Annual monitoring report on the cultivation of MON 810 in 2012

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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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 July 01, 2013)

[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 July 01, 2013)

[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 July 01, 2013)

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 2012, MON 810 was planted in the EU on approximately 129 042 hectares across five countries: Czech Republic (3052 ha), Portugal (9278 ha), Romania (217 ha), Slovakia (189 ha) and Spain (116 306 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, 2012).

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

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[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 July 01, 2013)

[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 July 01, 2013)

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 2012 - July 2013

1.6 Other monitoring reports have been submitted in respect of:

- **Import and Processing** .... Yes (September 2012)
- **Food/Feed** .... Not applicable

2. EXECUTIVE SUMMARY

In 2012, MON 810 was planted in the EU on approximately 129 042 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, as well as a voluntary General Surveillance monitoring program. The adherence to these stewardship measures in the context of the 2012 cultivation of MON 810 maize in Europe is detailed in this report.

The planting of MON 810 in the 2012 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 2012 and the success of these initiatives was reflected in the high levels of compliance with requirements for refuge implementation observed in the 2012 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 2012.

In 2012, 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 five European countries in 2012 did not reveal any unexpected adverse effects that could be associated with the genetic modification in MON 810. Furthermore, a detailed analysis of 37 publications related to MON 810 and/or Cry1Ab did not reveal any new scientific evidence that would invalidate the conclusions of the risk assessment concluding that MON 810 is as safe to human and animal health as its conventional counterpart, and confirms that there is negligible impact from the cultivation of MON 810 on biodiversity, abundance or survival of non-target species, and the environmental risk of MON 810 is considered to be negligible compared to conventional maize. Also, company stewardship activities and issue alerts did not reveal any adverse effects related to MON 810 cultivation in 2012. Taken together, these results demonstrate that there are no adverse effects attributed to the cultivation of MON 810 in Europe in 2012.

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.

Monsanto acknowledges the fact that EFSA made several recommendations to improve the methodology on how to perform General Surveillance, *i.e.*, in their general guidance document for post-market environmental monitoring (PMEM) of GM crops in August 2011 (EFSA, 2011[6]) and two specific opinions on MON 810 monitoring in the 2009 and 2010 growing seasons (EFSA, 2011[7]; 2012[8]). 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 2012 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). 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, 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 2010 and 2011 growing seasons and that the outcomes of the

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[6] http://www.efsa.europa.eu/en/efsajournal/pub/2316.htm (Accessed July 01, 2013)

[7] http://www.efsa.europa.eu/en/efsajournal/pub/2376.htm (Accessed July 01, 2013)

[8] http://www.efsa.europa.eu/en/efsajournal/pub/2610.htm (Accessed July 01, 2013)

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 2012, 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 2012 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[6]. 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 2012 (Czech Republic, 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 2012, 22 farmers in the Czech Republic, 41 farmers in Portugal, 10 farmers in Romania, 1 farmer in Slovakia, and 175 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 2012 and March 2013. 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].

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. 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.

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

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 Technical User Guides (TUGs), (see Appendix 3.1 to Appendix 3.5). 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].

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 2012).

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[12] EuropaBio info for operators webpage - http://www.europabio.org/information-operators-contact-point (Accessed July 01, 2013)

[13] Monsanto product stewardship webpage - http://www.monsanto.com/ourcommitments/Pages/product-stewardship.aspx (Accessed July 01, 2013)

[14] Channels of communication to external stakeholders include the Monsanto website - http://www.monsanto.com/newsviews/Pages/Issues-and-Answers.aspx (Accessed July 01, 2013)

[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.

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. More information on the approach was shared in previous MON 810 PMEM reports. As stated before, once an agreed-upon harmonized approach is reached, Monsanto will implement it upon MON 810 (re-)approval.

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 2012 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.

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 five European countries during the 2012 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 confirmed adverse effects related to MON 810 were reported in 2012.

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 2012 – 2013

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 2012, and beginning of June 2013, 37 publications related to MON 810 and/or Cry1Ab were published in high quality journals. In order to be able to cite scientific work with the highest credibility, Monsanto uses to the extent possible publications from journals that are included in the Web of Science^{SM} database[16]**Error! Bookmark not defined.**, 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 (DNA fate; Animal feeding study - *see* Appendix 5.1) and environment (Non-Target Organisms (NTO); Effects on soil organisms; Effects on biochemical processes in soil and Insect Resistance Management (IRM) - *see* Appendix 5.2).

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[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 July 01, 2013)

[17] http://isiwebofknowledge.com (Accessed July 01, 2013)

