Annual monitoring report
on the
cultivation of MON 810 in 2015

Czech Republic, Portugal,
Romania, Slovakia, and Spain

Submitted by

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September 2016

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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

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® 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 30 August 2016)

[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.

In 2015, MON 810 was planted in the EU on approximately 116 867 hectares across five countries: Czech Republic (997 ha[3]), Portugal (8 017 ha[4]), Romania (2.5 ha[5]), Slovakia (104 ha[6]) and Spain (107 749 ha[7]).

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 the GMO 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). Since 2010, the reports follow the format as laid out in Annex I to Commission Decision 2009/770/EC (Commission Decision, 2009).

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[3] Ministry of Agriculture of the Czech Republic, 2015 - http://eagri.cz/public/web/mze/zemedelstvi/gmo-geneticky-modifikovane-organismy/ (Accessed 30 August 2016)

[4] Anpromis, 2015 - http://www.anpromis.pt/dados-estatisticos/ (Accessed 30 August 2016)

[5] Ministry of Agriculture and Rural Development of Romania, 2015 - http://www.madr.ro/docs/agricultura/suprafete-cultivate-porumb-modificat-genetic-mon-810-anul-2015.pdf (Accessed 30 August 2016)

[6] Ministry of Agriculture and rural development of the Slovak Republic, 2015 - http://www.mpsr.sk/index.php?navID=764&navID2=764&sID=40&id=9573 (Accessed 30 August 2016)

[7] Ministry of Agriculture, Food and Environment of Spain, 2015 - http://www.magrama.gob.es/es/calidad-y-evaluacion-ambiental/temas/biotecnologia/2015_tcm7-399015.pdf (Accessed 30 August 2016)

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 2015 - July 2016

1.6 Other monitoring reports have been submitted in respect of:

- **Import and Processing** .... Yes, voluntary (September 2016)
- **Food/Feed** .... Not applicable

2. EXECUTIVE SUMMARY

In 2015, MON 810 was planted in the EU on approximately 116 867 hectares across five 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 Cry protein. The adherence to this stewardship measure in the context of the 2015 cultivation of MON 810 maize in Europe is detailed in this report.

The planting of MON 810 in the 2015 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 2015 and the success of these initiatives was reflected in the high levels of compliance with requirements for refuge implementation observed in the 2015 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 2015. Not a single MON 810 performance complaint allegedly caused by reduced target pest susceptibility was received from farmers in 2015.

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 2015, 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 261 questionnaires from a survey of farmers cultivating MON 810 in two European countries in 2015 did not reveal any adverse effects associated with the genetic modification in MON 810. Furthermore, a detailed analysis of 22 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 2015. Taken together, these results demonstrate that there are no adverse effects attributed to the cultivation of MON 810 in Europe in 2015.

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. Nevertheless, Monsanto has been reporting on its activities for this non-hypothesis based monitoring on a voluntary basis since 2005. Over a number of years, several approaches to monitor unanticipated adverse effects were developed and their methodologies improved substantially. A number of the complementary approaches initially developed by Monsanto were taken up by EuropaBio in an effort to harmonize proportional 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 11 PMEM reports covering 13 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 (Monsanto Europe S.A., 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2015, 2016). The resulting weight of 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 likely 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 2015 growing season, the results of the farmer questionnaires conducted in 2015 are provided. None of the reports, for which the results were statistically analyzed, identified a statistically meaningful effect that was adverse to human or animal health, or the environment. Only beneficial effects were reported 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 Post-Market Environmental Monitoring (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 data will be conducted. Provided that this analysis would not identify any adverse effect of MON 810 cultivation on human or animal health, or the environment, the monitoring plan and associated methodology for conducting farmer questionnaires should be modified and adapted based on the results of the monitoring information collected.

In addition to the results from the farmer questionnaires conducted in 2015, Monsanto's company-internal processes for issues and complaint handling could not identify any adverse effect caused by the MON 810 event. Furthermore, as a third GS approach activity, 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 390 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 11 monitoring reports, covering 13 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, Honduras, Japan, the Philippines, South Africa, Uruguay and the US. 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), Monsanto's monitoring reports (EFSA, 2011b, 2012d, 2013c, 2014a, 2015c, 2016c) 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 unanticipated 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 the same 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 19 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 target pests,
- absence of any confirmed 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 would be disproportional to the available weight of evidence demonstrating the safety of MON 810.

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 2015 GS activities are included in this report in order to meet the European Commission's request. Nevertheless, 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 Post-Market Environmental Monitoring (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, 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 always secured the balance between information maximization at the one hand, and implementation practicality 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 post-market environmental monitoring (PMEM) of GM crops in August 2011 (EFSA, 2011a) and six specific opinions on MON 810 monitoring in the 2009, 2010, 2011, 2012, 2013 and 2014 growing seasons (EFSA, 2011b, 2012d, 2013c, 2014a, 2015c, 2016c). Monsanto has adapted its monitoring approaches to be in line with EFSA recommendations where possible and feasible, taking into consideration the gained expertise on MON 810 monitoring and already established methodologies, in order to report on the results for the 2015 growing season. As previously highlighted, GS monitoring for MON 810 cultivation is conducted by Monsanto on a voluntary basis. The consent allowing MON 810 cultivation in the EU does not contain obligatory GS monitoring conditions (Commission Decision, 1998). As long as no authorization decision has been reached on the MON 810 renewal application (pending since 2007) containing GS monitoring as a condition of the consent, Monsanto elects to continue, in general, its current practices, which, as mentioned before, are not static but have improved over the years. Further to the dynamic improvement, Monsanto collaborates within EuropaBio towards a harmonized post-market environmental monitoring plan, which, once agreed with the different stakeholders including the European Commission, will be implemented when different GM crops are (re-)approved for cultivation. Finally, 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 or 2014 growing seasons and that the outcomes of the monitoring reports did not invalidate the previous risk assessment conclusions (EFSA, 2011b, 2012d, 2013c, 2014a, 2016c). 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 2015, Monsanto continued the GS monitoring program initiated in 2005 on a voluntary basis. The objective of GS is to identify 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 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 evidence of a potential 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 not be possible 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 2015 consisted of four elements:

- a farmer questionnaire designed to assess unusual observations in the areas where MON 810 has been cultivated;
- data collected from 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 value 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 easily understood and not to be too burdensome. Also, it had to be 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 2015 GS for MON 810 focused on the Iberian geographical regions where the majority of MON 810 was grown in 2015 (Portugal and Spain, countries accounting for approximately 99.1% of the MON 810 plantings in the EU in 2015), 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 2015, 49 farmers in Portugal and 212 farmers in Spain were asked to complete the questionnaire (261 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 December 2015 and February 2016. 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.

