Annual monitoring report on the cultivation of MON 810 in 2011

Czech Republic, Poland, Portugal, Romania, Slovakia, and Spain

Submitted by

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TABLE OF CONTENTS

LIST OF APPENDICES

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)[2]. 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)[3] 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

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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/food/fs/sc/scp/out02_en.html (Accessed June 14, 2012)

[2] Commission Decision (98/294/EC) of 22 April 1998 concerning the placing on the market of genetically modified maize (*Zea mays* L. line MON 810), pursuant to Council Directive 90/220/EEC - http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31998D0294:EN:NOT (Accessed June 14, 2012)

[3] 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 - http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32003R1829:EN:NOT (Accessed June 14, 2012)

part of an EFSA overall opinion on 30 June 2009[4]). According to the legal framework, these authorised products remain lawfully on the market until a decision on re-authorisation is taken.

In 2011, MON 810 was planted in the EU on approximately 114 508 hectares across six countries: Czech Republic (5090 ha), Poland (ca. 3 000 ha), Portugal (7723 ha), Romania (588 ha), Slovakia (761 ha) and Spain (97 346 ha) (see Appendix 1).

Results of Insect Resistance Management (IRM) are provided to the European Commission on an annual basis (*i.e.* this report) along with the results of the General Surveillance monitoring. Monsanto also reports annually on General Surveillance activities associated with the handling and use of viable MON 810 maize grain imported into the EU in a General Surveillance Import Monitoring Report. In both cases, if the investigation established that MON 810 is the cause of an adverse effect, Monsanto shall 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, shall 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).

The present report follows the format as laid out in Annex I to Commission Decision 2009/770/EC[5].

[4] EFSA scientific opinion on Applications (EFSA-GMO-RX-MON810) for renewal of authorisation for the continued marketing of (1) existing food and food ingredients produced from genetically modified insect resistant maize MON 810; (2) feed consisting of and/or containing maize MON 810, including the use of seed for cultivation; and or (3) food and feed additives, and feed materials produced from maize MON 810, all under Regulation (EC) No. 1829/2003 from Monsanto - http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902628240.htm (Accessed June 14, 2012)

[5] Commission Decision of 13 October 2009 establishing standard reporting formats for presenting the monitoring results of the deliberate release into the environment of genetically modified organisms, as or in products, for the purpose of placing on the market, pursuant to Directive 2001/18/EC of the European Parliament and of the Council (notified under document C(2009) 7680) - http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32009D0770:EN:NOT (Accessed June 14, 2012)

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 2011 - July 2012

1.6 Other monitoring reports have been submitted in respect of:

- **Import and Processing** Yes (September 2011)
- **Food/Feed** Not applicable

2. EXECUTIVE SUMMARY

In 2011, MON 810 was planted in the EU on approximately 114 508 hectares across six 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, as well as a voluntary General Surveillance monitoring program. The adherence to these stewardship measures in the context of the 2011 cultivation of MON 810 maize in Europe is detailed in this report.

The planting of MON 810 in the 2011 season was accompanied by a rigorous IRM plan involving three main elements: farmer education, refuge implementation, and monitoring. The initiatives developed to educate farmers about the importance of the implementation of IRM measures were continued in 2011 and the success of these initiatives was reflected in the high levels of compliance with requirements for refuge implementation observed in the 2011 season. A comprehensive IRM program demonstrated that there were no changes in resistance of *O. nubilalis* or *S. nonagrioides* to the Cry1Ab protein in the major MON 810 growing regions in Europe in 2011.

In 2011, 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 249 questionnaires from a survey of farmers cultivating MON 810 in six European countries in 2011 did not reveal any unexpected adverse effects that could be associated with the genetic modification in MON 810. Furthermore, a detailed analysis of 32 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 2011. Taken together, these results demonstrate that there are no adverse effects attributed to the cultivation of MON 810 in Europe in 2011.

3. MONITORING RESULTS

3.1 General Surveillance

In 2005, Monsanto initiated, on a voluntary basis, a General Surveillance monitoring program in anticipation of the mandatory requirement for post market environmental monitoring in all applications or renewals for deliberate release submitted under Directive 2001/18/EC and Regulation (EC) No. 1829/2003 (including the renewal of the MON 810 consent[2]).

The types of General Surveillance 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 General Surveillance 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.

Since the submission of the MON 810 monitoring report in July 2011, EFSA published several documents that provide additional guidance on how to conduct General Surveillance monitoring. Firstly, it published a general guidance document for post-market environmental monitoring (PMEM) of GM crops in August 2011 (EFSA, 2011[6]). Following this publication, two specific opinions were generated on the work Monsanto did monitoring the occurrence of potential adverse effects related to MON 810 cultivation in the 2009 and 2010 growing seasons (EFSA, 2011[7]; 2012[8]). Those were published by EFSA in October 2011 and April 2012, respectively.

Monsanto acknowledges the fact that EFSA makes several recommendations to improve the methodology on how to perform General Surveillance. However, Monsanto chose to pursue its gained expertise on MON 810 monitoring and already established methodologies in order to report on the results for the 2011 growing season, and this decision has been taken for several reasons. Firstly, as said before, General Surveillance monitoring for MON 810 cultivation is conducted by Monsanto on a voluntary basis. Currently, the consent allowing MON 810 cultivation in the EU does not contain obligatory General Surveillance 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 General Surveillance 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).