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;*

Eleven publications were evaluated in terms of food/feed safety, most of them dealing with feeding MON 810 maize to economically important animal species such as quail, chicken, pig and cow (Buzoianu *et al.*, 2012a; Buzoianu *et al.*, 2012b; Buzoianu *et al.*, 2012c; Buzoianu *et al.*, 2012d; Gu *et al.*, 2013; Guertler *et al.*, 2012; Reichert *et al.*, 2012; Sartowska *et al.*, 2012; Walsh *et al.*, 2013; Walsh *et al.*, 2012). The impact of MON 810 maize on the health, performance and nutritional value of quail was analysed after exposure over two generations (Sartowska *et al.*, 2012). These preliminary results from a 9 generation study suggest no impact on health and growth, reproduction or laying performance. Some differences were noted in chemical composition of breast muscle and egg yolk but no clear tendency was seen for or against any of the diets used in the study. Reichert *et al.* (2012) conducted feeding experiments on broiler chickens, laying hens, fattening pigs and calves. The studies revealed morphological changes in many organs, however, the statistical analysis showed no significant differences between treatments. The authors concluded that MON 810 maize did not cause adverse effects on morphology and structure of internal organs and muscles, as assessed histologically. Buzoianu, Walsh and collaborators published a series of papers looking at pigs and sows fed MON 810 maize for various lengths of time. *Bt* maize was well tolerated by the porcine intestinal microbiota following 31 days of exposure (Buzoianu *et al.*, 2012c). Feeding MON 810 maize to pigs from 12 days post weaning up to slaughter did not adversely affect growth, carcass characteristics, bone health or body composition (Buzoianu *et* *al.*, 2012d). There were also no changes in counts of cultural bacteria enumerated in the feces, ileum or cecum or in the composition of caecal microbiota, with the exception of a minor increase in the genus *Holdemania (Buzoianu et al., 2012a)*. Walsh *et al.* (2012) evaluated the effects of this feeding regime on the peripheral immune response and determined the digestive fate of the *cry1Ab* gene and truncated *Bt* toxin. Perturbations in peripheral immune response were not thought to be age-specific and were not indicative of allergenic or inflammatory responses. There was no evidence of *cry1Ab* gene or *Bt* toxin translocation to organs or blood. Histological examination indicated the absence of an adverse effect in the small intestine, the main site of nutrient digestion and absorption. Finally, feeding transgenic maize to sows during gestation and lactation did not result in any adverse effects on immunity and no Cry1Ab or Cry1Ab-specific antibodies were detected in the blood of sows or their offspring (Buzoianu *et al.*, 2012b). Walsh *et al*. (2013) further concluded that this exposure regime did not affect body composition, as determined by back-fat depth. Some differences in bodyweight were observed between the treatments at mid-gestation, but these were no longer present in late gestation. There was a minimal effect on maternal and offspring serum biochemistry and haematology at birth and bodyweight at weaning. In a study on cows conducted by Guertler *et al*. (2012), animals fed MON 810 maize did not show any differences in the gene expression of biomarkers for apoptosis, inflammation and cell cycle in liver and the gastrointestinal (GI) tract compared to controls fed with a near-isogenic maize variety. However, Gu et al. (2013) found that Atlantic salmon exposed to MON 810 maize used feed less efficiently. *Bt* maize seemed to potentiate oxidative cellular stress in the distal intestine of immune-sensitised fish. According to the authors, Cry1Ab protein or other antigens produced due to the genetic modification have potential local immunogenic effects in the GI tract and may function as biomarkers for MON 810 maize exposure for this species. They suggest that long-term observations and more in-depth studies on immune response and nutrient utilisation may be needed to confirm the results. On a different subject, Fernandes *et al.* (2013) traced DNA from MON 810 maize through the process of broa breadmaking[18]. The results confirmed that DNA degradation occurred, however, DNA from the transgenic event could still be detected in the bread at the end of the process (Fernandes *et al.*, 2013).

Twenty-two publications were reviewed in terms of environmental safety (Alcantara, 2012; Atsumi *et al.*, 2012; Barriuso *et al.*, 2012; Bowers *et al.*, 2013; Burkness and Hutchison, 2012; Cotta *et al.*, 2013; Dutra *et al.*, 2012; Grabowski and Dabrowski, 2012; Gryspeirt and Gregoire, 2012; Hansen *et al.*, 2013; Holst *et al.*, 2013; Kim *et al.*, 2012; Kruger *et al.*, 2012; Londono *et al.*, 2013; Lupwayi and Blackshaw, 2013; Meissle *et al.*, 2012; Perez-Hedo *et al.*, 2012; Rios-Diez *et al.*, 2012; Sander *et al.*, 2012; van der Merwe *et al.*, 2012; Verbruggen *et al.*, 2012; Wang *et al.*, 2012).

A series of studies dealt with non-target organisms, showing no harmful effects of MON 810 maize on the leaf beetle *Oulema melanopus* (Meissle *et al.*, 2012) and the termite *Coptotermes formosanus* (Wang *et al.*, 2012) under laboratory conditions, on the behaviour of

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[18] Broa is a type of cornbread traditionally made in Portugal, Galicia and Brasil.

honeybees in semi-fields experiments (Grabowski and Dabrowski, 2012), or on non-target arthropod communities of commercial farms and their adjacent riparian areas in the Philippines (Alcantara, 2012). *Mythimna unipuncta* and *Helicoverpa armigera*, two maize pests poorly susceptible to Cry1Ab protein, demonstrated changes in weight gain and morphology of gut epithelium when fed on *Bt* maize. However, once exposed to non-*Bt* maize, there was rapid recovery and overcompensation mechanisms set in Perez-Hedo *et al.* (2012). Kim *et al.* (2012) established that MON 810 maize caused no adverse effects on survival and growth of yellow mealworm *(Tenebrio molitor)* larvae following long-term exposure although the larvae retained Cry toxin in the body, which could act as an exposure route to predators of higher trophic levels. MON 810 maize did not impact emergence or development time of the maize weevil pest *Sitophilus zeamais* but apparently caused a decrease in emergence of females of its natural enemy *Lariophagus distinguendos* (Hansen *et al.*, 2013). The authors concluded that '*It seems that hosts developing in Bt maize constitute some developmental challenge to parasitoid larvae and the compatibility of biological control of stored-product pests and transgenic crop cultivation is not straightforward*.