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 3.5). Alternatively, EuropaBio[10] and Monsanto[11] websites offer a contact point.

[10] EuropaBio contact webpage - http://www.europabio.org/contact (Accessed 30 August 2016)
[11] Monsanto product stewardship webpage - http://www.monsanto.com/products/pages/product-stewardship.aspx (Accessed 30 August 2016)

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*

[12] Channels of communication to external stakeholders include the Monsanto website - http://www.monsanto.com/newsviews/Pages/Issues-and-Answers.aspx (Accessed 30 August 2016)

pertaining to ESNs such as limited data accessibility, data reporting format and data connectivity with GMO registers" (EFSA, 2014b).

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 2015 season can be found in Appendix 3 (see Appendix 3.1 to Appendix 3.5). Additional details on growers education in the context of refuge implementation is given in Section 3.2.1.3.

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 261 questionnaires from the survey of farmers cultivating MON 810 in Spain and Portugal during the 2015 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 any adverse effects directly associated with MON 810 cultivation that require risk mitigation, these will be reported immediately.

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

Peer reviewed publications on the safety of MON 810 and/or the Cry1Ab protein published in 2015 – 2016

Following the recommendations of the EFSA opinion on the MON 810 PMEM report on the 2014 cultivation season (EFSA, 2016c), Monsanto has revised 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 an additional scientific literature database (CABI CAB Abstracts®).

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 2015, and beginning of June 2016, 22 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 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 12 000 of the highest impact journals worldwide, including Open Access journals and over 160 000 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 800 million 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 nine 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® databases for performing the scientific literature searches. The Web of Science™ core collection database 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® database includes literature capture under the 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[14].

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

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[13] Web of Science™; http://wokinfo.com/products_tools/multidisciplinary/webofscience/ (Accessed 30 August 2016)

[14] Thomson Reuters; http://thomsonreuters.com/en/products-services/scholarly-scientific-research/scholarly-search-and-discovery/science-citation-index-expanded.html; http://thomsonreuters.com/en/products-services/scholarly-scientific-research/scholarly-search-and-discovery/conference-proceedings-citation-index.html; http://thomsonreuters.com/en/products-services/scholarly-scientific-research/scholarly-search-and-discovery/cab-abstracts.html (Accessed 30 August 2016)

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 Thomson Reuters' Web of Science™ database[15]. 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[16], which is why Thomson Reuters 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 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

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[15] Web of Knowledge; http://apps.webofknowledge.com/ (Accessed 30 August 2016) (*Note* access to the database requires a subscription).

[16] Web of Science™; http://wokinfo.com/essays/journal-selection-process/ (Accessed 30 August 2016)

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 (Animal feeding study; Allergenicity studies of the protein or the whole food/feed and Crop compositional studies; – see Appendix 5.1) and environment (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 Thomson Reuters 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® 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 22 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 the Web of Science™ Core Collection database was conducted every month covering in total the period of June 2015 to May 2016, whereas the literature search in the CABI CAB Abstracts® database was performed once on 27 May 2016 (see Appendix 5.3 and Appendix 5.4).

Five publications were evaluated in terms of food/feed safety, three dealing with exposing avian species (chicken and quail) to MON 810, one addressing allergenicity and one related to molecular characterisation (Czerwiński *et al.*, 2015 -a; Czerwiński *et al.*, 2015 -b; Mathur *et al.*, 2015; Sartowska *et al.*, 2015; Vidal *et al.*, 2015).

Czerwiński and colleagues (Czerwiński *et al.*, 2015 -a; Czerwiński *et al.*, 2015 -b) exposed broiler chickens for ca. 28 days to diets containing MON 810 maize and Roundup Ready® GTS 40-3-2 soybean meal. They found no significant effects compared to controls (fed non-GM diets) on feed efficiency and blood lymphocyte subpopulations of the birds, or on the functional development and maturation of the small intestinal epithelium. Sartowska *et al.* (2015) fed MON 810 maize and Roundup Ready® GTS 40-3-2 soybean in different combinations to Japanese quail (*Coturnix cot. japonica*) for 10 generations. The experimental results suggested that there were no negative effects of the GM diets on bird performance indices, including reproduction, survival rate, growth, egg production, body composition and the basic chemical composition of breast muscle and egg yolk. The group of Mathur *et al.* (2015) assessed the allergenicity of GM maize seeds containing Cry1Ab, Cry1Ac and Cry1C protein sequences using *in silico* searches and *in vitro* methods. The researchers concluded that, based on *in silico* tools, Cry1Ab, Cry1Ac and Cry1C protein sequences were non-allergenic, with no cross reactivity to known allergens. Also, the protein sequences presented no appreciable changes in endogenous protein expression of GM and non-GM maize seeds as analysed by specific IgE and immunoblot using native maize-allergic patient sera. Finally, a paper by Vidal *et al.* (2015) explored the use of proteomics to identify compositional differences between a sample of MON 810 maize and its non-GM counterpart. The differences in protein levels between the samples were hypothesised to arise from the genetic modification or as a result of an environmental influence pertaining to the commercial sample. The major functional category of proteins identified was related to disease/defence and, although differences were observed between samples, no toxins or allergenic proteins were found.