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[6] http://www.efsa.europa.eu/en/efsajournal/pub/2316.htm (Accessed June 14, 2012)

[7] http://www.efsa.europa.eu/en/efsajournal/pub/2376.htm (Accessed June 14, 2012)

[8] http://www.efsa.europa.eu/en/efsajournal/pub/2610.htm (Accessed June 14, 2012)

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. As an additional reason for the *status quo*, most of EFSA's recommendations (certainly those specific to MON 810) came several months after the closure of the 2011 growing season. Since contracts with third parties to conduct parts of the General Surveillance monitoring were already signed or about to be signed, it was difficult to implement changes in methodology. Finally, it needs to be repeated that EFSA concluded that no adverse effects on the environment, human or animal health were identified due to MON 810 cultivation during the 2009 and 2010 growing seasons and that the outcomes of the monitoring reports did not invalidate the previous risk assessment conclusions (EFSA, 2011[7]; 2012[8]). This confirms that Monsanto's methodologies are fit for the purpose of identifying adverse effects and no immediate action to improve the methodology is warranted. Anyhow, 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 2011, Monsanto continued the General Surveillance monitoring program initiated in 2005 on a voluntary basis.

The objective of General Surveillance 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 General Surveillance 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[6].

General Surveillance 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 General Surveillance monitoring program performed by Monsanto in 2011 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, or plant health) 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 General Surveillance 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**Error! Bookmark not defined.**. 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. This General Surveillance for MON 810 focused on the geographical regions

within the EU where MON 810 was grown in 2011 (Czech Republic, Poland, Portugal, Romania, Slovakia and Spain) and thus was performed in areas 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 2011, 29 farmers in the Czech Republic, 10 farmers in Poland, 42 farmers in Portugal, 15 farmers in Romania, 3 farmers in Slovakia, and 150 farmers in Spain were asked to complete the questionnaire (249 in total). The farmers/fields were randomly selected between the countries 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 2011 and February 2012. In Spain, which represented the largest market, the survey was performed by Markin[9] while in Portugal, it was performed by Agro.Ges[10]. In Romania, Monsanto´s field representatives assisted the farmers in filling in the questionnaires. In the Czech Republic and Slovakia, the surveys were performed by the Czech Agriculture University[11]. In Poland, the farmers were interviewed by an independent consultant.

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.

[9] Instituto Markin, Spain.
[10] Agro.Ges - Sociedade de Estudos e Projectos, Portugal.
[11] Czech Agricultural University, Czech Republic.

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. It includes *1)* assessment of the safety and sustainability of the products, *2)* absolute respect of all the regulations in place, and *3)* support to the products by explaining and promoting the proper and responsible use of those products and technologies.

As part of product stewardship and responsible use, Monsanto urges user/licensees 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 Technology User Guides (TUGs), (see Appendix 3.1 to Appendix 3.6). Alternatively, EuropaBio[12] and Monsanto[13] 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)[14].

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[12] EuropaBio info for operators webpage - http://www.europabio.org/information-operators-contact-point (Accessed July 5, 2012)

[13] Monsanto product stewardship webpage - http://www.monsanto.com/ourcommitments/Pages/product-stewardship.aspx (Accessed June 14, 2012)

[14] Channels of communication to external stakeholders include the Monsanto website - http://www.monsanto.com/newsviews/Pages/Issues-and-Answers.aspx (Accessed June 14, 2012)

Alerts on environmental issues by existing networks

An initial effort to categorize, evaluate and select Existing Environmental Surveillance (EES) networks was presented by BioMath GmbH (contracted by Monsanto) in frame of Post Market Environmental Monitoring (PMEM) for MON 810 in Germany[15]; it illustrated a structured and systematic approach, focused on Germany. An example of the German EES monitoring report, entitled *2008 German Network Monitoring*, can be found in the monitoring report submitted in 2010 (Note that similar to last year, such report was not developed this year as MON 810 was not planted in Germany in 2011).

In anticipation of the mandatory request for post market environmental monitoring in all applications or renewals for deliberate release submitted under Directive 2001/18/EC and Regulation (EC) No. 1829/2003 (including the renewal for the MON 810 consent), based on the MON 810 example in Germany, the EuropaBio Working Group on monitoring coordinated a more general effort to map EES networks in Europe and to set up a unique reporting system. This effort was taken as a project by EuropaBio since it would allow a harmonized approach on the matter which would allow improving the quality of the data collection and reporting, bringing consistency across criteria for the selection of networks, methodology in the assessment of data and translations of surveillance reports and having a single interface with networks. In what follows, a general approach is described on how these EES networks could be selected and used in PMEM of GM crops.

A list of EES networks should be identified operating in the field of environmental monitoring. These networks can be selected from a pool of national or EU-wide obligatory monitoring activities (according to EU directives 92/43/EEC and 2004/35/EC) and other existing national or EU-wide environmental monitoring programs. This approach was also proposed by Bartsch *et al.* (2008). Once agreed upon by the different stakeholders, the publications from those networks should be reviewed on a regular basis in order to determine whether adverse effects to protection goals have been detected.