However, this conclusion is speculative and unsupported as no empirical data is provided that clearly establishes a cause and effect relationship for the observed difference in the number of female offspring and presence or absence of the Bt trait in the host food source. Further, it is well known that several factors influence the sex ratio and clutch size of parasitic wasps. A paper by Holst *et al.* (2013) assessed the potential effects of Cry protein in pollen from Bt maize on larvae from the herbivorous lepidopteran butterfly *Inachis io* in European farmland conditions, using the object-oriented model BtButTox. The model used was not spatially explicit and its application was directed only to nettles growing in worse case proximity to maize with assumed worse case inputs for pollen deposition, exposure, efficacy, and phenology. Furthermore, previous assessments have been provided concluding on negligible risk to the Nymphalidae feeding on leaves of the host plant *Urtica dioica* in the European farming landscape (Perry *et al.*, 2010; Schuppener *et al.*, 2012).

Cultivation of *Bt* maize did not change soil rhizobacterial communities when compared to soils where non-*Bt* maize was planted (Barriuso *et al.*, 2012; Cotta *et al.*, 2013). The same conclusion was reached with regard to soil microbial biomass or diversity in a 5 year study by Lupwayi and Blackshaw (2013). Arbuscular mycorrhizal fungi communities were comparable in the roots of MON 810 maize and non-*Bt* maize plants (Verbruggen *et al.*, 2012). Further studies contributed to the current scientific data suggesting that cultivating MON 810 maize is unlikely to result in any significant change in rates of residue turnover or cause significant changes in soil decomposer activity or community composition (Londono *et al.*, 2013) and that Cry1Ab protein is likely to strongly adsorb to apolar organic matter in agricultural soils (Sander *et al.*, 2012). Repeated exposure of earthworms to MON 810 maize did not affect cocoon production, hatching success or neutral red retention time (a cellular metal-stress biomarker) of the earthworm *E. andrei* although the authors suggested that further studies should be conducted to investigate multi-generation effects on the sub-organismal and organismal level (van der Merwe *et al.*, 2012). Bowers *et al.* (2013) underlined once again the positive effect of *Bt* maize on fumonisin contamination of grains due to improved pest control. In the area of insect resistance management (IRM), Atsumi *et al.* (2012) conducted some mechanistic research, demonstrating that mutation of the ATP-binding cassette (ABC) transporter gene *ABCC2* is causally related to Cry1Ab protein resistance in the silkworm (*Bombyx mori*). The results of Dutra *et al.* (2012) confirmed the predatory status of the coleopteran *Harmonia axyridis* on the lepidopteran maize pest *Spodoptera frugiperda* and showed that *H. axyridis* adults and larvae have no preference between prey exposed to *Bt* maize or not. The lack of preference between *Bt*- and non *Bt*-fed prey should act in favour of IRM strategies using mixtures of GM and non-GM maize seed. Maize and rice strains of *S. frugiperda* showed different susceptibility to Cry1Ab and Cry1Ac proteins (Rios-Diez *et al.*, 2012). In central Colombia, integrated pest management of fall armyworm should therefore be different between maize and rice. According to Gryspeirt and Gregoire (2012), synchronous emergence of adults of *Plodia interpunctella* in *Bt* and non-*Bt* maize could result in faster development of resistance due to failure in IRM strategies based on the high dose-refuge strategy. However, the high control mortality in these laboratory tests, the diet used (ground maize with non-uniform particle sizes) and the methodology used (potential shortcomings discussed by the authors) make the results from this study questionable and their relevance in field situations unclear, especially when *Bt* maize is not targeted for control of stored grain pests such as *Plodia interpunctella* and therefore an IRM plan (e.g. including refugia) is not required. Kruger *et al.* (2012) compared life history characteristics as well as fecundity and longevity of *Busseola fusca* moths of field-collected *Bt* resistant and susceptible populations. The study showed that the general fitness of a *Bt*-resistant summer generation (pupae and moths) on *Bt* maize was poorer compared to that of a susceptible summer generation on non-*Bt* maize. A study in the USA established that, as is already well known, cross pollination between *Bt* and refuge maize occurs at a high percentage in the first rows of refuge maize, decreasing with distance (Burkness and Hutchison, 2012). The authors hypothesise that cross pollination rates can be reduced by temporal isolation or by using hybrids of different maturity in the *Bt* and refuge areas. The conclusion is that, based on the nature of pollen dispersal and cross pollination in maize, there is a high probability that non-*Bt* ears of maize will be cross pollinated by *Bt* pollen under a ‘refuge in a bag’ scenario. More research is needed to fully measure the impact of *Bt* pollen on lepidopteran pests for sustainable IRM practices in *Bt* maize.