Thirteen publications were reviewed in terms of environmental safety, on the subject of non-target organisms, fate of Cry protein in water and soil, gene flow, insect resistance management and fumonisin contamination in maize (Arias-Martin *et al.*, 2016; Böttger *et al.*, 2015; Crespo *et al.*, 2015; García *et al.*, 2015; Holderbaum *et al.*, 2015; Resende *et al.*, 2016; Rocha *et al.*, 2016; Shu *et al.*, 2015; Szénási and Markó, 2015; Taverniers *et al.*, 2015; Valldor *et al.*, 2015; Xu *et al.*, 2015; Zeng *et al.*, 2015).

Under field conditions, the incidence of *Spodoptera frugiperda*, the primary target pest of maize infesting the whorls and ears, and the insect community (non-target insect species, secondary pests and natural enemies) was evaluated by Resende *et al.* (2016) in conventional and Bt maize expressing different proteins (Cry1Ab, Cry1F, Cry1A.105 and Cry2Ab2) in seven counties of Minas Gerais, Brazil. The results did not support the hypothesis that Bt protein affects insect biodiversity. The richness and diversity data of insects studied were dependent on the location and other factors, such as the use of insecticides. Holderbaum *et al.* (2015) looked into the effects of chronic, high-dose dietary exposure of *Daphnia magna* to MON 810 maize leaves on fitness parameters. The bioassays showed a resource allocation on the production of resting eggs and early fecundity in *Daphnia* fed GM maize, with adverse

® Roundup Ready is a registered trademarks of Monsanto Technology LLC effects on body size and fecundity later in life (higher rate of mortality, less offspring and ephippia). However, survival of *Daphnia* provided with GM and control maize diets did not differ significantly. Despite these findings, the results presented in this study consistently show no differences between maize treatments for survival, growth, and reproduction during the first 21 days and within the accepted guideline study duration. Conclusions of adverse effects based on results and observations after day 21, and simultaneous with control mortality greater than 20% indicating poor testing conditions, should be interpreted with caution. Assertions that multiple endpoints should be included over the full life cycle of model organisms, and that testing should be conducted using plant-produced proteins or plant material in place of the microbially-produced proteins are not supported by the results from this study.

There were four publications dealing with soil organisms. The potential effects of MON 810 maize straw return on the earthworm *Eisenia fetida* were investigated using traditional, biochemical and molecular endpoints under laboratory conditions (Shu *et al.*, 2015). Negative, no and positive effects on growth and reproduction of adult earthworms were observed in the 1st, 2nd and 3rd generations, respectively. Negative and positive effects were seen on the growth of juveniles produced from 1st- and 2nd-generation adults. No significant differences in superoxide dismutase (SOD) activity were noted compared to controls, while the glutathione peroxidise (GSH-PX) activity of earthworms from Bt-maize treatments was significantly higher than that of controls on Day 90. The *tctp* and *sod* genes were up-regulated and *ann* and *hsp 70* genes down-regulated in treated worms. Cry1Ab released from maize straw degraded rapidly in the first 30 days and had a slow decline during the remaining time. Cry1Ab concentrations in the soil, casts and guts of earthworm significantly decreased over the course of the experiment. In Arias-Martin *et al.* (2016), continuous cultivation of MON 810 maize over four years in an experimental field in Spain did not negatively affect soil micro-arthropods, indicating that Bt maize could be compatible with this community. Using the terminal restriction fragment length polymorphism (T-RFLP) analysis of 28S ribosomal DNA and sequencing methods, Zeng *et al.* (2015) also found that five seasons of continuous MON 810 maize cultivation at one site in China did not negatively impact the colonisation and community structure of arbuscular mycorrhizal fungi (AMF) in maize roots, bulk soils and rhizospheric soils. Szénási and Markó (2015) examined the potential effects of MON 810 on non-target flea beetles (Chrysomelidae, Alticinae) in an isolated maize stand located near Budapest, Hungary. The researchers found no impact on flea beetle assemblages and species, or on their abundance and species richness.

A study by Böttger *et al.* (2015) assessed the effect of litter from MON 810 maize on leaf litter decomposition rates in the aquatic environment, under controlled temperature and dissolved oxygen conditions. Cry1Ab protein had no effect on litter mass loss or lignin and phenol contents, while it affected nitrogen and total protein content of leaf litter during decomposition process and therefore was hypothesised to affect carbon turnover and nutrient spiralling in freshwater ecosystems in the long-term. Furthermore, a slightly decreased cellulose content of leaves during aquatic decomposition was found. Nevertheless, the compositional differences observed are marginal and their predicted consequences are not supported by the reported data. More specifically, the results of the publication show no consistent or significant differences between test and control for nitrogen and protein content, cellulose degradation or lignin accumulation throughout the course of the experiment, which discredits the conclusion that "*a relevant year by year input of protein and therefore nitrogen rich Bt maize litter into aquatic environments may affect the balanced nutrient turnover in aquatic ecosystems*". Further, as also highlighted by the authors, no reference samples were tested to determine the natural variability in the components tested. Finally, to support the conclusion provided, changes in nutrient content within the water phase should be measured. Valldor *et al.* (2015) characterized the fate of Cry1Ab protein in two agricultural soils with contrasting amounts of clay but high similarities in other parameters. The researchers traced ^{14}C-labelled Cry1Ab protein in the background of natural soil proteins and indigenous microbial activities and quantified its mineralization, adsorption and incorporation into soil microbial biomass. Their work suggests that ecological risk assessment of Bt crops should consider that the very low concentrations of extractable Cry1Ab do not reflect the actual elimination of the protein from the soils but that desorbed proteins mineralize quickly due to efficient microbial degradation.

On the topic of gene flow, Taverniers *et al.* (2015)[17] investigated the influence of sampling stage on GM DNA quantification from different samples of MON 810 maize, taken from a field in Belgium at various periods during the growth season. When examining the outcrossing into a neighbouring non-GM maize field, decreasing rates of GM cross-pollination percentage are to be expected with increasing distance. Based on empirical testing, different models could be composed to follow the decrease of GM percentage from the border to the centre of the field.