Identification of the EES networks

Firstly, the initial list of available EES networks should be classified according to the protection goals they are addressing and to their geographical coverage. No specific protection goals are defined in Directive 2001/18/EC or other GMO legislation. Directive 2002/811/EC only states that 'the monitoring plan should […] incorporate general surveillance for unanticipated or unforeseen adverse effects'. Therefore, protection goals for GM monitoring have to be sought in other EU and national legislation as already

[15] On 27 April 2007, the German Competent Authority (CA), the Federal Office of Consumer Protection and Food Safety, temporarily suspended the authorisation to distribute MON 810 maize seeds for commercial planting in Germany until Monsanto submitted an 'appropriate' monitoring plan for MON 810 cultivation in Germany. An agreement on this monitoring plan, which included both Farmer Questionnaires and the use of available information from defined existing networks as key components of general surveillance, was the basis for the lifting of the German suspension. An analysis of these networks was carried out and reported to the German CA for the 2008 cultivation season.

mentioned above, such as Directives 92/43/EEC and 2004/35/EC. Examples of these protection goals could be biodiversity, human health, animal health, plant health, soil function, water quality, and sustainable agriculture. These should form the basis for categories of EES networks identified.

Inclusion of the EES networks

In a second step, the EES networks should be analyzed for their relevance and usability for GM cultivation monitoring. To that end, an EES network datasheet can be developed and used to find relevant information about the applicability of the network in accordance with set criteria, *i.e.*, frequency of data collection, quality of data, availability of report, willingness to collaborate, etc. On the basis of information collected by these EES network datasheets, networks can be analyzed, scored and ranked. Based on this analysis, EES networks should be included in the final list of EES networks.

Analysis of the reports from the included EES networks

On a regular basis, the reports of selected EES networks should be analysed by a third party which has experience in environmental monitoring in general, and more specifically, in assessing adverse effects to environmental protection goals. However, it should be noted that the "non-hypothesis" driven observation of the networks can only provide insights on important unanticipated adverse effects (or lack thereof) and cannot deliver final conclusions.

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 2011 season can be found in Appendix 3. Additional details on growers education in the context of refuge implementation is given in section 3.2.1.3.

In the context of the farmer questionnaire initiative (see Sections 3.1.2.1 and 3.1.4.1), 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).

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 249 questionnaires from the survey of farmers cultivating MON 810 in six European countries during the 2011 growing

season did not reveal any unexpected 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 potential adverse effects related to MON 810 were reported in 2011.

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 2011 – 2012

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 2011, and beginning of June 2012, 32 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[SM] database[16], accessible through the Web of Knowledge[SM] platform[17], 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 #10 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 (Molecular characterization; Animal feeding study; Composition/Nutrition study - *see* Appendix 5.1) and

[16] 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 June 14, 2012)

[17] http://isiwebofknowledge.com (Accessed June 14, 2012)

environment (Non-target Organisms; Insect resistance management, protein/DNA fate in soil and Ecology - *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[SM] database.

Set	Search criteria
#10	#7 NOT #9 *DocType=All document types; Language=All languages;*
#9	#8 NOT (#4 OR #5 OR #6) *DocType=All document types; Language=All languages;*
#8	TS= (BT176 OR BT11 OR BT-176 OR BT-11 OR CRY1A.105 OR CRY1A105 OR CRYIA105 OR CRYIA 105 OR CRYIA.105 OR CRY2AB2 OR CRYIIAB2 OR CRY2-AB2 OR CRYII-AB2 or Cry1F or Cry1Ac OR Cry3Bb1 OR Cry11* OR Cry4* OR Roundup-ready OR ((Yieldg* OR Yield-g*) SAME (rootworm OR VT OR PLUS OR PRO OR RR OR roundup)) OR (bt SAME (cotton OR soy* OR rape OR potato OR brinjal OR rice)) OR herculex OR MON-89034 OR MON89034 OR TC1507 OR 59122 OR MON88017 OR MON-88017 OR MON-863 OR MON863 OR MIR604 OR DBT418 OR 15985) *DocType=All document types; Language=All languages;*
#7	#6 OR #5 OR #4 OR #3 *DocType=All document types; Language=All languages;*
#6	TS= (Bt-Maize OR Bt-corn OR Yieldg* OR Yield-gard OR Yield-guard) *DocType=All document types; Language=All languages;*
#5	TS=(MON810 OR MON-810) *DocType=All document types; Language=All languages;*
#4	TS=(Cry1Ab OR CryIab OR Cry-1Ab OR CryI-Ab OR Cry1A-B OR CryIA-B) *DocType=All document types; Language=All languages;*
#3	#2 AND #1 *DocType=All document types; Language=All languages;*
#2	TS=(((TOLERAN* OR RESISTAN* OR PROTEC*) SAME (LEPIDOPTERA* OR CORN-BORER* OR Ostrinia* OR nubilalis*)) AND (Genetically-modified OR modified-genetically OR transgenic* OR GM OR GMO OR MONSANTO)) *DocType=All document types; Language=All languages;*
#1	TS=(MAIZE OR CORN OR ZEA-MAYS) *DocType=All document types; Language=All languages;*