Finally, four review papers on *Bt* maize were identified in the search output (Carroll *et al.*, 2012; Carstens *et al.*, 2012; Nedelnik *et al.*, 2012; Romeis *et al.*, 2013). Carstens *et al.* (2012) use *Bt* maize as a case study to demonstrate how comprehensive problem formulation can be used to develop a conceptual environmental risk assessment (ERA) model and identify potential environmental exposure pathways. A paper by Romeis *et al.* (2013) details a proposal on how to derive criteria for the selection of arthropod species to test in the laboratory in the context of the ERA of genetically modified crops. The influence of growing *Bt* maize on *Fusarium* infection and mycotoxin content is reviewed by Nedelnik and collaborators (Nedelnik *et al.*, 2012). In conclusion, where insect larvae damage is a major factor in mycotoxin contamination, *Bt* maize can lower mycotoxin levels in many cases. The protection of maize plants against insect damage (European corn borer) through the use of *Bt* technology seems to be one of the ways to reduce the contamination of maize by *Fusarium* species and mycotoxins. Carroll *et al.* (2012)use modelling to determine that, across a range of conditions, seed mix refugia provide an effective alternative IRM tactic to stuctured refuges for delaying resistance evolution. Under some conditions, use of seed mix refugia may be a superior IRM tactic leading to longer delays to resistance, and greater durability, compared to structured refugia and is a risk adverse tactic in situations when no refuge is planted.

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

Meanwhile, EFSA published an updated guidance document on post-market environmental monitoring of GM crops as well two specific opinions on the monitoring conducted by Monsanto on MON 810 in the 2009 and 2010 growing seasons (EFSA, 2011[21]; 2011[22];

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

[20] SCP (1999), Opinion of the Scientific Committee on Plants on Bt resistance monitoring (Opinion expressed on March 04, 1999), *Document SCP/GMO/094-Rev.5* - http://ec.europa.eu/food/fs/sc/scp/out35_en.print.html (Accessed July 01, 2013)

[21] http://www.efsa.europa.eu/en/efsajournal/pub/2316.htm (Accessed July 01, 2013)

[22] http://www.efsa.europa.eu/en/efsajournal/pub/2376.htm (Accessed July 01, 2013)

2012[23]). One of the elements described in the original plan was to maintain it updated in view of the findings and new scientific information. Taking into account the opinions from EFSA on the matter, the large amount of data generated in the past growing seasons, data in the scientific literature, and the experience gained from IRM plans established in other regions, the EuropaBio Monitoring working group has updated the IRM plan in September 2012 to anticipate approvals for the cultivation in the EU of different *Bt* maize products (see Appendix 6). The purpose of the IRM plan is to proactively avoid where possible, and in all cases delay the potential development of pest resistance to the Cry protein 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.

3.2.1.1 Refuge

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

Many initiatives have been taken to educate the farmers on the importance of implementing IRM measures (see Section 3.2.1.3). For cultural reasons, certain farming communities are reluctant to accept 'signed agreements' requiring them to adhere to particular agricultural practices. Moreover, seeds are usually sold through distributors and farmer cooperatives, which adds another 'step' in the commercial chain. The absence of direct sales between end-users and seed companies makes signed agreements very difficult to manage. As a consequence, the seed industry has put particular emphasis on the development of communication tools.

In Spain, farmer satisfaction and monitoring of use conditions (including IRM communication and effective refuge implementation) was assessed at the end of the 2012 planting season, through a survey sponsored by ANTAMA (Spanish Foundation supporting the use of new technologies in agriculture[24]). The survey, as in previous years, was carried out in the Ebro Valley (Huesca, Lérida and Zaragoza and Navarra), which is where most of MON 810 is currently planted in Spain. The survey involved 110 farmers which had all planted MON 810 maize. They collectively planted 2755 hectares. The conclusions from the answers delivered by the 110 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

[23] http://www.efsa.europa.eu/en/efsajournal/pub/2610.htm (Accessed July 01, 2013)
[24] ANTAMA - http://fundacion-antama.org/ (Accessed July 01, 2013)

plant a refuge. To the question whether the farmer knew that a 20% refuge had to be planted if the collective *Bt* maize area exceeds 5 ha, 95% of the farmers responded positively.

The survey also revealed a high level of compliance with refuge requirements indicating that 94% of the farmers planted conventional maize as well as Bt maize in their farms and 99% of the decisions to have conventional maize were based on refuge compliance. In other words, 93% of the farmers planted 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, *(2)* the sowing is more complicated, or *(3)* corn borers cause significant losses. It has to be noted that, as found in previous seasons, small farms showed less compliance with refuge requirements than larger farms. The compliant farms planting both Bt maize and conventional maize have an average size of 35.8 ha of maize, whereas those planting only Bt maize have an average size of 12.2 ha. The survey indicated as well that 97% of the farmers are very satisfied or quite satisfied and only 3% little satisfied. Further, 94% of the interviewed farmers confirmed their willingness to plant in 2013 season at least the same Bt maize area as is 2012.

In Portugal, a Monitoring Report on the planting of MON 810 varieties (including IRM communication and refuge implementation) during the 2012 growing season was prepared by the Portuguese authorities[25]. In addition to the farmers trained in previous seasons, and in compliance with the Portuguese law, 79 new farmers were trained in 2012 on national and EU legislations that regulate the cultivation of GM varieties and to learn about the main characteristics of MON 810 maize. Furthermore, 125 inspections were performed of farmers planting MON 810 maize (out of the total 278 notifications received in 2012). 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, 58 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 the context of Monsanto's 2012 General Surveillance, 249 farmers across five 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.