Three publications were identified relating to insect resistance management. In Garcia *et al.* (2015), the inheritance of resistance, associated fitness costs and the existence of incomplete resistance in the moth *Mythimna unipuncta* were investigated in an experimental field in Spain. The potential of this secondary pest to develop resistance and the implications for resistance monitoring were discussed. The work concluded that both resistant and heterozygous larvae of *M. unipuncta* survive the Cry1Ab toxin expressed in Bt maize, with a weak fitness cost for the homozygous larvae, indicating the potential risk of field-evolved resistance and its relevance to resistance monitoring. However, no field-related adverse effects are expected. *M. unipuncta* is a secondary pest with no observed change in field performance since the introduction of MON 810 maize in 1998. As demonstrated in the publication, *M. unipuncta* has very low susceptibility to Cry1Ab, meaning that selection pressure by feeding in MON 810 maize should be low. In the Southern United States, Crespo *et al.* (2015) looked at whether a blended refuge with maize containing events which express Cry1F, Cry1Ab and

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[17] This paper was selected, assessed and summarized based on the full text (see Appendix 5.2). It was 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 publication. The PDF version of this publication, however, could not be included with this report due to limited access permissions.

Vip3Aa proteins causes changes in survival and development of the ear-feeding pest *Helicoperva zea*. The reduction in survival of *H. zea* due to blending was variable and the authors stated that a sensitivity analysis including all possible scenarios of reduction in survival should be considered. Finally, work conducted in central China by Xu *et al.* (2015) compared the transcriptional profile of an Asian corn borer (ACB) strain resistant to Cry1Ab toxin (ACB-AbR) with a susceptible strain (ACB-BtS) using high throughput RNA sequencing (RNA-seq) and bioinformatics tools. The study identified differentially expressed unigenes related to general Bt resistance in ACB. The assembled, annotated transcriptomes are thought to provide a valuable genomic resource for further understanding the molecular basis of ACB Bt resistance mechanisms.

On the topic of fumonisins (FUM), the group of Rocha *et al*. (2016) in Brasil measured the levels of this toxin produced by *Fusarium verticillioides* in GM (MON 810) and non-GM maize, post-harvest, in different periods of incubation and under controlled conditions. The authors concluded that wounded maize grains possibly facilitated *F. verticillioides* penetration, leading to higher fumonisin production in non-Bt hybrids during the period of incubation due to the increase of fungal biomass. Management of insects through the use of Bt maize was considered to greatly reduce insect damage, thus indirectly controlling fungal and mycotoxin contamination in the field and the accumulation of mycotoxin during storage. A gene expression analyses demonstrated that further studies should be conducted to determine an acceptable qualitative genetic marker for identifying fumonisin production in early stages of infection by *F. verticillioides* in maize.

For the 2015-2016 period, a total of four review papers on Bt maize were identified in the search output (Carrière *et al.*, 2016; Rahman *et al.*, 2015; Singh and Dubey, 2016; van Capelle *et al.*, 2016). No reviews were identified in the area of food/feed safety.

Environmental reviews included a paper by van Capelle *et al.* (2016) in which focal earthworm species, typical for arable soil under crop rotation with (GM) maize and/or potatoes within three European regions, were selected based on literature data in a four step procedure. As a result of the analysis, the earthworm species *Aporrectodea caliginosa* and *Lumbricus terrestris* were selected as focal species and recommended by the authors to be included in a standardized laboratory risk assessment test system based on life-history traits. A review by Singh and Dubey (2016) summarised the results of laboratory studies and field trials to assess the Bt toxin proteins (including MON 810 maize) in soil microbes and the processes determining the soil quality in conjunction with the existing hypothesis and molecular approaches to elucidate the risk posed by GM crops. Singh and colleagues concluded that ecological perturbations hinder the risk aspect of soil microbiota in response to GM crops, therefore extensive research based on *in vivo* and interpretation of results using high-throughput techniques such as Next Generation Sequencing (NGS) on risk assessment are imperative to evaluate the impact of Bt crops and resolve the controversy related to their commercialization. Rahman *et al.* (2015) looked into the safety assessment of *cry* genes in GM crops. Potential harmful effects of B crops on non-target organisms were assessed before their release into the environment and, most commonly, cultivation was found safe. There is however a continuing debate on the need for conducting extensive laboratory as well as field trials and also discussions on methods and procedures of calculating ecological risks. It is generally accepted that procedures, methods and protocols for evaluating the potential risks of GM crops and foods should be standardized for building confidence of all stakeholders. They concluded that efforts should be exerted in deploying genes of interest, marker genes and regulatory sequences invoking no or little issues of potential risks to the ecosystem.

In the insect resistance management area, Tabashnik and Carrière (2015) reviewed the successes and failures of transgenic Bt crops. The authors reviewed data monitoring resistance to seven Bt proteins in 13 major pest species targeting Bt maize and Bt cotton on six continents. Twenty seven sets of monitoring data were analyzed. Seven indicated severe field-evolved resistance within 2 - 8 years, with practical consequences for pest control. Eight showed less severe field-evolved resistance and 12 showed no evidence of decreased susceptibility after 2 - 15 years. The surge in cases of practical resistance since 2005 was associated with increased planting of Bt crops and increased monitoring. In addition, practical resistance to Bt crops was associated with a scarcity of refuges. Another review by Carrière *et al.* (2016) discussed whether pyramids and seed mixtures delay resistance to Bt crops. The primary strategy for delaying the evolution of pest resistance entails non-Bt refuges, Bt crop ‘pyramids’, and planting seed mixtures yielding random distributions of pyramided Bt and non-Bt maize plants within fields. The authors conclude that conditions often deviate from those favouring the success of pyramids and seed mixtures, particularly against pests with low inherent susceptibility to Bt toxins. For these problematic pests, promising approaches include using larger refuges and integrating Bt crops with other pest management tactics. In the case of MON 810 cultivation in the EU, the target pests do not have a low inherent susceptibility to the expressed Cry1Ab protein. Huang (2015) described resistance management for Bt maize and above-ground Lepidopteran targets in the USA, with a shift from first generation maize containing only a single Bt gene for a target to second generation maize, as of 2005, expressing two or more pyramided Bt proteins. Analysis of the available data showed that all corn borer species remain susceptible to Bt proteins and that no field resistance has occurred after nearly two decades of intensive use of Bt maize in the continent. Pyramided Bt maize is effective in controlling corn earworm and fall armyworm, although recent studies indicate that field resistance to single gene *cry1F* maize in the fall armyworm has occurred in the south-east coastal areas of the USA mainland. A paper by Van den Berg and Campagne (2015) looked into the evolution of *Busseola fusca* resistance to MON 810 maize in South Africa and the numerous challenges to insect resistance management in Africa, ranging from landscape heterogeneity to poor knowledge on *B. fusca* biology.