Twelve publications were evaluated in terms of food/feed safety (Balsamo *et al.*, 2011; Coll *et al.*, 2011; Frank *et al.*, 2012; Kamota *et al.*, 2011; Neumann *et al.*, 2011; Rossi *et al.*, 2011; Sissener *et al.*, 2011; Stadnik *et al.*, 2011; Swiatkiewicz *et al.*, 2011; Walsch *et al.*, 2011; Walsch *et al.*, 2012; Zhou *et al.*, 2011). MON 810 maize seeds from several fields in Germany were analysed by PCR to investigate genetic stability (Neumann *et al.*, 2011). None of the selected samples showed alterations in the sequence of the 5' plant-to-insert or 3' insert-to-plant junction, supporting the notion that no post-transformational changes occurred and that the MON 810 construct was stable. Several animal feeding studies were conducted in chicken, pig and fish. Stadnik *et al.* (2011) showed that MON 810 maize did not affect the physico-chemical parameters of broiler meat such as pH, water holding capacity, oxido-reduction potential and meat colour. Further, broilers fed GM diets exhibited improved lipid stability of breast and thigh muscles. Weaned pigs also performed better than piglets fed conventional maize in a study by Rossi *et al.* (2011). This was related to a lower Fumonisin B1 content in the MON 810 maize. Swiatkiewicz *et al.* (2011) fed MON 810 maize to swine and found no effects on fattening results and carcass and meat quality. Transgenic DNA was not detected in any tissues and content of distal parts of alimentary tract. The presence of transgenes only in the content of the stomach and duodenum confirmed the efficiency of

digestion process in the gastrointestinal tract. Finally, the GM feeds did not cause any histopathological changes in examined issues. Walsh *et al.* (2012) demonstrated that short-term feeding of MON 810 maize to weanling pigs had no adverse effect on growth performance or intestinal morphology. There were also no changes in organ weights except for an increase in kidney weight, which was not associated with histopathological or blood biochemical changes. Authors speculated on changes in carbohydrate fractions between GM and non-GM diets and how they might impact performance, however, the relevance of the findings is questionable and conclusions of adverse effects cannot be drawn from this study. . In a previous article, Walsh *et al.* (2011) described the effects of short-term feeding of MON 810 maize on the peripheral and systemic immune responses of pigs and investigated the in vivo fate of transgenic DNA. Maize-derived DNA, either of intrinsic or recombinant origin, was largely degraded in the gastro-intestinal tract (GIT). There was no evidence of cry1Ab gene or Cry1Ab protein translocation to organs or plasma and Cry1Ab protein was detected in GIT digesta but only at very low concentrations. Exposure to MON 810 maize induced some alterations in localized and peripheral immune responses in weanling pigs which, according to the authors, require further investigation. However, the lack of Cry1Ab-specific Ig production suggests that the immune response was not allergenic. Authors indicate that there were some significant differences in cytokines but these were inconsistent and authors conclude they may be due to endotoxins. They did not feel there was a negative effect on health. Sissener *et al.* (2011) followed up on an earlier study in which Atlantic salmon fed MON 810 maize showed differences in growth, relative organ sizes, cellular stress and immune function compared to fish consuming non-GM parental maize. The study was not conclusive but a potential explanation may have been the presence of a fungal toxin, dioxynivalenol, in the MON 810 maize. In compositional studies, proteomic profiling of leaves (Balsamo *et al.*, 2011) and grain (Coll *et al.*, 2011) showed no significant differences between MON 810 maize and non-GM counterpart varieties. The same was true for key forage or grain nutritional components of MON 810 maize or multi-trait maize hybrids containing the MON 810 event compared to conventional controls (Zhou *et al.*, 2011). A study by Frank *et al.* (2012) suggested that environmental factors (i.e. growing locations and seasons) were dominant parameters driving the variability of MON 810 maize metabolite profiles. Finally, Kamota *et al.* (2011) evaluated the effects of ensiling MON 810 maize on Cry1Ab protein degradation and silage compositional quality. The presence of the MON 810 gene did not essentially influence compositional quality. However, a significant proportion of the initial concentration of Cry1Ab protein remained in silage at the end of the 42 d observation period. This article reconfirms what has already been shown in other studies of Cry1Ab - that ensilement does not lead to complete degradation of the Cry1Ab protein or its activity. These previous studies are noted by the authors in the body of the article.

Ten publications were reviewed in terms of environmental safety (Bell et al., 2012; George et al., 2012; Gruber et al., 2011; Gruber et al., 2012; Hendriksma et al., 2011; Kruger et al., 2011; Pérez-Hedo et al., 2011; Razze et al., 2011; Shu et al., 2011; Tan et al., 2011). Studies relating to non-target organisms confirmed that there are no harmful effects of MON 810 maize pollen on honeybees (Hendriksma *et al.*, 2011) or of MON 810 plants on earthworms

