B and Sender G, 2016. Health status and potential uptake of transgenic DNA by Japanese quail fed diets containing genetically modified plant ingredients over 10 generations. British Poultry Science, 57, 415-423.

Kotey DA, Obi A, Assefa Y, Erasmus A and Van den Berg J, 2017. Monitoring resistance to Bt maize in field populations of Busseola fusca (Fuller) (Lepidoptera: Noctuidae) from smallholder farms in the Eastern Cape Province of South Africa. African Entomology, 25, 200-209.

Lee MS and Albajes R, 2016. Monitoring carabid indicators could reveal environmental impacts of genetically modified maize. Agricultural and Forest Entomology, 18, 238-249.

Mashiane RA, Ezeokoli OT, Adeleke RA and Bezuidenhout CC, 2017. Metagenomic analyses of bacterial endophytes associated with the phyllosphere of a Bt maize cultivar and its isogenic parental line from South Africa. World J Microbiol Biotechnol, 33, 1-12.

Monsanto Company, 1995. Submission to the French Commission du Génie Biomoléculaire. Application to place on the market genetically modified higher plants: insect-protected maize (MON810). Monsanto Report, 1.

Monsanto Europe S.A., 2005. Report on the implementation of the Insect Resistant Managment plan for MON 810 in the European Union - MON 810 cultivation in Spain in 2003 and 2004. Monsanto Report, 1-88.

Monsanto Europe S.A., 2006. Monitoring report - MON 810 cultivation - Czech Republic, France, Germany, Portugal and Spain - 2005. Monsanto Report, 1-290.

Monsanto Europe S.A., 2007. Monitoring report - MON 810 cultivation - Czech Republic, France, Germany, Portugal, Slovakia and Spain - 2006. Monsanto Report, 1-16.

Monsanto Europe S.A., 2008. Monitoring report - MON 810 cultivation - Czech Republic, France, Germany, Poland, Portugal, Romania, Slovakia and Spain - 2007. Monsanto Report, 1-18.

Monsanto Europe S.A., 2009. Monitoring report - MON 810 cultivation - Czech Republic, Germany, Poland, Portugal, Romania, Slovakia and Spain - 2008. Monsanto Report, 1-28.
Monsanto Europe S.A., 2010. Monitoring report - MON 810 cultivation - Czech Republic, Portugal, Slovakia, Poland, Romania and Spain - 2009. Monsanto Report, 1-27.
Monsanto Europe S.A., 2011. Monitoring report - MON 810 cultivation - Czech Republic, Poland, Portugal, Romania, Slovakia, and Spain - 2010. Monsanto Report, 1-30.
Monsanto Europe S.A., 2012. Monitoring report - MON 810 cultivation - Czech Republic, Poland, Portugal, Romania, Slovakia, and Spain - 2011. Monsanto Report, 1-31.
Monsanto Europe S.A., 2013. Monitoring report - MON 810 cultivation - Czech Republic, Portugal, Slovakia, and Spain - 2012. Monsanto Report, 1-60.
Monsanto Europe S.A., 2015. Revised monitoring report - MON 810 cultivation - Czech Republic, Portugal, Romania, Slovakia, and Spain - 2014. Monsanto Report, 1-63.
Monsanto Europe S.A., 2016. Monitoring report (updated version) - MON 810 cultivation - Czech Republic, Portugal, Romania, Slovakia and Spain - 2014. 1-38.
Monsanto Europe S.A., 2017. Monitoring report - MON 810 cultivation - Czech Republic, Portugal, Romania, Slovakia, and Spain - 2016. Monsanto Report, 1-72.
Niu Y, Head GP, Price PA and Huang F, 2016. Performance of Cry1A.105-selected fall armyworm (Lepidoptera: Noctuidae) on transgenic maize plants containing single or pyramided Bt genes. Elsevier, 88, 79-87.
OECD (OECD), 2012. OECD guideline for the testing of chemicals No. 211: *Daphnia magna* Reproduction Test. 1-25.
Omoto C, Bernardi O, Salmeron E, Sorgatto R, Dourado PM, Crivellari A, Carvalho RA, Willse A, Martinelli S and Head GP, 2016. Field-evolved resistance to Cry1Ab maize by *Spodoptera frugiperda* in Brazil. Pest Manag Sci 2016, 72, 1727-1736.
Osborne SL, Lehman RM and Rosentrater KA, 2016. Grain and biomass nutrient uptake of conventional corn and their genetically modified isolines. Journal of Plant Nutrition, 39, 2047-2055.
Peterson JA, Obrycki JJ and Harwood JD, 2016. Spiders from multiple functional guilds are exposed to Bt-endotoxins in transgenic corn fields via prey and pollen consumption. Biocontrol Science and Technology, 1230-1248.
Roush RT and Miller GL, 1986. Considerations for design of insecticide resistance monitoring programs. Journal of Economic Entomology, 79, 293-298.
Schmidt K, Döhring J, Kohl C, Pla M, Kok EJ, Glandorf DCM, Custers R, van der Voet H, Sharbati J, Einspanier R, Zeljenková D, Tulinská J, Spök A, Alison C, Schrenk D, Pöting A, Wilhelm R, Schiemann J and Steinberg P, 2016. Proposed criteria for the evaluation of the scientific quality of mandatory rat and mouse feeding trials with whole food/feed derived from genetically modified plants. Arch Toxicol, 90, 2287-2291.
Shu Y, Zhang Y, Zeng H, Zhang Y and Wang J, 2017. Effects of Cry1Ab Bt maize straw return on bacterial community of earthworm *Eisenia fetida*. Elsevier, 173, 1-13.
Smets G, Alcalde E, Andres D, Carron D, Delzenne P, Heise A, Legris G, Parrilla MM, Verhaert J, Wandelt C, Ilegems M and Rudelsheim PR, 2014. The use of existing environmental networks for the post-market monitoring of GM crop cultivation in the EU. Environmental Science-Processes & Impacts, 16, 1754-1763.
Sousa FF, Mendes SM, Santos-Amaya OF, Araujo OG, Oliveira EE and Pereira EJG, 2016. Life-History Traits of *Spodoptera frugiperda* Populations Exposed to Low-Dose Bt Maize. PLOs, 11, 1-18.
Stenekamp D, Pringle K and Addison M, 2016. Effect of Genetically Modified Bt Maize in an Artificial Diet on the Survival of *Cydia pomonella* (Lepidoptera: Tortricidae). Florida Entomologist, 99, 200-205.
Sumerford DV, Head GP, Shelton A, Greenplate J and Moar W, 2015. Field-Evolved Resistance: Assessing the Problem and Ways to Move Forward. Journal of Economic Entomology, 106, 1-10.
Tabashnik BE, Brevault T and Carriere Y, 2013. Insect resistance to *Bt* crops: lessons from the first billion acres. Nature Biotechnology, 31, 510-521.