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 considered to be 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, both publications were not retrieved in the literature search as they were only included in the Web of Science™ Core Collection or the CABI CAB Abstracts® databases after this years' search period.

The lack of relevance of these papers for the risk assessment of MON 810 is described in the EFSA assessments (EFSA, 2016a), concluding that "*As the evidence reported by Bøhn et al. (2016) is insufficient to indicate the necessity to revise the environmental risk assessment conclusions for maize MON 810 and Bt11, EFSA considers that the risk assessment conclusions on maize MON 810 and Bt11 for cultivation made by the Panel on Genetically Modified Organisms remain valid and applicable*" and "*EFSA considers that there are no data in Hofmann et al. (2016) that indicate the necessity to revise the previous environmental risk assessment conclusions and risk management recommendations for Bt-maize made in EFSA (2015)*" and "*EFSA considers that the previous risk assessment conclusions and risk management recommendations on maize MON 810, Bt11 and 1507 made by the Panel on Genetically Modified Organisms remain valid and applicable*" (EFSA, 2016b).

As EFSA conducts a literature search to identify additional relevant scientific publications related to MON 810 maize on a yearly basis in the context of the review of the yearly MON 810 PMEM report, the abovementioned publications would have been assessed by EFSA, if deemed relevant for the risk assessment of MON 810. This provides for an efficient screening of the available literature in one search instead of numerous, separate mandates for assessing a specific publication. Furthermore, if any publication relevant for the risk assessment of MON 810 would become available throughout the monitoring period of a growing season, it would be in EFSA's remit to initiate a self-task mandate in order to assess the impact on the safety conclusions of the newly available data.

[18] EFSA Register of Questions; http://registerofquestions.efsa.europa.eu/roqFrontend/login?0 (Accessed 30 August 2016)

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, 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 post-market environmental monitoring of GM crops as well as six specific opinions on the monitoring conducted by Monsanto on MON 810 in the 2009, 2010, 2011, 2012, 2013 and 2014 growing seasons (EFSA, 2011b, 2012d, 2013c, 2014a, 2015c, 2016c). 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 opinions from EFSA, the large amount of data generated in the past growing seasons, 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 September 2012 to anticipate approvals for the cultivation in the EU of different *Bt* maize products (see Appendix 6). The purpose of the IRM plan is to proactively avoid where possible, and in all cases delay the potential development of pest resistance to the Cry protein(s) expressed in *Bt* maize. This harmonized IRM plan contains guidance on the following key elements:

- Refuge;
- Baseline studies and monitoring of the target pests;
- Communication and education;

In addition to the elements above, Monsanto has a robust farmer complaint system. Such systems provide a means for farmers to report any complaint related to Monsanto products

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[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 30 August 2016)

and services. This farmer complaint system is the most appropriate venue for the farmer to record any unexpected effect when cultivating Bt maize in their field. Farmers are first in line to detect a change in product performance, including reduced target pest insect control. Farmer complaint systems are not limited to a subsample of growers but are available for the entire farming community and for every field where MON 810 is cultivated commercially.

Therefore, the farmer complaint system serves as the primary tool to detect insect resistance development (Sumerford *et al.*, 2015) (see Section 3.2.1.3).

3.2.1.1 Refuge

According to the *Harmonised insect resistance management (IRM) plan for cultivation of Bt maize (single insecticidal traits) 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 3.2.1.3). 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 to manage. As a consequence, the seed industry has put particular emphasis on the development of communication tools.

In the context of Monsanto's 2015 GS, 261 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.

95.4% of the farmers indicated that they followed the technical guidelines regarding the implementation of a refuge (80.8 % planted a refuge and 14.6 % 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 2015 Farmer Questionnaire Survey show that 94.3% of the farmers in Spain were compliant with refuge planting while 12 farmers out of 212 (*i.e.*, 5.7%) indicated they did not plant a refuge. The farmers gave two main reasons for not being compliant with the refuge requirements: (1) lack or not enough information about the technical guidelines (8/12, 66.6%) and (2) the refuge implementation complicates the sowing and other agronomic practices (4/12, 33.3%).

In Portugal, an independent Monitoring Report on the planting of MON 810 varieties (including IRM communication and refuge implementation) during the 2015 growing season

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

was prepared by the Portuguese authorities[22]. In addition to the farmers trained in previous seasons, and in compliance with the Portuguese law, 51[23] new farmers were trained in 2015 on national and EU legislations that regulate the cultivation of GM varieties and to learn about the main characteristics of MON 810 maize. Furthermore, 66 inspections were performed of farmers planting MON 810 maize (out of the total 216 notifications received in 2015). These inspections showed good compliance in general terms, with minor changes compared to the declared information, and no sanctions were needed. Full compliance with refuge and labelling requirements was found. In addition, 49 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 2015 season are consistent and do show a high level of compliance, probably due to the high effectiveness of the grower education. Regardless of these results, the message on the importance of refuge implementation is being repeated in countries growing MON 810 in the 2016 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.

3.2.1.2 Baseline studies and monitoring of 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 would justify 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* in 2005 and again for the French samples of *S. nonagrioides* in 2006 (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 to the above, 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

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[22] Direção General de Agricultura e Desenvolvimento - http://www.dgv.min-agricultura.pt/portal/page/portal/DGV/genericos?generico=3665233&cboui=3665233#5 (Accessed 30 August 2016)

[23] So far, 1699 farmers have been trained on national and EU legislations since 2005.

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).