(Shu *et al.*, 2011) and arbuscular mycorrhizal fungi communities colonising maize roots and rhizoshere soils (Tan *et al.*, 2011). Related to insect resistance management, Razze *et al.* (2011) explored feeding behaviour to understand how *Ostrinia nubilalis* larvae detect MON 810 maize and how detection affects dispersal behavior over time. The effect of ingesting sublethal amounts of Cry1Ab protein from MON 810 maize on the development and hormonal balance of *Sesamia nonagrioides* larvae was evaluated by Pérez-Hedo *et al.* (2011). The larvae of this lepidopteran pest that survived after feeding on MON 810 maize leaves or high levels of Cry1Ab protein had higher concentrations of juvenile hormone (JH), whereas their ecdysteroids did not increase sufficiently to allow pupation, leading to a longer larval development and more larval moults. This may be considered a defense mechanism that allows some larvae to survive toxin ingestion. Changes in the hormone levels in diapausing larvae were undetectable, probably masked by a higher level of JH in hemolymph and because of lack of ecdysteroid titer increase, a phenomenon that is usually observed a few days before pupation in non-diapausing larvae. The authors concluded that these results should be taken into account in the establishment of non-GM refuges to prevent development of resistance in *S. nonagrioides* populations. The lepidopteran pest *Busseola fusca* was the subject of two investigations. The first confirmed toxicity of MON 810 maize to larvae in the laboratory (George *et al.*, 2012) while the second describes a case of resistance in the Vaalharts area of South Africa (Kruger *et al.*, 2011). According to the authors, further research was needed on possible fitness costs associated with resistance evolution as well as insect resistance management and the high and dose refuge strategy to limit the development and spread of *Bt*-resistant populations to other maize production regions. Nevertheless, the level of resistance appears to be low, with appreciable fitness costs such as reduced growth rate. This could be quite important as insects collected from the Christiana region showed no detectable resistance, suggesting that fitness costs or other measures have reduced resistance in this area. Additionally, there is no data on what has happened to resistance levels in 2009 - 2011. Regarding protein/DNA fate in soil, Gruber *et al.* (2011) describes the fate of recombinant Cry1Ab protein in a field receiving liquid manure from dairy cows fed with MON 810 maize. Extensive and, compared to other proteins, rapid degradation of recombinant Cry1Ab protein occurred, leading to non-detectable levels in soil and the following crop. In 2012, Gruber *et al.* (2012) published a study looking into the amount of Cry1Ab protein in agricultural soils from four different experimental field sites in a nine-year field trial with MON 810 maize. No accumulation or persistence of Cry1Ab protein over time was seen. In the area of *O. nubilalis* ecology, Bell *et al.* (2012) analysed population dynamics between the US states of Wisconsin and Minnesota and evaluated if landscape-level manipulations could be used to restrict the cycle amplitude of this pest.

Finally, ten review papers on *Bt* maize were identified in the search output (Glaum *et al.*, 2012; Huang *et al.*, 2011; Kruger *et al.*, 2012; Meissle *et al.*, 2011; Peterson *et al.*, 2011; Rizzi *et al.*, 2012; Snell *et al.*, 2012; Viktorov, 2011; Yu *et al.*, 2011; Zhang and Shi, 2011). Rizzi *et al.* (2012) reviewed the fate of dietary DNA from genetically modified (GM) crops in the GIT of animals and the potential for horizontal gene transfer (HGT) to bacteria or animal cells. Mammals have been shown to take up dietary DNA from the GIT but *in vivo* transfer of

dietary DNA to bacteria in the intestine has not been detected in the experimental studies conducted so far. The publication of Snell *et al.* (2012) summarised 12 long-term and 12 multi-generation studies looking at the effects of diets containing GM maize, potato, soybean, rice or triticale on animal health. Results from the studies did not suggest any health hazards. A number of statistically significant differences were observed in measured parameters but these fell within the normal variation ranges and were considered to have no biological significance. Zhang and Shi (2011) specifically discussed the effects of GM crops on animal reproduction. They conclude that a number of controversial studies have been published in the past years, so that further long-term and multi-generation studies are needed to clarify the issues. However, the combined weight of evidence gained through the years by independent scientists feeding MON 810 maize to animals seem to suggest otherwise. Yu *et al.* (2011) summarize the data regarding the development and commercial use of *Bt* varieties. A mass of laboratory and field studies have shown that currently available crops have no direct detrimental effects on non-target organisms due to their narrow spectrum of activity. *Bt* crops are increasing the abundance of some beneficial insects and improving the natural control of specific pests. The use of *Bt* crops results in significant reductions of insecticide application and clear benefits on the environment and farmer health. Consequently, they can be a useful component of integrated pest management systems. Peterson *et al.* (2011) focus on spiders. Meta-analysis reveals that, in existing studies, *Bt* corn has not affected foliar or soil-dwelling spider abundance. According to the authors, *Bt* crops have become a prominent and increasingly dominant part of the agricultural landscape; understanding their interactions with spiders, a diverse and integral component of agroecosystems, is therefore essential. Viktorov (2011) deals with the pathways of *Bt* corn by-product entry into the environment. Special attention was given to decomposition of corn detritus in fresh water and migration of *Bt* toxins to stream ecosystems. The authors assess potential exposure from *Bt* corn by-products (harvested material or pollen) on stream detritophages, summarize field and laboratory experiments and conclude that a cascade of effects is possible and that the long-term ecological consequences of *Bt* plant usage are unpredictable. Original publications relevant to the environmental risk assessment (ERA) cited in Viktorov (2011) have been presented and analyzed by Monsanto in previous submissions to the EU authorities (*e.g.*, in the frame of annual monitoring reports). Given that no new data related to the ERA is presented this review does not change the conclusion of negligible risk of MON 810 to the environment. Insect resistance management is the subject of two papers, Glaum *et al.* (2012) and Huang *et al.* (2011). In the first, the potential impact of contamination on the success of high dose refuge strategy is discussed. Results from general models of resistance evolution suggest that contamination has the potential to undermine the efficacy of the high-dose/refuge strategy, yet depending upon the particular pest and situation; this may not be a concern. In the second paper, the authors note the long-term success of the high dose refuge strategy in North America and cite some factors associated with observed cases of field resistance, including failure to use high-dose *Bt* cultivars and lack of sufficient refuge. Kruger *et al.* (2012) illustrate a case of poor compliance with refuge requirements in South Africa, leading to stem borer infestations of 5 to 95% in *Bt* maize. Awareness of the importance of planting a correct refuge, indicated as the major driver for resistance development of *Busseola fusca* in the

