Annual monitoring report on the cultivation of MON 810 in 2014

Czech Republic, Portugal, Romania, Slovakia, and Spain

Submitted by

MONSANTO EUROPE S.A.

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

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Data protection.

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

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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.

® 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/food/fs/sc/scp/out02_en.html (Accessed 24 August 2015)

[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.

In 2014, MON 810 was planted in the EU on approximately 143 015 hectares across five countries: Czech Republic (1 754 ha[3]), Portugal (8 542 ha[4]), Romania (711 ha[5]), Slovakia (411 ha[6]) and Spain (131 537 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 our 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). Since 2010, our 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, 2014 - http://eagri.cz/public/web/mze/tiskovy-servis/tiskove-zpravy/x2014_plochy-s-geneticky-modifikovanou.html (Accessed 24 August 2015)

[4] Ministry of Agriculture and Sea of Portugal, 2014 - http://www.drapal.min-agricultura.pt/drapal/images/servicos/ogm/DADOS_NACIONAIS_2014_setembro.pdf (Accessed 24 August 2015)

[5] Ministry of Agriculture and Rural Development of Romania, 2014 - http://www.madr.ro/docs/agricultura/suprafete-cultivate-porumb-modificat-genetic-mon-810-anul-2014.pdf (Accessed 24 August 2015)

[6] Ministry of Agriculture and rural development of the Slovak Republic, 2014 - http://www.mpsr.sk/index.php?navID=764&navID2=764&sID=40&id=8629 (Accessed 24 August 2015)

[7] Ministry of Agriculture, Food and Environment of Spain, 2014 - http://www.magrama.gob.es/es/calidad-y-evaluacion-ambiental/temas/biotecnologia/ESTIMACI%C3%93N_DE_LA_SUPERFICIE_TOTAL_DE_VARIEDADES_OMG_CULTIVADAS_EN_ESPA%C3%91A_tcm7-345334.pdf (Accessed 24 August 2015)

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 2014 - July 2015

1.6 Other monitoring reports have been submitted in respect of:

- **Import and Processing** Yes, voluntary (September 2015)
- **Food/Feed** Not applicable

2. EXECUTIVE SUMMARY

In 2014, MON 810 was planted in the EU on approximately 143 015 hectares across five countries. As part of stewardship of the technology, industry has implemented an Insect Resistance Management (IRM) plan to proactively avoid and/or delay the potential development of pest resistance to the Cry protein. The adherence to this stewardship measure in the context of the 2014 cultivation of MON 810 maize in Europe is detailed in this report.

The planting of MON 810 in the 2014 season was accompanied by a rigorous IRM plan involving four main elements: 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 2014 and the success of these initiatives was reflected in the high levels of compliance with requirements for refuge implementation observed in the 2014 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 2014. Not a single MON810 performance complaint allegedly caused by reduced target pest susceptibility was received from farmers in 2014.

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 2014, Monsanto continued its General Surveillance monitoring program, 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 2014 did not reveal any adverse effects associated with the genetic modification in MON 810. Furthermore, a detailed analysis of 25 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 and issue alerts did not reveal any adverse effects related to MON 810 cultivation in 2014. Taken together, these results demonstrate that there are no adverse effects attributed to the cultivation of MON 810 in Europe in 2014.

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 ten 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 (Monsanto Europe S.A., 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2015). 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. Since the first implementation of farmer interviews, more than 2300 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. 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. In addition, 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 370 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, reports of EENs were used to confirm the safety of GM crops in general and MON 810 in particular, 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 10 monitoring reports, covering 11 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, 2015c), Monsanto's monitoring reports (EFSA, 2011b, 2012d, 2013c, 2014a, 2015b) 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 11 years of experience with MON 810 cultivation in the EU
- more than 18 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 98/294/EC), 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 2014 GS activities are included in this report in order to meet the European Commission's request.