Monitoring of the target pests

Monitoring for changes in susceptibility to Cry1Ab in *O. nubilalis* and *S. nonagrioides* across the Ebro Valley, central Spain and Extremadura-Andalusia 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 the European Union Working Group on Insect Resistance Management. Different geographical areas with considerable commercial plantings of MON 810 varieties were selected. The monitoring studies performed with *O. nubilalis* and *S. nonagrioides* showed that the susceptibility of the collected insect samples to Cry1Ab were within what is considered a normal range, demonstrating no change in susceptibility.

In the 2012 growing season Monsanto revised its IRM plan in view of the related opinions from EFSA, the large amount of historical data generated since commercial introduction, data in the scientific literature, and the experience gained from IRM plans established in other world areas. The elements that changed since the 2012 growing season compared to previous seasons are all reflected in the updated IRM plan from EuropaBio Monitoring working group of September 2012 (Appendix 6). A change in the sampling approach was introduced in order to address EFSA's guidelines; the approach as defined in Table 4 of the EuropaBio harmonized IRM plan was implemented to be able to connect sampling frequency to the MON 810 adoption rate and the ecology of the target pests (*i.e.*, multivoltine versus univoltine life cycles).

MON 810 adoption in the areas covering the Czech Republic, Romania and Slovakia was well below 20%. The three areas identified in the entire EU where adoption of MON 810 in 2015 was expected to be greater than 20% are the Ebro valley (defined in earlier reports as Northeast Iberia), Central Iberia (particularly the province of Albacete) and the Southwest Iberia area (Southwest of Spain and south Portugal). Since adoption in those areas is below 80% Monsanto samples them every two years. Therefore, monitoring activities in 2015 were concentrated in Spain and Portugal, more specifically in Northeast Iberia for *Sesamia* and Northeast and Central Iberia for *Ostrinia*. Central Iberia was not sampled for *Sesamia* and Southwest Iberia was neither sampled for *Sesamia* nor *Ostrinia* since those collections and analyses were conducted during the 2014 growing season, and reported in previous year's monitoring report (Monsanto Europe S.A., 2016). Following the EuropaBio harmonized IRM plan, the objective of the sampling efforts is to collect a minimum of 100 larvae for both *Sesamia* and *Ostrinia* per field. However, from the experience gained in 10 years of MON 810 PMEM, it was demonstrated that such collections are not always possible (see Appendix 7). The reason for this difficulty is because pest pressure in MON 810 cultivation regions has drastically decreased since the introduction of the crop[24], which makes it very difficult and in some cases impossible to sample sufficient larvae.

Monsanto acknowledges the fact that EFSA made several recommendations to improve the methodology on how to perform case-specific monitoring, *i.e.* IRM, in their specific opinions on MON 810 monitoring in the 2009, 2010, 2011, 2012, 2013 and 2014 growing seasons (EFSA, 2011b, 2012d, 2013c, 2014a, 2015c, 2016c) and most recently in their technical report on the EFSA GMO Panel recommendations on IRM for MON 810 and the opinion of the 2014 growing season (EFSA, 2015a, 2016c). In the aforementioned documents, EFSA provides recommendations for the sampling frequency of target pests that are not in line with the approach followed by Monsanto for IRM for MON 810, outlined in the updated IRM plan from EuropaBio (Appendix 6). Nevertheless, EFSA concluded that no adverse effects related to IRM were identified due to MON 810 cultivation during the 2009, 2010, 2011, 2012, 2013 and 2014 growing seasons and that the outcomes of the monitoring reports did not invalidate the previous risk assessment conclusions (EFSA, 2011b, 2012d, 2013c, 2014a, 2015c, 2016c). This confirms that Monsanto's methodologies are fit for the purpose of identifying adverse effects. Furthermore, the absence of resistance development of *S. nonagroides* against the Cry1Ab protein after 16 years was also described in scientific literature (Castañera *et al.*, 2016).

In their most recent opinion on the 2014 MON 810 growing season, EFSA recommends to detect a resistance allele frequency of 3% and to monitor the target pest populations exclusively in Northeast Iberia (*i.e.* the Ebro valley). The first recommendation would require a collection of 1 000 *Sesamia* and *Ostrinia* larvae per area. As described above, collection of 300 larvae (*i.e.* 100 larvae per sampled field) can already be challenging. Therefore, it will not be possible for Monsanto to commit to implementing this recommendation, as the success of sampling will depend on environmental conditions of the particular season. As an alternative, EFSA proposes the use of F_2 screening as this requires the use of fewer larvae. However, where resistance alleles are not recessive, concentration-response or diagnostic assays of F_1 larvae are highly effective in detecting resistance alleles. Therefore, these assays remain the standard approach for Bt resistance monitoring of corn borers in the EU. F_2 screens can be a more sensitive approach for resistance monitoring when resistance alleles are functionally recessive. However, the main limitation of F_2 screens is that the sensitivity is limited by the number of sibling families that can be obtained from a single collection. Therefore, this method has rarely been used to look at differences in susceptibility among populations or at changes in susceptibility over time (Siegfried *et al.*, 2007). In any case, non-recessive resistance alleles pose a greater threat than recessive alleles (they will increase faster in frequency and refuge programs are less effective in slowing their evolution), meaning that the primary purpose of resistance monitoring programs should be to detect the former rather than the latter.

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[24] Catalunya Research Institute, IRTA, 2014; https://www.ruralcat.net/c/document_library/get_file?uuid=52ce0d40-0c2f-42c8-ac9f-3609cc656237&groupId=10136 (Accessed 30 August 2016)

With regards to the second recommendation, based on experience with MON 810 cultivation in Iberia, this region has demonstrated to have the highest MON 810 adoption rate since the start of the cultivation in 2003. Insect resistance against the Cry1Ab protein is more likely to evolve in areas where the selection pressure is the highest. Therefore, Monsanto will implement the EFSA recommendation of focusing the sampling efforts exclusively in Northeast Iberia on a yearly basis as from the 2016 growing season, as the sampling of the target pests was already performed at the time of publication of the latest EFSA opinion (EFSA, 2016c).