Republic of South Africa, has increased through frequent training of seed representatives from Monsanto and seed company licensees, as well as growers. Finally, the review of Meissle *et al.* (2011) deals with *Bt* maize and integrated pest management (IPM). In an IPM context, *Bt* maize is regarded as a preventive (host plant resistance) or responsive pest control measure. In any case, it is a highly specific tool that efficiently controls the main pests and allows combination with other preventive or responsive measures to solve other agricultural problems including those with secondary pests.

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 if the use of those products is not managed appropriately. 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 effective Insect Resistance Management (IRM) strategies for insect-protected maize.

Following this example, Monsanto along with three other companies[18] has established the European Union Working Group on Insect Resistance Management (EUWGIRM). This group, formed in 2001, has developed a harmonised IRM plan specific for the EU (see Appendix 6), that 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). The harmonised IRM plan has been based on published research, current EU legislation, the European Commission's Scientific Committee on Plants (SCP) opinion on IRM[19] and practical experience gained during the implementation of IRM plans in other parts of the world. 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 expressed in *Bt* maize. This harmonised IRM plan contains guidance on the following key elements:

- Refuge;

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

[19] 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 June 14, 2012)

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

The IRM plan as implemented for the 2011 growing season does not yet take into account the recommendations provided by EFSA specifically on the monitoring conducted by Monsanto on MON 810 in the 2009 and 2010 growing seasons (EFSA, 2011[20]; 2012[21]) since the contracts were signed and the field work was already finished at the time the opinions were published. Hence, the recommendations could not be implemented in a timely manner.

3.2.1.1 Refuge

According to the *Harmonised insect resistance management (IRM) plan for cultivation of Bt maize in the EU* (see Appendix 6), farmers planting more than 5 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 Spain, farmer satisfaction and monitoring of use conditions (including IRM communication and effective refuge implementation) was assessed at the end of the 2011 planting season, through a survey sponsored by ANTAMA (Spanish Foundation supporting the use of new technologies in agriculture[22]). The survey, as in previous years, was carried out in the Ebro Valley (Huesca, Lérida and Zaragoza), which is where most of MON 810 is currently planted in Spain. The survey involved 200 farmers and half of them had planted MON 810 maize. The 100 farmers planting MON 810 maize collectively planted 3 372 hectares. The conclusions from the answers delivered by the 100 farmers growing MON 810 maize are detailed below.

Farmer responses demonstrated the effectiveness of communication regarding IRM requirements. 100% of the farmers planting MON 810 knew about the recommendation to plant a refuge. In this group, 89% considered themselves to be "well informed" and 11% "somehow informed". The farmers responses regarding the clarity of the recommendations about the implementation of refuges were as follows: 99% considered the recommendations "very clear/quite clear", 1% considered the recommendations "little clear/not clear"; 80% of

[20] http://www.efsa.europa.eu/en/efsajournal/pub/2376.htm (Accessed June 14, 2012)
[21] http://www.efsa.europa.eu/en/efsajournal/pub/2610.htm (Accessed June 14, 2012)
[22] ANTAMA - http://fundacion-antama.org/ (Accessed June 14, 2012)

the interviewees considered that it is "very easy/quite easy" to follow the recommendations while 20% considered that it is "little easy/not easy".

The survey also revealed a high level of compliance with refuge requirements indicating that 93 of the 100 farmers that were included in the final survey planted a conventional maize refuge on their farm. The remaining farmers surveyed (*i.e.* 7%) did not plant a refuge. Reasons given by the farmers for not planting a refuge were: *(1)* they consider their farms as small farms (*i.e.* less than 5 hectares and therefore no refuge required) (5%), *(2)* the sowing is more complicate (3%), or *(3)* corn borers (*Ostrinia nubilalis*) cause significant economic losses (3%).

In addition, this survey analysed the satisfaction of the growers. The survey indicated that 81% of the farmers are very satisfied, 17% quite satisfied and 2% little satisfied. The main advantage/benefit, reported by 99% of the farmers, was the effective protection against corn borers, followed by the plant health (plants/ear of maize do not collapse) (56%), good yield (53%), higher profitability (39%) , healthier plants (37%), and less preoccupation (31%).

In Portugal, a Monitoring Report on the planting of MON 810 varieties (including IRM communication and refuge implementation) during the 2011 growing season was prepared by the Portuguese authorities[23]. A total of 65 new farmers were trained in 2011 on national and EU legislations that regulate the cultivation of GM varieties and to learn about the main characteristics of MON 810 maize. Furthermore, 104 inspections were performed of farmers planting MON 810 maize (out of the total 248 notifications received in 2011)).