90.4% of the farmers indicated that they followed the technical guidelines regarding the implementation of a refuge (81.5% planted a refuge and 8.8% had less than 5 ha planted with

[25] http://www.dgadr.pt/ (Accessed July 01, 2013)

MON 810 on their farm[26]). Most countries reported a very high level of compliance with refuge requirements. The farmers in the Czech Republic, Slovakia, Romania and Portugal were in full compliance with refuge requirements. Responses of the Monsanto 2012 Farmer Questionnaire Survey show that 86.3% of the farmers in Spain were compliant with refuge planting while 24 farmers out of 175 (*i.e.*, 13.7%) indicated they did not plant a refuge. The farmers gave two main reasons for not being compliant with the refuge requirements: (1) lack or not enough information about the technical guidelines (10/24, 41.7%) and (2) the refuge implementation complicates the sowing and other agronomic practices (10/24, 41.7%).

The compliance in Spain as reported through the Monsanto 2012 Farmer Questionnaire Survey (86.3%) was comparable but slightly lower than the one from the ANTAMA survey (93%). The data from the Monsanto 2012 Farmer Questionnaires came from different areas across Spain, including some areas with a less experience in the growing of Bt maize when compared to the Ebro Valley area. Hence, these differences in experience and awareness might explain the higher compliance in ANTAMA survey which was focused on EbroValley. In order to improve the compliance in those areas, the companies selling Bt maize seeds in Spain have jointly decided to increase the communication and education activities during the 2013 season on Bt growing areas out of Ebro Valley (see 3.2.1.3 for activities in 2012).

In conclusion, the results from the presented surveys (ANTAMA, Portuguese authorities and Monsanto) during the 2012 season are consistent and do show a rather 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 2013 growing 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 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.

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

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* species 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 after the commercialisation of varieties including Bt176 maize from Syngenta, that also expresses a 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.

For the 2012 growing season, Monsanto revised its IRM plan in view of the opinions from EFSA on the matter, 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 world areas. The elements that changed for the 2012 growing season compared to previous seasons are all reflected in the updated IRM plan from the EuropaBio Monitoring working group of September 2012 (Appendix 6). A significant change in the sampling approach was introduced in order to implement EFSA's advice; 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 2012 was expected to be greater than 20% are the Ebro valley (defined in earlier reports as Iberia Northeast), 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 2012 were concentrated in Spain and Portugal, more in particular in Southwest Iberia (Southwest of Spain and south Portugal) for *Sesamia* and *Ostrinia*, and Central Iberia for *Sesamia*. Iberia Central was not sampled for *O. nubilalis* and Iberia Northeast was neither sampled for *S. nonagrioides* nor *O. nubilalis* since those collections and analyses were conducted during the 2011 growing season, and reported in previous year's monitoring report (Monsanto Europe S.A., 2012).

1. Sesamia nonagrioides

In 2012, susceptibility to the Cry1Ab toxin of *S. nonagrioides* has been assessed from collections in Southwest Iberia (Southwest of Spain and south Portugal) and Central Iberia (see Appendix 7). As it was established by the results of the 2009 season, only values of molting inhibition concentration (MIC) have been used to assess the susceptibility of this species to Cry1Ab.

The results of MIC_{50} (29 ng Cry1Ab/cm² for Southwest Iberia and 15 ng Cry1Ab/cm² for Central Iberia) and MIC_{90} (158 ng Cry1Ab/cm² for Southwest Iberia and 160 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 29 ng Cry1Ab/cm² (Southwest Iberia in the present season). These results evidenced a magnitude variation of 4.1-fold. Likewise, values of MIC_{50} of laboratory strains were also very uniform, ranging between 7 and 19 ng Cry1Ab/cm², which means a magnitude variation of 2.7-fold. In the light of these results, MIC_{50} values obtained during this campaign for the field collected populations and for the laboratory strain are within the range of values obtained in the past years. 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 would be the use of the diagnostic dose (DD), which facilitates the monitoring execution (Halliday and Burnham, 1990; Roush and Miller, 1986).

In order to conclude on the dose that will be used in the following growing seasons, the MIC_{99} was determined for field populations of *S. nonagrioides* from the past growing seasons. This will help to diminish the variation expected. Thus, the diagnostic dose (DD) was defined to cause 99% of moulting inhibition to first instar larvae (MIC_{99}) and determined for the field populations of *S. nonagrioides* collected in Iberia from 2008 to 2012. The DD was determined to be 726 ng Cry1Ab/cm². This value represents the response of 6,646 neonates.

2. Ostrinia nubilalis

In 2012, 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 mortality and larval moult inhibition data at the different concentrations of Cry1Ab tested were analyzed. Moulting inhibition concentrations at 50% (MIC_{50}) and 90% (MIC_{90}) for *O. nubilalis* collected in Southwest Iberia were 4.08 and 8.69 ng Cry1Ab/cm², respectively. Variation in Cry1Ab susceptibility (MIC_{50} and MIC_{90}) of *O. nubilalis* collected in the field during the 2012 growing season was 1.4-fold in both cases. 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 2005–2012 could not be detected.