1. Sesamia nonagrioides

In 2015, susceptibility of *S. nonagrioides* to the Cry1Ab toxin has been assessed from collections in Northeast (see Appendix 7). Values of moulting inhibition concentration (MIC) have been used to assess the susceptibility of this species to Cry1Ab. In addition, a diagnostic dose (DD) was used as an alternative approach to test the dose-mortality for monitoring the susceptibility of *S. nonagrioides* to Cry1Ab.

The results of MIC_{50} (17 ng Cry1Ab/cm²) and MIC_{90} (84 ng Cry1Ab/cm²) are in the range of those obtained in previous years. Bioassays of susceptibility performed in the laboratory with the progenies of the field populations of *S. nonagrioides* since 2004 have yielded low variability in MIC_{50} and MIC_{90} values. MIC_{50}s ranged between 9 ng Cry1Ab/cm² (Northeast Iberia in 2005) and 22 ng Cry1Ab/cm² (Northeast Iberia in 2009). These results evidenced a magnitude variation of 2.4-fold. Likewise, values of MIC_{50} of laboratory strains were also very uniform, ranging between 5 and 28 ng Cry1Ab/cm², which means a magnitude variation of 5.7-fold. These measured differences and oscillations in susceptibility values to the Cry1Ab toxin reflect the common natural variations in *S. nonagrioides* previously reported (Farinós *et al.*, 2004).

Another approach to test the dose-mortality for monitoring the susceptibility to Cry1Ab is the diagnostic dose (DD), which facilitates the monitoring execution (Halliday and Burnham, 1990; Roush and Miller, 1986). The DD is here defined to cause 99% of moulting inhibition to first instar larvae (MIC_{99}) and was determined to be 726 ng Cry1Ab/cm², based on data obtained from larvae collected in different locations of Southwest, Central and Northeast Iberia between 2008 and 2012 (Monsanto Europe S.A., 2013). This protein concentration was applied to the population of *S. nonagrioides* collected in Southwest and Northeast Iberia in 2015. A moult inhibition of 100% was observed on neonates exposed to this concentration for Northeast Iberia.

2. Ostrinia nubilalis

In 2015, susceptibility to the Cry1Ab toxin of *O. nubilalis* has been assessed from collections in Northeast and Central Iberia (see Appendix 8). Values of moulting inhibition concentration (MIC) have been used to assess the susceptibility of this species to Cry1Ab. In addition, a diagnostic dose (DD) was used as an alternative approach to test the dose-mortality for monitoring the susceptibility of *S. nonagrioides* to Cry1Ab.

The results of the MIC_{50} (2.12 ng Cry1Ab/cm² for Northeast Iberia and 1.88 ng Cry1Ab/cm² for Central Iberia) and MIC_{90} (5.43 ng Cry1Ab/cm² for Northeast Iberia and 3.38 ng Cry1Ab/cm² for Central Iberia) are in the range of those obtained in previous years. Variation in the Cry1Ab susceptibility (MIC_{50}) of *O. nubilalis* collected in the Northeast and Central Iberian fields during the campaign 2015 growing season was 1.68-fold and 1.64-fold, respectively. Variation in the Cry1Ab susceptibility (MIC_{90}) of *O. nubilalis* collected in the Northeast and Central Iberian fields during the campaign 2015 growing season was 2.85-fold and 1.49-fold, respectively. Variation in Cry1Ab susceptibility (MIC_{50} and MIC_{90}) of field samples in comparison with the reference strain G.04 was up to 0.65-fold and 1.11-fold, respectively. Variation in Cry1Ab susceptibility (MIC_{50} and MIC_{90}) of field samples in comparison with the reference strain ES.ref was up to 1.44-fold and 2.65-fold, respectively. Significant differences in Bt susceptibility between ECB from Northeast Iberia and the reference strain G.04 were found. The results indicate that the observed variation in susceptibility reflects natural variation in Bt susceptibility among ECB origins. Any evidence for a decrease of Cry1Ab susceptibility of *O. nubilalis* during the monitoring duration from 2005–2015 could not be detected.

Like for *S. nonagrioides*, a DD was applied to *O. nubilalis*. The same definition was used and the DD was determined to be 28.22 ng Cry1Ab/cm². This value was based on MIC_{99} values obtained from larvae collected in 2005-2012 in fields from Czech Republic, France, Germany, Italy, Panonia, Poland, Portugal, Romania and Spain (Monsanto Europe S.A., 2013). Not a single larva tested in the 2015 growing season survived this dose.

During the bioassays used for Bt resistance monitoring, the situation can occur that some of the larvae have not fed at all on the artificial diet. Consequently, a portion of these larvae often survive to the end of the assay because the assay conditions are otherwise relatively benign. Counting larvae that have not grown significantly during the course of the assay as dead (*i.e.*, use of Moulting Inhibition Concentration (MIC) rather than Lethal Concentration (LC) as the response variable) gives a more consistent assessment of larval Bt susceptibility and the mortality estimate also is more reflective of what would occur under field conditions (or with a longer assay duration) where those very small larvae would not be expected to survive and develop to the adult stage.

For these reasons, the response criteria used by Siegfried (2007) for over 15 years of monitoring Bt susceptibility in *O. nubilalis* in the USA consider larvae that have not grown beyond first instar and weigh <0.1 mg to be dead. A comparable approach is used for resistance monitoring of other Bt maize target species world-wide. For the same reasons, the use of MIC is preferred to LC for Bt resistance monitoring of corn borers in the EU.

Furthermore, based on the experience gained, the use of a Diagnostic Dose (DD) assay rather than the use of MIC doses is appropriate, which can increase sensitivity (Roush and Miller, 1986). In such assays, a single concentration of the protein would be used in the bioassays with the target pests. This concentration would be determined based on experience with MIC values determined on baseline collections. Therefore, as sufficient additional experience is now available, Monsanto will use a DD assay for monitoring the Cry1Ab susceptibility of both *S. nonagrioides* and *O. nubilalis* as from the 2016 growing season.