Tefera T, Mugo S, Mwimali M, Anani B, Tende R, Beyene J, Gichuki S, Oikeh SO, Nang'ayo F, Okeno J, Njeru E, Pillay K, Meisel B and Prasanna BM, 2016. Resistance of *Bt*-maize (MON 810) against the stem borers *Busseola fusca* (Fuller) and *Chilo partellus* (Swinhoe) and its yield performance in Kenya. Crop Protection, 89, 202-208.

Thieme T, Buuk C, Gloyna K, Ortego F and Farinós G, 2017. Ten years of MON 810 resistance monitoring of field populations of Ostrinia nubilalis in Europe. Journal of Applied Entomology, 00, 1-9.

Van den Berg J, 2016. Resistance of *Busseola fusca* Insect Resistance Management in Bt Maize: Wild Host Plants of Stem Borers Do Not Serve as Refuges in Africa. Journal of Economic Entomology, 110, 221-229.

Venter HJ and Bøhn T, 2016. Interactions between Bt Crops and Aquatic Ecosystems: a review. Environmental Toxicology and Chemistry, 35, 2891-2902.

Waquil MS, Pereira EJG, De Sousa Carvalho SS, Pitta RM, Waquil JM and Mendes SM, 2016. Fitness index and lethal time of fall armyworm on Bt corn. Pesquisa Agropecukiria Brasileira, 5, 563-570.

Wilhelm R, Beissner L, Schmidt K, Schmidtke J and Schiemann J, 2004. Monitoring des Anbaus gentechnisch veränderter Pflanzen - Fragebögen zur Datenerhebung bei Landwirten. Nachrichtenbl. Deut. Pflanzenschutzd., 56, 184-188.

Wolt JD, Keese P, Raybould A, Fitzpatrick JW, Burachik M, Gray A, Olin SS, Schiemann J, Sears M and Wu F, 2010. Problem formulation in the environmental risk assessment for genetically modified plants. Transgenic Research, 19, 425-436.

Yang G, Niu Y, Head GP, Price PA and Huang F, 2016. Performance of Cry1Ab-susceptible and -heterozygous resistant populations of sugarcane borer in sequential feedings on non-Bt and Bt maize plant tissue. Entomologia Experimentalis Et Applicata, 162, 51-59.

Yao J, Zhu Y, Lu N, Buschman LL and Zhu KY, 2017. Comparisons of Transcriptional Profiles of Gut Genes between Cry1Ab-Resistant and Susceptible Strains of *Ostrinia nubilalis* Revealed Genes Possibly Related to the Adaptation of Resistant Larvae to Transgenic Cry1Ab Corn. International Journal of Molecular Sciences, 18, 1-17.

Yinghua S, Yan D, Jin C, Jiaxi W, Wei J and Jianwu W, 2017. Responses of the cutworm *Spodoptera litura* (Lepidoptera: Noctuidae) to two Bt corn hybrids expressing Cry1Ab. Scientific Reports, 7,

Zeljenková D, Alácová R, Ondrejková J, Ambrušová K, Bartušová M, Kebis A, Kovrižnych J, Rollerová E, Szabová E, Wimmerová S, Černák M, Krivošíková Z, Kuricová M, Líšková A, Spustová V, Tulinská J, Levkut M, Révajová V, Ševcíková3 Z, Schmidt K, Schmidtke J, Schmidt P, La Paz J, Pla M, Kleter G, Kok E, Sharbati J, Bohmer M, Bohmer N, Einspanier R, Adel‐Patient K, Spök A, Pöting A, Kohl C, Wilhelm R, Schiemann J and Steinberg P, 2016. One‐year oral toxicity study on a genetically modified maize MON810 variety in Wistar Han RCC rats (EU 7th Framework Programme project GRACE). Arch Toxicol, 90, 2531-2562.