The survey showed good compliance in general terms, with minor changes compared to the declared information, and no sanctions were needed. Full compliance with refuge and labeling requirements was found. No fields of organic maize were found close to those of GM maize.

In addition, 56 farmer questionnaires were completed by farmers growing MON 810 maize. 100% of them declared that no adverse effect of the GM crop was observed. The survey also indicated that 96.4% of the farmers considered themselves to be "well informed", while 3.6% as "not sufficiently informed". Nevertheless, 100% of the growers stated that the technical information on the seed bags was sufficient and clear.

In the context of Monsanto's 2011 General Surveillance, 249 farmers across six countries where MON 810 was commercially cultivated were surveyed for their implementation of a refuge (see Appendix 1). This General Surveillance took place in representative environments, reflecting the range and distribution of farming practices and environments exposed to MON 810 plants and their cultivation.

97.6% of the farmers indicated that they followed the technical guidelines regarding the implementation of a refuge (92.0% planted a refuge and 5.6% had less than 5 ha planted with

[23] http://www.dgadr.pt/ (Accessed June 14, 2012)

MON 810 on their farm[24]). Most countries reported a very high level of compliance with refuge requirements. The results of this survey are discussed in further detail in Section 3.1.4.1. In general, the farmers in the Czech Republic, Slovakia, Romania, Poland and Portugal, were in full compliance with refuge requirements. Responses of the Monsanto 2011 Farmer Questionnaire Survey show that in Spain six farmers (*i.e.,* 6 of 150) indicated they did not plant a refuge. Four farmers explained that implementing a refuge complicates the sowing, whereas the two others had different reasons, *i.e.*, the resulting yield losses due to ECB attacks and not reading the label recommendations. The compliance in Spain as reported through the Monsanto 2011 Farmer Questionnaire Survey (*i.e.*, 134 of 150 farmers, 97.6%) therefore is similar as surveyed by ANTAMA.

In conclusion, the results from the presented surveys (ANTAMA, Portuguese authorities and Monsanto) during the 2011 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 all countries growing MON 810 in the 2012 growing season. It is important to continue educating the farmers on the necessity to implement refuges.

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 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 Bt176 maize cultivation (Farinós *et al.*, 2004; Gonzalez-Nunez *et al.*, 2000), prior to MON 810 introduction.

In addition to the above, a German lab (BTL Bio-Test Labor GmbH) explored the baseline susceptibility of *O. nubilalis* to Cry1Ab from 2005 to 2007 in other major European maize growing regions based on the MON 810 adoption. During this period, levels of susceptibility to Cry1Ab have been determined for one laboratory colony and several populations collected 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

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

Monitoring for resistance to Cry1Ab in *O. nubilalis* and *S. nonagrioides* populations across the Ebro Valley, central Spain and Extremadura-Andalusia since 1999 was in place after the commercialisation of varieties including Bt176 maize from Syngenta, that also expresses a Cry1Ab protein (Farinós *et al.*, 2004).

During 2004-2010, monitoring for *O. nubilalis* and *S. nonagroides* resistance to Cry1Ab expressed in MON 810 was performed. Different geographical areas with considerable commercial growing of MON 810 varieties were selected. The monitoring studies performed with *O. nubilalis* and *S. nonagrioides* showed that the susceptibility of the population samples to Cry1Ab were within what is considered a normal range, demonstrating no development of resistance.

In 2011, susceptibility to the Cry1Ab toxin of the *S. nonagrioides* populations from Northeast Iberia has been assessed for the fourth time since 2004. Differences found in the susceptibility to the toxin are within the range of variability expected for field populations of this corn borer. Further, the analyses of historical series of susceptibility data of *S. nonagrioides* to Cry1Ab did not reveal signs of development of resistance to this toxin by field populations from the sampling areas considered (see Appendix 7).

The susceptibility of the *O. nubilalis* laboratory strain to the Cry1Ab toxin was comparable with values of susceptibility obtained for laboratory strains in previous years. Molting Inhibition Concentrations (MIC) values evidenced consistency through time, showing around 6-fold variation in MIC_{50} values (see Appendix 8).

In addition to the baseline results described above, BTL Bio-Test Labor GmbH determined the susceptibility of *O. nubilalis* to Cry1Ab from 2005 to 2010 in major European maize growing regions. The susceptibility of 15 populations with 111 samples of *O. nubilalis* was analysed. Thus far, susceptibility to Cry1Ab have been assessed for one laboratory colony and populations collected in maize fields in Czech Republic, France, Germany, Italy, Hungary, Slovakia, Poland, Portugal, Romania, and Spain. *O. nubilalis* larvae were exposed to artificial diet treated with increasing Cry1Ab concentrations, and growth inhibition was evaluated after 7 days (see Appendix 8).