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

3.2.1.3 Communication and education

An extensive grower education program is essential for the successful implementation of the IRM plan. Each purchaser of MON 810 receives a Technical User Guide (see Appendix 3). 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 2015 planting season in Spain, 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 3.5), 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 Asociación Nacional de Obtentores Vegetales (ANOVE, the National Breeder Association in Spain) 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. In 2015, 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).

    b. A postcard 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. Presentation by sales and marketing teams of IRM requirements in farmer meetings/farmer talks to reinforce the need for refuge compliance (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 39 visits at planting time in MON 810 growing areas to inform, distribute material and ensure that farmers are well informed on refuge implementation when buying MON 810 seeds. These visits were focussed in Andalucía and Extremadura as the non-compliance refuge rates were higher in previous seasons. For the Ebro Valley and Castilla-La Mancha, letters reminding of refuge obligations were sent to the point of sales visited in previous seasons.

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

4) Farmer complaint system

Monsanto has a robust farmer complaint system. Such systems provide a means for farmers to report any complaint related to Monsanto products and services. This farmer complaint system is the most appropriate venue for the farmer to record any unexpected effect when cultivating Bt maize in their field. Farmers are first in line to detect a change in product performance, including reduced target pest insect control. Farmer complaint systems are not limited to a subsample of growers but are available for the entire farming community and for every field where MON810 is cultivated commercially.

Farmers can complain to Monsanto about any issue via the local sales representatives or customer service routes. Once a product-specific complaint is received, an internal procedure for verification and potential analysis, registration and follow up is triggered via the local Monsanto sales representative. In case the analysis of the complaint indicates potential insect resistance development, an internal procedure will be followed that includes on-site follow-up by a Monsanto representative. If this assessment would confirm insect-resistance development, a remedial plan as described in the EuropaBio harmonized plan will be implemented.

During the 2015 growing season, Monsanto representatives did not receive any complaint related to MON 810 efficacy. Nevertheless, the effectiveness of the system was demonstrated because in total Monsanto received and assessed more than 300 complaints that season, related to any issue going from seed germination issues to complaints related to meteorological conditions.

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 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.

3.2.2 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 2015, the percentage of farmers implementing refuges in their fields was very high.

The results of the analysis of 2015 farmer questionnaires did not identify any potential adverse effects that might be related to MON 810 plants and their cultivation. Company stewardship activities, systems and issue alerts did not reveal any 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 2015 cultivation season as confirmed by the absence of farmer complaints related to allegedly reduced MON 810 product performance.

A comprehensive insect resistance monitoring program demonstrated that there were no changes in susceptibility of either *O. nubilalis* or *S. nonagrioides* to the Cry1Ab protein in the MON 810 growing regions in Europe in 2015. This is in line with the observation that also on a global level no resistance is found for *O. nubilalis* and *S. nonagrioides* (Tabashnik *et al.*, 2013), which confirms the appropriateness of the implemented IRM plan.

Monsanto has not conducted monitoring activities specifically addressing the presence of teosinte detections in Spain. In line with Article 13(6) of Directive 2001/18/EC and Articles 9 and 21 of Regulation (EC) No 1829/2003, Monsanto assesses if any new information has become available with regard to the risk of MON 810 to human health or the environment, or any new scientific or technical information which might influence the evaluation of the safety in use of food or feed. However, for the reasons laid down in our letter to the European Commission of 1 July 2016, Monsanto is of the opinion that the presence of teosinte in Spain cannot be classified as information that regards the risk of MON 810 to human health or the environment, nor can it be regarded as information that influences the evaluation of the safety in the use of food or feed. In addition, further information can be found in the two EuropaBio documents "*Teosinte in the EU*" and "*Additional information concerning the presence of teosinte in the EU in response to questions of the European Commission*" provided to the European Commission on 13 May 2016 and 19 July 2016, respectively.

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 eleven post-market environmental monitoring (PMEM) reports covering twelve 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 continuing to support the safety conclusions 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 the same 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 twelve years of experience with MON 810 cultivation in the EU, more than 19 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 any confirmed adverse effect related to the event. All together, these results demonstrate that there are no adverse effects attributed to the cultivation of MON 810 in Europe. The result of the 2015 monitoring concurs 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 now about thirteen years, and discussions with different stakeholders such as the European Commission, Member States, independent experts and other biotech industries. Based on these discussions, this report also includes information on the adaptations of the current monitoring strategy that will be implemented as from the 2016 cultivation season, based on gained experience and knowledge. Furthermore, 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. This PMEM plan includes a proposal for a harmonized approach towards case-specific monitoring (IRM), which is currently a condition of the MON 810 authorization in the EU.

Feyza Selcuk Meers

Signed: ____________________

Date: 01/09/2016

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Appendix 1. Post Market Monitoring of insect protected *Bt* maize MON 810 in Europe – Conclusions of a survey with Farmer Questionnaires in 2015

Appendix 2. MON 810 Farmer Questionnaire: 2015

Appendix 3. Examples of Technical User Guides

Appendix 3.1 Czech Republic

Appendix 3.2 Portugal

Appendix 3.3. Romania

Appendix 3.4. Slovakia

Appendix 3.5. Spain

Appendix 4. Insect Protected Maize Farmer Questionnaire – User's Manual

Appendix 4.1 User manual annexes Portugal

Appendix 4.2. User manual annexes Spain

Appendix 5. MON 810 Literature Review (June 2015 – May 2016)

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 2015 – May 2016) – Web of Science^{TM} Core Collection database

Appendix 5.4 MON 810 Literature Review – List of all hits (June 2015 – May 2016) – Cabi Cab Abstracts® database

Appendix 6. EuropaBio Harmonised insect resistance management (IRM) plan for cultivation of *Bt* maize (single insecticidal traits) in the EU, September 2012

Appendix 7. Insect Resistance Monitoring in Iberian collections of *Sesamia nonagrioides*: 2015 Season

Appendix 8. Insect Resistance Monitoring in Iberian collections of *Ostrinia nubilalis* (ECB): 2015 Season

Appendix 9. Iberian Refuge Implementation Communication Materials

Appendix 9.1 Good Agricultural Practices Leaflet

Appendix 9.2 IRM advertisement

Appendix 9.3 Refuge postcard

Appendix 9.4 Refuge presentation

Appendix 9.5 IRM Poster

Appendix 9.6 YieldGard Technical Guide PT