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 five specific opinions on MON 810 monitoring in the 2009, 2010, 2011, 2012 and 2013 growing seasons (EFSA, 2011b, 2012d, 2013c, 2014a, 2015b). Monsanto pursues its gained expertise on MON 810 monitoring and already established methodologies in order to report on the results for the 2014 growing season. Firstly, 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 98/294/EC). 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 its current *modus operandi* (which, as mentioned before, is not static but has 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,

human or animal health were identified due to MON 810 cultivation during the 2009, 2010, 2011 and 2012 growing seasons and that the outcomes of the monitoring reports did not invalidate the previous risk assessment conclusions (EFSA, 2011b, 2012d, 2013c, 2014a). 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 2014, 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 2014 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 2014 GS for MON 810 focused on the Iberian geographical regions where the majority of MON 810 was grown in 2014 (Portugal and Spain, countries accounting for approximately 98% of the MON 810 plantings in the EU in 2014), 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 2014, 48 farmers in Portugal and 213 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 2014 and March 2015. 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).

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.

[8] Instituto Markin, Spain.

[9] Agro.Ges - Sociedade de Estudos e Projectos, Portugal.

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.

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. More information on the approach was shared in previous MON 810 PMEM reports. As a result, 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). 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 identified several limitations to the use of these networks and concluded that a more complex data analysis would be required in order to allow the possibility to combine data from different EENs networks (EFSA, 2014b).

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[10] EuropaBio info for operators webpage - http://www.europabio.org/information-operators-contact-point (Accessed 24 August 2015)

[11] Monsanto product stewardship webpage - http://www.monsanto.com/products/pages/product-stewardship.aspx (Accessed 24 August 2015)

[12] Channels of communication to external stakeholders include the Monsanto website - http://www.monsanto.com/newsviews/Pages/Issues-and-Answers.aspx (Accessed 24 August 2015)

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 2014 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 2014 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 2014.

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 2014 – 2015

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 2014, and beginning of June 2015, 24 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 to the extent possible publications from journals that are included in the Web

of Science™ database[13], accessible through the Web of Science™ platform[14], a product of Thomson Reuters. The web-based interface allows for a customized search using key words in a certain combination. The key words used for this search and the operators to combine them are provided in Table 1. All publications that resulted from the search as described in set #7 in Table 1 were screened, and relevant publications to the risk assessment were subsequently assessed. 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 and Molecular characterisation; – see Appendix 5.1) and environment (Non-Target Organisms (NTO); Insect Resistance Management (IRM); Agronomy and Environmental Safety – see Appendix 5.2).

[13] http://apps.webofknowledge.com/WOS_GeneralSearch_input.do?SID=R2COEh8dkg4AFJkLed8&product=WOS&search_mode=GeneralSearch&preferencesSaved= (Note that access to the database requires a subscription) (Accessed 24 August 2015)

[14] http://isiwebofknowledge.com (Accessed 24 August 2015)

Table 1. List of key words and operators used to obtain relevant publications related to MON 810 in Thomson Reuters Web of Science[SM] 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;*

Seven publications were evaluated in terms of food/feed safety, two dealing with exposing economically important animal species to MON 810, three addressing allergenicity of MON 810 and the Cry1Ab protein, and two related to molecular characterisation (Andreassen *et al.*, 2015a; Andreassen *et al*., 2015b; Furgal-Dierzuk *et al*., 2014; Gu *et al*., 2014; La Paz *et al.*, 2014; Reiner *et al*., 2014; Trtikova *et al*., 2015).

The Polish group of Furgal-Dierzuk *et al*. (2014) looked at milk parameters, serum metabolite profiles and transfer of transgenic DNA into the milk of cows fed MON 810 from the third week before parturition to the 305th day of lactation. There were no significant differences between transgenic and non-transgenic feeds with respect to milk yield and composition, dry matter intake, body weight and blood metabolite profiles. Although numerically small differences were observed in the composition of the feed, they were within the normal expected range and comparable to the conventional feed used in Poland. Transgenic DNA sequences from the GM maize were not detected in the cow milk. Gu *et al*. (2014) exposed Atlantic salmon (*Salmo salar* L) to MON 810 or conventional maize for 99 days via feed. Histo-morphological, radiographic and mRNA expression evaluations did not reveal any biologically relevant effects of MON 810 in the gastrointestinal tract, liver and skeleton. The authors concluded that the Cry1Ab protein or other compositional differences in MON 810 maize may cause minor alterations in intestinal responses in juvenile salmon, but without affecting overall survival, growth performance, development or health. Andreassen *et al*. (2015a; 2015b) published two papers on studies in which mice were exposed intranasally to MON 810 (pollen / leaf material) or Cry1Ab protein (trypsinised or not). In a first study, MON 810 plant material did not elicit humoral immune responses in mice after airway exposure. However, the production of specific IgG1 against the two purified protein versions indicated that Cry1Ab protein as such is capable of inducing immune responses, and the production of specific IgE further indicated the ability of Cry1Ab to trigger pro-allergic