Results for *O. nubilalis* populations were pooled according to geographic and climatic conditions. These pooled populations correspond to homogenous regions based on available knowledge of insect biology and geography. This approach follows the IRM industry working group guidelines (see Appendix 6). The results of the *O. nubilalis* populations pooled according to geographic and climatic conditions were similar and differed 1.8-fold, 6.6-fold, 2.6-fold, 4.2-fold, 3.2-fold, 2.04-fold and 5.1-fold for *O. nubilalis* collected in 2005, 2006, 2007, 2008, 2009, 2010 and 2011 respectively. A similar degree of variability was reported for *O. nubilalis* susceptibility to Cry1Ab for populations from three broad geographic areas in the US, chosen based on market penetration for *Bt* maize. Similar levels of variability were also observed in a study that included populations of different voltine ecotypes and pheromone strains (Marçon *et al*., 1999). For the current study, the pheromone races were not distinguished (see Appendix 8).

These results indicate that the observed population variation in susceptibility reflects natural variation in *Bt* susceptibility among *O. nubilalis* populations. Any evidence for a decrease of Cry1Ab susceptibility of populations during the monitoring duration from 2005–2011 could not be detected. In the future, other regional sources may be added to ensure that the monitoring program continues to represent the Cry1Ab maize market in Europe.

In conclusion, data collected on *S. nonagrioides* and *O. nubilalis* indicate that the IRM plan proposed by the industry is still valid since no change in susceptibility to Cry1Ab was observed.

These results are aligned with the conclusions of independent studies conducted in Spain and summarized last year in the review published by the Spanish Ministry of Environment, Rural and Marine Affairs (MARM). It is concluded that monitoring results from 13 years of *Bt* maize cultivation in Spain (including MON 810 since 2003) indicate no evidence of increased susceptibility of target pests to the Cry1Ab protein[25].

3.2.1.3 Communication and education

An extensive grower education program is essential for the successful implementation of the IRM plan. As stated in Section 3.1.3, 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, other initiatives on communication are taken. For the 2011 planting season in Spain, a number of initiatives were taken, as in previous seasons, to emphasise the importance of refuge implementation. A comprehensive program to raise awareness of refuge requirements and educate personnel, dealers, cooperatives and individual farmers was implemented. Activities included:

1) Ensuring continuous communication about IRM implementation in all sales tools (leaflets, brochures, catalogues, etc.). Some examples include the good agricultural practices (GAP) leaflet (see Appendix 9.1) and Guía Técnica YieldGard® (YieldGard Technical Guide) (see Appendix 3.6) that are attached to each MON 810 bag sold in Spain.
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. Thus, in 2011, the following actions were taken:

[25] http://www.marm.es/es/calidad-y-evaluacion-ambiental/temas/biotecnologia/organismos-modificados-geneticamente-omg-/notificaciones-y-autorizaciones/comercializacion.aspx (Accessed June 14, 2012)

a. Advertisement about refuge compliance 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 and stickers distributed among seed distributors and point of sales to be used with invoices and letters (see Appendix 9.5 and Appendix 9.6)

e. Communication plan for cooperatives, small points of sales outlets and farmers: trained ANOVE inspectors completed several visits in MON 810 growing areas to inform, distribute material and ensure that farmers are well informed on refuge implementation when buying MON 810 seeds.

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

The ANTAMA survey conducted in Spain, and referred to in Section 3.2.1.1, demonstrates the effectiveness of the education program to raise awareness of refuge implementation. 100% of the farmers surveyed acknowledged they were made aware of the fact that they are required to plant a refuge. This is corroborated by the results of Monsanto's farmer questionnaires describing that 98.8% of the farmers reported to be informed on the good agricultural practices applicable to MON 810. Similarly in Portugal, the second largest MON 810 area growing country in the EU, users have received information through different materials (see Appendix 9.7) and the mandatory training sessions according to the Portuguese law. The high level of acknowledge and commitment with these requirements is reflected in the conclusions of the monitoring report performed by Portugal and referred to in Section 3.2.1.1 of this report.

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 those learnings. The commercial planting of MON 810 in Europe has been accompanied by a rigorous Insect Resistance Management (IRM) plan, involving three main elements: refuge implementation, monitoring, and farmer education.

Following the establishment and reinforcement of an effective education and communication program in countries where MON 810 was grown in 2011, the percentage of farmers implementing refuges in their fields was very high.

The results of the analysis of 2011 farmer questionnaires did not identify any potential adverse effects that might be related to MON 810 plants and their cultivation. Company stewardship activities 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 2011 planting season.

A comprehensive insect resistance monitoring program demonstrated that there were no changes in resistance of *O. nubilalis* or *S. nonagrioides* to the Cry1Ab protein in the major MON 810 growing regions in Europe in 2011.

All together, these results demonstrate that there are no adverse effects attributed to the cultivation of MON 810 in Europe. The result of the 2011 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 nine 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 is collaborating with the other applicants towards an harmonized approach for environmental monitoring of these GM maize varieties. This PMEM plan will include a proposal for a harmonized approach towards case-specific monitoring (IRM), which is currently a condition of the MON 810 authorization in the EU, as well as a proposal for harmonizing the General Surveillance for cultivated GM crops in the EU. Although General Surveillance is not yet mandatory for MON 810 in the EU, it is anticipated that it will become a general request in all authorizations for applications or renewals for deliberate release of GM crops submitted under Directive 2001/18/EC and Regulation (EC) No. 1829/2003, hence, harmonization is advisable. This harmonized approach towards GM crop monitoring can be implemented once several GM crops (including MON 810) are (re-)approved for cultivation and when the different stakeholders including the European Commission agree with it.

Signed: 

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