Like for *S. nonagrioides*, another approach to test the dose-mortality for monitoring the susceptibility of *O. nubilalis* to Cry1Ab would be the use of the diagnostic dose (DD). For the calculation of the DD the data for all experiments using *O. nubilalis* collected from 2005-2012 in fields from Czech Republic, France, Germany, Italy, Panonia, Poland, Portugal, Romania and Spain were used and represented the responses of 11,502 larvae. Using the average of the moulting inhibition concentrations (MIC) for 99% (MIC_{99}) the DD for *O. nubilalis* larvae from Europe is 28.22 ng/cm^2 using the most recent batch of Cry1Ab protein.

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.

These results are aligned with the conclusions of independent studies conducted in Spain and summarized in the review published by the Spanish Ministry of Environment, Rural and Marine Affairs (MARM). It is concluded that monitoring results from 10 years of *Bt* maize cultivation in Spain (1999-2009) and including MON 810 since 2003, indicate no evidence of altered susceptibility of target pests to the Cry1Ab protein[27].

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 2012 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.). Also, in addition to the TUG (Appendix 3.5),

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[27] http://www.marm.es/es/calidad-y-evaluacion-ambiental/temas/biotecnologia/organismos-modificados-geneticamente-omg-/notificaciones-y-autorizaciones/comercializacion.aspx (Accessed July 01, 2013)

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. Thus, in 2012, 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 outlets and farmers: trained ANOVE inspectors completed 85 visits in MON 810 growing areas mainly in the Ebro Valley 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 in the Ebro Valley 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 96.4% 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 the TUG attached to the seed bags and other different materials (see Appendix 9.6) and went through 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 2012, the percentage of farmers implementing refuges in their fields was very high.

The results of the analysis of 2012 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 2012 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 2012. 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.

All together, these results demonstrate that there are no adverse effects attributed to the cultivation of MON 810 in Europe. The result of the 2012 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 ten 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: [redacted]

Date: 31/07/2012

REFERENCES

Alcantara EP, 2012. Postcommercialization monitoring of the long-term impact of *Bt* corn on non-target arthropod communities in commercial farms and adjacent riparian areas in the Philippines. Environmental Entomology, 41, 1268-1276.

Atsumi S, Miyamoto K, Yamamoto K, Narukawa J, Kawai S, Sezutsu H, Kobayashi I, Uchino K, Tamura T, Mita K, Kadono-Okuda K, Wada S, Kanda K, Goldsmith MR and Noda H, 2012. Single amino acid mutation in an ATP-binding cassette transporter gene causes resistance to *Bt* toxin Cry1Ab in the silkworm, *Bombyx mori*. Proceedings of the National Academy of Sciences of the United States of America, 109, E1591-E1598.

Barriuso J, Valverde JR and Mellado RP, 2012. Effect of Cry1Ab protein on rhizobacterial communities of *Bt*-maize over a four-year cultivation period. PLoS ONE, 7,

Bowers E, Hellmich R and Munkvold G, 2013. Vip3Aa and Cry1Ab proteins in maize reduce *Fusarium ear rot* and *fumonisins* by deterring kernel injury from multiple *Lepidopteran* pests. World Mycotoxin Journal, 6, 127-135.

Burkness EC and Hutchison WD, 2012. *Bt* pollen dispersal and *Bt* kernel mosaics: integrity of non-*Bt* refugia for *Lepidopteran* resistance management in maize. Journal of Economic Entomology, 105, 1773-1780.

Buzoianu SG, Walsh MC, Rea MC, Cassidy JP, Ross RP, Gardiner GE and Lawlor PG, 2012a. Effect of feeding genetically modified *Bt* MON810 maize to similar to 40-day-old pigs for 110 days on growth and health indicators. Animal, 6, 1609-1619.

Buzoianu SG, Walsh MC, Rea MC, O'Donovan O, Gelencser E, Ujhelyi G, Szabo E, Nagy A, Ross RP, Gardiner GE and Lawlor PG, 2012b. Effects of feeding *Bt* maize to sows during gestation and lactation on maternal and offspring immunity and fate of transgenic material. PLoS ONE, 7, e47851-e47851.

Buzoianu SG, Walsh MC, Rea MC, O'Sullivan O, Cotter PD, Ross RP, Gardiner GE and Lawlor PG, 2012c. High-throughput sequence-based analysis of the intestinal microbiota of weanling pigs fed genetically modified MON810 maize expressing *Bacillus thuringiensis* Cry1Ab (*Bt* maize) for 31 days. Applied and Environmental Microbiology, 78, 4217-4224.

Buzoianu SG, Walsh MC, Rea MC, O'Sullivan O, Crispie F, Cotter PD, Ross RP, Gardiner GE and Lawlor PG, 2012d. The effect of feeding Bt MON 810 maize to pigs for 110 days on intestinal microbiota. PLos, 7, 1-9.

Carroll MW, Head G and Caprio M, 2012. When and where a seed mix refuge makes sense for managing insect resistance to *Bt* plants. Crop Protection, 38, 74-79.

Carstens K, Anderson J, Bachman P, De Schrijver A, Dively G, Federici B, Hamer M, Gielkens M, Jensen P, Lamp W, Rauschen S, Ridley G, Romeis J and Waggoner A, 2012. Genetically modified crops and aquatic ecosystems: considerations for environmental risk assessment and non-target organism testing. Transgenic Research, 21, 813-842.