responses in mammals. Airway exposure to MON 810 was therefore concluded to be a route of practical relevance. In a second study, MON 810 plant material, Cry1Ab protein or trypsinised Cry1Ab protein did not exert adjuvant effects after airway exposure in a mouse model. The authors suggested that further experiments with purified plant proteins, as well as long-term testing should be conducted to evaluate exposure experienced in real-life situations. Despite the authors' findings it should be noted that the observed IgE and IgG production in mice could be a result of Cry1Ab protein over exposure and do not represent relevant levels of exposure for MON 810. Furthermore, the source organism (*B. thuringiensis*) is not known to be allergenic, the Cry1Ab protoxin expression in MON 810 is very low and has no sequence similarity to know allergens, and the protein is rapidly digested in simulated gastric and intestinal fluids. Finally, it must be noted that the BALB-C mice used in the studies are prone to produce allergenic responses to proteins and therefore the significance of the observations in humans is questionable. Based on the above, the relevance of the findings in the studies are not conclusive and do not pose any changes to the initial safety conclusions. Reiner *et al.* (2014) assessed the adjuvant effect of MON 810 maize, via diet, on the initiation and relapse of ovalbumin (OVA)-induced allergic airways disease in experimental mice. Feeding MON 810 did not affect airway and lung inflammation, mucus secretion in lung and OVA-specific antibody production at initiation or relapse of OVA-induced allergic asthma. This indicates that MON 810 has no adjuvant effect on allergic responses in the mouse model of allergic asthma. In the area of molecular characterisation, La Paz *et al.* (2014) compared the immature embryo transcriptome of MON 810 with the one of non-GM near-isogenic varieties. Their results suggested that overall transcription was similar in embryos of MON 810 and the corresponding non-GM near-isogenic variety 20 days after pollination. Nevertheless, about 140 genes had altered transcription levels, which was very likely due to small differences in seed development in MON 810 *versus* conventional comparators. These differences in transcription were most probably linked to the MON 810 event but were not associated to undesirable changes in the phenotype and plant behaviour, nor in the chemical and nutritional composition. Moreover, while most expression changes in MON 810 immature embryos were maintained in other transgenic varieties, some gene expression was found to be modulated by the genetic background in which the transgene was introduced through conventional breeding programs. This is in line with results from a study by Batista *et al.*, describing that observed alteration in gene expression of untargeted genes was more extensive in mutagenized than in transgenic plants (Batista *et al.*, 2008). Trtikova *et al.* (2015) explored the relationship between *cry1Ab* gene expression and Cry1Ab protein content in two MON 810 varieties (yellow and white maize) and tested whether abiotic environmental stress conditions influenced this relationship. The authors found large variations in gene expression and protein content caused by plant genetic background and environmental conditions. They concluded that field-grown MON 810 plants might therefore not always produce high enough dose of Cry1Ab protein to kill the intermediate (heterozygous) resistant insect pests. Thus, assessment of MON 810 could be proposed to include transgene expression in conjunction with Bt protein content and efficacy. Despite this opinion of the authors, it must be noted that the target species of MON 810 in the EU (*i.e.* the European and Mediterranean corn borer) are very susceptible to the Cry1Ab protein. The data presented in this study indicate that even

under the stress-induced changes, the Cry1Ab expression levels can still be considered a high dose for the European target species and therefore heterozygotes will exhibit sufficient mortality. In addition, industry implements farmer complaint systems that register reduced product performance at producer level when occurring. There have been no MON 810 performance complaints since the launch of the product in Europe.

Eighteen publications were reviewed in terms of environmental safety, many of them on the subject of non-target organisms, non-target pests, pests or insect resistance management. There were also a few papers on fumonisin contamination in maize (Bowen et al., 2014; Bowers et al., 2014; Campos and Hernandez, 2015; Čerevková and Cagan, 2015; Cotta et al., 2014; Cruz and Eizaguirre, 2015; da Silva et al., 2014; Erasmus and Van den Berg, 2014; Giron-Perez et al., 2014; Grabowski et al., 2014; Gulli et al., 2015; Habustova et al., 2015; Hurej et al., 2014; Leite et al., 2014; Meissle et al., 2014; Reisig et al., 2015; Truter et al., 2014; Zeng et al., 2014).

Meissle *et al.* (2014) looked at the suitability of pollen from various maize varieties (including MON 810) as a food source for larvae of green lacewing (*Chrysoperla carnea*). Complete development was only possible when larvae were provided with pollen complemented by eggs. Lacewing performance was not affected by maize cultivar. In Grabowski *et al.* (2014), tritrophic bioassays involving MON 810 maize, the herbivore *Tetranychus urticae* koch and the predatory ladybird beetle *Adalia bipunctata* L. showed that Cry1Ab protein concentrations decreased through the food chain. The authors however concluded that an accurate environmental risk assessment of GM plants could not be made since all potential Bt protein sources in coccinellids via multi-trophic food webs in agro-ecosystems are not known. Truter *et al*. (2014) described the biodiversity of arthropods in maize in South-Africa, and compared the diversity and abundance of arthropods and the functional groups on *Bt* and non-*Bt* maize. Results from this short-term study demonstrated that abundance and diversity of arthropods were not significantly affected by *Bt* maize. The differences in dung beetle community composition and structure in forest fragments next to conventional versus Bt maize crops, and possible impacts caused by these environmental changes in organisms via trophic cascade interactions were investigated by Campos and Hernandez (2015) in Brazil. Based on their findings, the authors concluded that the observed impact of transgenic crops on functional group dynamics within dung beetle communities could potentially lead to impaired capacity for faeces removal, seed dispersal, edaphic aeration, and incorporation of organic matter in the soil in these areas, as such ecosystem services are not performed by the dominant, over-represented functional group (*i.e.* dwellers). These findings have to be balanced by adding that no empirical evidence to substantiate this conclusion was provided. In the case of insecticidal traits in maize, the assessment of the potential hazard to a non-target beneficial insect population such as the dung beetle population, is typically carried out within the context of an ecological risk assessment whereby knowledge of the insecticidal activity spectrum of the toxin is combined with data on the environmentally relevant levels and routes of exposure. Additionally, no information is provided in this study with regard to the growth stages of the various matrices of maize fields, crop management regime, and pesticide or nutrient application in either transgenic or non-transgenic maize fields. Therefore it is impossible to

place the results of this study into any context in relationship to the maize crop, regardless of the presence of the transgenic maize varieties. Habustova *et al*. (2015) examined the effects of MON 810 maize on a range of species that could be considered as bioindicators for the post-market environmental monitoring (PMEM) defined in Directive 2001/18/EC. The study supports previous laboratory and field studies demonstrating no adverse effects of the Cry1Ab protein expressed in MON 810 on ground dwelling arthropods, including ground beetles, rove beetles and spiders (Balog *et al*., 2010; Comas *et al*., 2014; Farinós *et al*., 2008; Leslie *et al*., 2007; Rezac *et al.*, 2006; Rose and Dively, 2007; Toschki *et al*., 2007; Twardowski *et al*., 2014). Nevertheless, the authors suggest total counts of ground beetles, rove beetles and spiders collected in the fields once or twice per season to serve as bioindicators for the PMEM. It should be noted that this suggestion is not supported by the weight of evidence of this study or previous laboratory and field studies, and it is made in relation to the existence Directive 2001/18/EC and improving PMEM techniques and is not a call for more monitoring based upon the identification of adverse effects of MON 810 on the examined arthropod communities.