Cotta SR, Franco Dias AC, Marriel IE, Gomes EA, van Elsas JD and Seldin L, 2013. Temporal dynamics of microbial communities in the rhizosphere of two genetically modified (GM) maize hybrids in tropical agrosystems. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology, 103, 589-601.

Dutra CC, Koch RL, Burkness EC, Meissle M, Romeis J, Hutchison WD and Fernandes MG, 2012. *Harmonia axyridis* (Coleoptera: Coccinellidae) exhibits preference between *Bt* and non-*Bt* maize fed *Spodoptera frugiperda* (Lepidoptera: Noctuidae). PLoS ONE, 7,

Farinós GP, de la Poza M, Hernandez-Crespo P, Ortego F and Castanera P, 2004. Resistance monitoring of field populations of the corn borers *Sesamia nonagrioides* and *Ostrinia nubilalis* after 5 years of Bt maize cultivation in Spain. Entomologia Experimentalis et Applicata, 110, 23-30.

Fernandes TJR, Oliveira M and Mafra I, 2013. Tracing transgenic maize as affected by breadmaking process and raw material for the production of a traditional maize bread, broa. Food Chemistry, 138, 687-692.

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

Grabowski M and Dabrowski ZT, 2012. Evaluation of the impact of the toxic protein Cry1Ab expressed by the genetically modified cultivar MON810 on honey bee (Apis mellifera L.) behavior. Medycyna Weterynaryjna, 68, 630-633.

Gryspeirt A and Gregoire J-C, 2012. Effects of two varieties of *Bacillus thuringiensis* maize on the biology of *Plodia interpunctella*. Toxins, 4, 373-389.

Gu J, Krogdahl A, Sissener NH, Kortner TM, Gelencser E, Hemre G-I and Bakke AM, 2013. Effects of oral *Bt*-maize (MON810) exposure on growth and health parameters in normal and sensitised Atlantic salmon, *Salmo salar* L. British Journal of Nutrition, 109, 1408-1423.

Guertler P, Brandl C, Meyer HHD and Tichopad A, 2012. Feeding genetically modified maize (MON810) to dairy cows: comparison of gene expression pattern of markers for apoptosis, inflammation and cell cycle. Journal Fur Verbraucherschutz Und Lebensmittelsicherheit-Journal of Consumer Protection and Food Safety, 7, 195-202.

Halliday WR and Burnham KP, 1990. Choosing the optimal diagnostic dose for monitoring insecticide resistance. Journal of Economic Entomology, 83, 1151-1159.

Hansen LS, Lovei GL and Szekacs A, 2013. Survival and development of a stored-product pest, *Sitophilus zeamais* (Coleoptera: Curculionidae), and its natural enemy, the parasitoid *Lariophagus distinguendus* (Hymenoptera: Pteromalidae), on transgenic *Bt* maize. Pest Management Science, 69, 602-606.

Holst N, Lang A, Lovei G and Otto M, 2013. Increased mortality is predicted of Inachis io larvae caused by *Bt*-maize pollen in European farmland. Ecological Modelling, 250, 126-133.

Kim YH, Hwang CE, Kim T-S and Lee SH, 2012. Risk assessment system establishment for evaluating the potential impacts of imported *Bacillus thuringiensis* maize on a non-target insect, *Tenebrio molitor*. Journal of Asia-Pacific Entomology, 15, 225-229.

Kruger M, Van Rensburg JBJ and Van den Berg J, 2012. Reproductive biology of *Bt*-resistant and susceptible field-collected larvae of the maize stem borer, *Busseola fusca* (Lepidoptera: Noctuidae). African Entomology, 20, 35-43.

Londono LM, Tarkalson D and Thies JE, 2013. In-field rates of decomposition and microbial communities colonizing residues vary by depth of residue placement and plant part, but not by crop genotype for residues from two Cry1Ab *Bt* corn hybrids and their non-transgenic isolines. Soil Biology & Biochemistry, 57, 349-355.

Lupwayi NZ and Blackshaw RE, 2013. Soil microbial properties in *Bt* (Bacillus thuringiensis) corn cropping systems. Applied Soil Ecology, 63, 127-133.

Meissle M, Knecht S, Waldburger M and Romeis J, 2012. Sensitivity of the cereal leaf beetle *Oulema melanopus* (Coleoptera: *Chrysomelidae*) to *Bt* maize-expressed Cry3Bb1 and Cry1Ab. Arthropod-Plant Interactions, 6, 203-211.

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

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

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

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

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

Monsanto Europe S.A., 2009. Monitoring report - MON 810 cultivation - Czech Republic, Germany, Poland, Portugal, Romania, Slovakia and Spain - 2008. Monsanto report,

Monsanto Europe S.A., 2010. Monitoring report - MON 810 cultivation - Czech Republic, Portugal, Slovakia, Poland, Romania and Spain - 2009. Monsanto report, Monsanto Europe S.A., 2011. Monitoring report - MON 810 cultivation - Czech Republic, Poland, Portugal, Romania, Slovakia, and Spain - 2010. Monsanto report,

Monsanto Europe S.A., 2012. Monitoring report - MON 810 cultivation - Czech Republic, Poland, Portugal, Romania, Slovakia, and Spain - 2011. Monsanto report,

Nedelnik J, Linduskova H and Kmoch M, 2012. Influence of growing *Bt* maize on *Fusarium* infection and mycotoxins content - a review. Plant Protection Science, 48, S18-S24.