Several authors worked with pests or non-target pests, in some occasions with an insect resistance management background. Leite *et al.* (2014) evaluated possible prey-mediated effects of the maize pest *Spodoptera frugiperda* (fall armyworm) larvae fed with MON 810 maize on the biology and behaviour of the predatory stinkbug *Podisus nigrispinus*. The assays demonstrated no indirect negative effects of MON 810 on the predator's performance and search behaviour. Plant damage was lowest if MON 810 was used concurrently with biological control by *P. nigrispinus* for managing *S. frugiperda*. Erasmus and Van den Berg (2014) determined the effects of MON 810 maize on non-target coleopteran (*Heteronychus arator* and *Somaticus angulatus*) and lepidopteran (*Helicoverpa armigera*) pests in the laboratory or greenhouse using larvae collected from non-Bt maize fields in South Africa or colonies established from field-collected insects. MON 810 had no effect on *H. arator* or *S. angulatus* mortality, mass, fertility or fecundity but no *H. armigera* larvae survived to the pupal stage when exposed to MON 810. The variability in Cry1Ab protein susceptibility among different populations of the sugarcane borer (*Diatraea saccharalis*) was assessed by Giron-Perez *et al.* (2014). Resistance of borer populations to Cry1Ab was variable, with LC50 and EC50 values reaching about 30-fold. The larvae responded positively to Cry1Ab selection, exhibiting a 55-fold increase in resistance after four generations. This suggests the suitability of using leaves containing *Bt*-expressing genes for selection and the existence of variability of *Bt* resistance in populations of the borer. The field relevance of this study is questionable because of the conduction of only a three-day bioassay together with the use of maize leaves instead of stalks for feeding. Cruz and Eizaguirre (2015) studied whether gravid females of the maize pest *S. nonagrioides* could discriminate between genetically modified insect protected Bt maize plants versus their respective near isogenic counterparts, in a dual-choice olfactometer assay. According to the authors, the possibility that gravid females of *S. nonagrioides* are less attracted to maize with an increased level of vitamins could have important unintended consequences for insect resistance management. The stacked genetically modified plant could be less attractive to gravid females than the refuge plants, which would

increase the value of refuge plants for managing resistance. Besides the fact that this study was conducted under laboratory conditions and therefore may not be representative for conditions in the field, a GM maize with increased vitamin levels has not been commercialized up to date and therefore this study is premature to determine what impact such a commercial event would have on preference. Reisig *et al.* (2015) compared plant injury from five species of Lepidoptera between single *Bt* traits, pyramided Bt traits and a blended or a structured refuge. Hybrids with pyramided *Bt* traits were more effective for managing *Spodoptera frugiperda* and *Helicoverpa zea*. Both single and pyramided *Bt* trait hybrids were effective against *Diatraea grandiosella*, *Diatraea saccharalis* and *Elasmopalpus lignosellus*. These data suggest that it is likely that the period for development of resistance to these traits would remain static compared to the traditional structured refuge in the southern US.

There were five publications dealing with soil/plant root organism communities. In a multi-year field study conducted in South China, Zeng *et al.* (2014) found that MON 810 maize had only minor effects on the diversity of arbuscular mycorrhizal fungi (AMF) communities in soil and roots of subsequently planted conventional maize, in soils where Bt maize had been grown for consecutive seasons. Plant growth stage was found to have a greater influence on AMF diversity than *Bt* traits. Cotta *et al.* (2014) looked at effects of transgenic and non-transgenic maize on nitrogen-transforming *Archaea* and bacteria in tropical soils (Brazil). All maize types tested, including MON 810, revealed similar growth rates during the cropping seasons and in the two soil types evaluated. Significant changes in the abundances of ammonia-oxidizing bacterial and archaeal communities occurred as a result of the maize host being genetically modified. In contrast, the structures of the total communities were mainly driven by factors such as soil type and season and not by plant genotype. It should be noted that this study and observations are limited in scope, and the lack of a pre-planting analysis as well as lack of real-time functional measurements to determine whether the changes in abundance could be detected at a functional level. da Silva *et al.* (2014) found no significant differences in endophytic microbial communities (bacteria, archae and fungi) between MON 810 maize or its near-isogen, in field studies in Brazil. Also, Hurej *et al*. (2014) found no influence of Bt maize on the number, quantity changes of fungi-infected aphids or on the spectrum of fungal species in a three year field study in Poland. Finally, two field trials by Čerevková and Cagan (2015) showed that soil nematode communities were not influenced by the cultivation of Bt maize hybrids, whereas fertilizers treatment, soil moisture and attack of the European corn borer larvae may have an effect.

Bowers *et al.* (2014) compared fumonisin contamination in *Bt* and near-isogenic maize hybrids infested with European corn borer (ECB) or Western bean cutworm (WBC). WBC-produced protein was capable of increasing fumonisin levels in maize. Under WBC infestation, Cry1F mitigated this risk more consistently than Cry1Ab or non-*Bt* hybrids. The authors concluded that transgenically expressed Bt proteins active against multiple lepidopteran pests could provide broad, consistent reductions in the risk of fumonisin contamination. Bowen *et al*. (2014) evaluated insect damage, aflatoxin content and yield of *Bt* maize in Alabama (US). *Bt* maize hybrids provided yield advantages in many situations, but did not impact aflatoxin concentrations under the conditions in this study. *Bt* hybrids

expressing multiple proteins provide greater protection from ear damage by lepidopteran pests than those with single *Bt* traits.

The group of Gulli *et al.* (2015) assessed the effect of water deficit on physiological parameters, the global transcriptional pattern and *Cry1Ab* gene expression in the MON 810 maize variety DKC6575, compared to the near-isogenic non-GM Tietar. The main photosynthetic parameters were affected by drought to a similar extent in both the GM and non-GM varieties. However, under controlled environmental conditions, DKC6575 was demonstrated to have a greater sensitivity to stress in the early phase with respect to Tietar. Nevertheless, these early phase differences did not extend to differences in the final biomasses of several plant tissues. A whole genome transcriptomic analysis demonstrated that the water deficit regimes determined the up-and down- regulation of many genes, but with an up-regulation of stress-responsive genes to a greater extent in Tietar, suggesting more efficient drought responses in this genotype than in DKC6575. Finally, the expression of the transgene Cry1Ab was not influenced by the water regime, being expressed at a constant level, suggesting that any eventual greater sensitivity to drought stress in the GM variety did not concern the level of transgene expression, which was stable. It cannot be ruled out that the differential drought response observed by the authors is caused by the difference in genetic background between DKC6575 and Tietar. This has been studied by Venkatesh *et al.* (2015), who demonstrated that differences between GM crops and their non-GM comparators can be attributable to minor genomic differences in near-isogenic lines.

For the 2014-2015 period, a total of six review papers on *Bt* maize were identified in the search output (Ostry *et al*., 2015; Swiatkiewicz *et al*., 2014; Tufarelli *et al*., 2015; Turrini *et al*., 2015; Van Eenennaam and Young, 2014; Zdziarski *et al*., 2014).

In the area of food/feed safety, Tufarelli *et al.* (2015) looked at published information on the impact of GM (including *Bt*) feed in poultry diet on safety, performance and product quality. All studies confirmed that GM feeds are substantially equivalent and as safe as existing conventional feeds. A review of Van Eenennaam and Young (2014) summarized the scientific literature on performance and health of animals consuming feed containing GM ingredients and composition of products derived from them. It also discussed the field experience of providing GM feed to commercial livestock populations and summarizes the suppliers of GM and non-GM animal feed in global trade. These field data sets, representing over 100 billion animals following the introduction of GM crops, did not reveal unfavourable or perturbed trends in livestock health and productivity. No study has revealed any differences in the nutritional profile of animal products derived from GM-fed animals. The authors question the need to perform additional long-term feeding studies since the amount of data collected is significant, unless there is a specific question to be addressed. Swiatkiewicz *et al*. (2014) reviewed recent experiments on the effects of GM feeds on the physiological and metabolic indices of livestock, poultry and fish. The review paper concluded that, since the results presented in the vast majority of experiments did not indicate any negative effects of GM materials, commercialised transgenic crops can be safely fed to target food-producing animals without affecting metabolic indices or the quality of such products as meat, milk and eggs.

The aim of a review by Zdziarski *et al.* (2014) was to examine the relationship between GM crops and health, based on the histopathological investigations of the digestive tract in rats. The search identified 21 studies on crops approved for human and/or animal consumption. In three of these studies, MON 810 was analyzed. Inconsistency in methodology and a lack of defined criteria for outcomes that would be considered toxicologically or pathologically significant were observed. In addition, there was a lack of transparency in the methods and results, which made comparisons between the studies difficult. In the case of MON 810, no histopathology was performed in two studies, while in the third there was no mention on the analysis of the digestive tract. The authors concluded that there is an incomplete picture regarding the safety of GM products consumed by humans and animals and each GM product should be assessed on merit, with appropriate studies performed to indicate the level of safety associated with them. Specifically, detailed guidelines should be developed which will allow for the generation of comparable and reproducible studies.