Perez-Hedo M, Lopez C, Albajes R and Eizaguirre M, 2012. Low susceptibility of non-target *Lepidopteran* maize pests to the *Bt* protein Cry1Ab. Bulletin of Entomological Research, 102, 737-743.

Perry JN, Devos Y, Arpaia S, Bartsch D, Gathmann A, Hails RS, Kiss J, Lheureux K, Manachini B, Mestdagh S, Neemann G, Ortego F, Schiemann J and Sweet JB, 2010. A mathematical model of exposure of non-target Lepidoptera to *Bt*-maize pollen expressing Cry1Ab within Europe. Proceedings of the Royal Society B-Biological Sciences, 277, 1417-1425.

Reichert M, Kozaczynski W, Karpinska TA, Bocian L, Jasik A, Kycko A, Swiatkiewicz M, Swiatkiewicz S, Furgal-Dierzuk I, Arczewska-Wlosek A, Strzetelski J and Kwiatek K, 2012. Histopathology of internal organs of farm animals fed genetically modified corn and soybean meal. Bulletin of the Veterinary Institute in Pulawy, 56, 617-622.

Rios-Diez JD, Siegfried B and Saldamando-Benjumea CI, 2012. Susceptibility of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) strains from entral colombia to Cry1Ab and Cry1Ac entotoxins of *Bacillus thuringiensis*. Southwestern Entomologist, 37, 281-293.

Romeis J, Raybould A, Bigler F, Candolfi MP, Hellmich RL, Huesing JE and Shelton AM, 2013. Deriving criteria to select arthropod species for laboratory tests to assess the ecological risks from cultivating arthropod-resistant genetically engineered crops. Chemosphere, 90, 901-909.

Roush RT and Miller GL, 1986. Considerations for design of insecticide resistance monitoring programs. Journal of Economic Entomology, 79, 293-298.

Sander M, Tomaszewski JE, Madliger M and Schwarzenbach RP, 2012. Adsorption of insecticidal Cry1Ab protein to humic substances. 1. Experimental approach and mechanistic aspects. Environmental Science & Technology, 46, 9923-9931.

Sartowska K, Korwin-Kossakowska A, Sender G, Jozwik A and Prokopiuk M, 2012. The impact of genetically modified plants in the diet of Japanese quails on performance traits and the nutritional value of meat and eggs - preliminary results. Archiv Fur Geflugelkunde, 76, 140-144.

Schuppener M, Muhlhause J, Muller A and Rauschen S, 2012. Environmental risk assessment for the small tortoiseshell Aglais urticae and a stacked Bt-maize with combined resistances against Lepidoptera and Chrysomelidae in central European agrarian landscapes. Molecular Ecology, 4646-4662.

Tabashnik BE, Brevault T and Carriere Y, 2013. Insect resistance to *Bt* crops: lessons from the first billion acres. Nature Biotechnology, 31, 510-521.

van der Merwe F, Bezuidenhout C, van den Berg J and Maboeta M, 2012. Effects of Cry1Ab transgenic maize on lifecycle and biomarker responses of the earthworm, *Eisenia Andrei*. Sensors, 12, 17155-17167.

Verbruggen E, Kuramae EE, Hillekens R, de Hollander M, Kiers ET, Roling WFM, Kowalchuk GA and van der Heijden MGA, 2012. Testing potential effects of maize expressing the *Bacillus thuringiensis* Cry1Ab endotoxin (*Bt* Maize) on mycorrhizal fungal communities via DNA- and RNA-based pyrosequencing and molecular fingerprinting. Applied and Environmental Microbiology, 78, 7384-7392.

Walsh MC, Buzoianu SG, Gardiner GE, Rea MC, O'Donovan O, Ross RP and Lawlor PG, 2013. Effects of feeding *Bt* MON810 maize to sows during first gestation and lactation on maternal and offspring health indicators. British Journal of Nutrition, 109, 873-881.

Walsh MC, Buzoianu SG, Rea MC, O'Donovan O, Gelencser E, Ujhelyi G, Ross RP, Gardiner GE and Lawlor PG, 2012. Effects of feeding *Bt* MON810 maize to pigs for 110 days on peripheral immune response and digestive fate of the cry1Ab gene and truncated *Bt* toxin. PLoS ONE, 7, Wang C, Henderson G, Huang F, Gautam BK and Zhu CQ, 2012. Survival rate, food consumption, and tunneling of the formosan subterranean termite (Isoptera: Rhinotermitidae) feeding on *Bt* and non-*Bt* maize. Sociobiology, 59, 1335-1350.

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

Appendix 1. Post Market Monitoring of insect protected *Bt* maize MON 810 in Europe – Conclusions of a survey with Farmer Questionnaires in 2012

Appendix 2. MON 810 Farmer Questionnaire: 2012

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 Czech Republic

Appendix 4.2. User manual annexes Portugal

Appendix 4.3. User manual annexes Romania

Appendix 4.4. User manual annexes Slovakia

Appendix 4.5. User manual annexes Spain

Appendix 5. MON 810 Literature Review (June 2012 - May 2013)

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*: 2012 Season

Appendix 8. Insect Resistance Monitoring in Iberian collections of *Ostrinia nubilalis* (ECB): 2012 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