Environmental review papers included a review by Turrini *et al.* (2015) in which available data on direct, indirect and pleiotropic effects of GM plants on soil microbiota, considering both the technology and the genetic construct utilized, were reported. Plants modified to express phytopathogen/ phytoparasite resistance, or traits beneficial to food industries and consumers, differentially affected soil microorganisms depending on transformation events, experimental conditions and taxa analyzed. In the case of MON 810, Cry1Ab protein was not detected in soil in one nine-year field trial but the protein was still found in soil after four years in another paper. The authors concluded that future studies should address the development of harmonized methodologies by taking into account the complex interactions governing soil life. Ostry and colleagues (2015) reviewed the results of 10 studies evaluating the difference in aflatoxin levels between *Bt* maize carrying the Cry1Ab insecticidal protein and the corresponding near isogenic lines. *Bt* maize had significantly lower (10-20-fold) levels of aflatoxins than non-*Bt* maize in five studies. One study showed no difference and for the remaining four studies the results were not conclusive.

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, and 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.

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 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[15] 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[16] 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 five specific opinions on the monitoring conducted by Monsanto on MON 810 in the 2009, 2010, 2011, 2012 and 2013 growing seasons (EFSA, 2011a, 2011b, 2012d, 2013c, 2014a, 2015b). 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 1). 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;

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

[16] 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://ec.europa.eu/food/fs/sc/scp/out35_en.print.html (Accessed 24 August 2015)

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 1), 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 2014 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.

95.8% of the farmers indicated that they followed the technical guidelines regarding the implementation of a refuge (86.6 % planted a refuge and 9.2 % had less than 5 ha planted with MON 810 on their farm[17]). Both countries reported a very high level of compliance with refuge requirements. The farmers in Portugal were in full compliance with refuge requirements. Responses of the Monsanto 2014 Farmer Questionnaire Survey show that 94.2% of the farmers in Spain were compliant with refuge planting while 11 farmers out of 189 (*i.e.*, 5.8%) 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 (7/11, 63.6%) and (2) the refuge implementation complicates the sowing and other agronomic practices (4/11, 36.4%).

In Portugal, an independent Monitoring Report on the planting of MON 810 varieties (including IRM communication and refuge implementation) during the 2014 growing season was prepared by the Portuguese authorities[18]. In addition to the farmers trained in previous seasons, and in compliance with the Portuguese law, 37[19] new farmers were trained in 2014 on national and EU legislations that regulate the cultivation of GM varieties and to learn about the main characteristics of MON 810 maize. Furthermore, 81 inspections were performed of

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

[18] Direção General de Agricultura e Desenvolvimento - http://www.dgv.min-agricultura.pt/portal/page/portal/DGV/genericos?generico=3665233&cboui=3665233#5 (Accessed 24 August 2015)

[19] So far, 1648 farmers have been trained on national and EU legislations since 2005.

farmers planting MON 810 maize (out of the total 238 notifications received in 2014). 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, 47 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 2014 season are consistent and do show a high level of compliance, probably due to the high effectiveness of the grower education. Anyhow, the message on the importance of refuge implementation will be repeated in countries growing MON 810 in the 2015 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 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 significant 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 2014 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 2014 were concentrated in Spain and Portugal, more in particular in Central and Southwest Iberia for *Sesamia* and Southwest Iberia for *Ostrinia*. Central Iberia was not sampled for *Ostrinia* and Northeast Iberia was neither sampled for *Sesamia* nor *Ostrinia* since those collections and analyses were conducted during the 2013 growing season, and reported in previous year's monitoring report (Monsanto Europe S.A., 2015).

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 and 2013 growing seasons (EFSA, 2011b, 2012d, 2013c, 2014a, 2015b) and most recently in their technical report on the EFSA GMO Panel recommendations on IRM for MON 810 (EFSA, 2015a). 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). Because of the wealth of experience with the product and the absence of any reported resistance cases showing the implemented IRM strategy is fit for purpose, Monsanto continued to choose pursuing its gained expertise on MON 810 monitoring and already established methodologies in order to report on the IRM

results. EFSA concluded that no adverse effects related to IRM were identified due to MON 810 cultivation during the 2009, 2010, 2011, 2012 and 2013 growing seasons and that the outcomes of the monitoring reports did not invalidate the previous risk assessment conclusions (EFSA, 2011b, 2012d, 2013c, 2014a, 2015b). This confirms that Monsanto's methodologies are fit for the purpose of identifying adverse effects.

1. Sesamia nonagrioides

In 2014, susceptibility of *S. nonagrioides* to the Cry1Ab toxin has been assessed from collections in Southwest and Central Iberia (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} (31 ng Cry1Ab/cm² for Southwest Iberia and 15 ng Cry1Ab/cm² for Central Iberia) and MIC_{90} (236 ng Cry1Ab/cm² for Southwest Iberia and 138 ng Cry1Ab/cm² for Central Iberia) 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 7 ng Cry1Ab/cm² (Central Iberia in 2006) and 31 ng Cry1Ab/cm² (Southwest Iberia in the present season). These results evidenced a magnitude variation of 4.4-fold. Likewise, values of MIC_{50} of laboratory strains were also very uniform, ranging between 5 and 19 ng Cry1Ab/cm², which means a magnitude variation of 3.8-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 Central Iberia in 2014. A moult inhibition of 96 (± 2)% and 96 (± 1)% was observed on neonates exposed to this concentration for Southwest and Central Iberia, respectively.

2. Ostrinia nubilalis

In 2014, susceptibility to the Cry1Ab toxin of *O. nubilalis* has been assessed from collections in Southwest Iberia (see Appendix 8). To determine the susceptibility to Cry1Ab, larval moult inhibition data at the different concentrations of Cry1Ab tested were analyzed, together with the dose-mortality by use of a DD. MIC_{50} and MIC_{90} values for *O. nubilalis* collected in Southwest Iberia were 1.32 and 3.80 ng Cry1Ab/cm², respectively. Variation in the Cry1Ab susceptibility (MIC_{50} and MIC_{90}) of *O. nubilalis*

collected in the field during the campaign 2014 growing season was 4.11-fold and 3.01-fold, respectively. Variation in Cry1Ab susceptibility (MIC_{50} and MIC_{90}) of *O. nubilalis* collected in the field during the 2014 growing season in comparison with the lab strain was 10.86-fold and 11.12-fold, respectively. Significant differences in susceptibility between the *O. nubilalis* from Southwest Iberia and the reference strain were found, however the susceptibility to the Cry1Ab protein of *O. nubilalis* in commercial field situations did not decrease in comparison to previous years. The observed variation in susceptibility reflects natural variation in Cry1Ab susceptibility among *O. nubilalis* collections. Any evidence for a decrease of Cry1Ab susceptibility of *O. nubilalis* during the monitoring duration from 2005–2014 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 2014 survived this dose.

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 2014 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 implemented. Activities included:

1) Ensuring continuous communication about IRM implementation in all sales tools (leaflets, brochures, catalogues, *etc.*). Also, in addition to the TUG (Appendix 3.5), which is included in seed bags and has been extensively distributed, other communication materials previously printed like the Guía Técnica YieldGard® (YieldGard Technical Guide) (see Appendix 9.1) will continue to be available.
2) Stewardship requirements and IRM compliance for MON 810 cultivation are reviewed with licensee companies and Monsanto sales teams every season in different training

sessions. After this annual review, a presentation on IRM was provided by ANOVE (the National Breeder Association in Spain) and by individual companies ensuring common messages across the market. In 2014, the following actions were taken:

a. Advertisement about refuge compliance, articles and references to the TUG published in key agricultural magazines (see Appendix 9.2)

b. Sending a postcard (on behalf of ANOVE) from each company to farmers in their database located in MON 810 growing areas reinforcing the key messages of refuge implementation (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 40 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.

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: 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 2014, the percentage of farmers implementing refuges in their fields was very high.

The results of the analysis of 2014 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 2014 cultivation season as confirmed by the absence of farmer complaints related to allegedly reduced MON810 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 2014. 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.

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 ten post-market environmental monitoring (PMEM) reports covering eleven 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 eleven years of experience with MON 810 cultivation in the EU, more than 18 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 2014 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 twelve years, and discussions with different stakeholders such as the European Commission, Member States, independent experts and other biotech industries. 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.

Signed: 

Date: 01/09/2015

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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 2014

Appendix 2. MON 810 Farmer Questionnaire: 2014

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 2014 – May 2015)

Appendix 5.1. MON 810 Literature Review – Food/Feed

Appendix 5.2 MON 810 Literature Review - Environment

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*: 2014 Season

Appendix 8. Insect Resistance Monitoring in Iberian collections of *Ostrinia nubilalis* (ECB): 2014 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