Annual Monitoring Report on the Cultivation of MON 810 in 2008

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

Submitted by

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

EXECUTIVE SUMMARY

In 2008, *Bt* maize was planted in the EU on 107,719 hectares across seven countries (James, 2008). 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 cultivation of MON 810 maize in Europe is detailed in the Annual Monitoring Report on the Cultivation of MON 810 in 2008.

The planting of MON 810 in the 2008 season was accompanied by a rigorous IRM plan involving three main elements: refuge implementation, monitoring and farmer education. A number of initiatives were taken to educate farmers about the importance of the implementation of IRM measures and the success of this program was reflected in results of surveys indicating high levels of compliance with requirements for refuge implementation observed in the 2008 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 2008.

In 2008, 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 297 questionnaires from a survey of farmers cultivating MON 810 in seven European countries in 2008, and the analysis of several German networks, did not reveal any unexpected adverse effects that could be associated with the genetic modification in MON 810. Furthermore, a detailed analysis of more than 50 publications related to MON 810 and/or Cry1Ab did not reveal any new scientific evidence that would invalidate the findings of the risk assessment which conclude 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. These conclusions were supported by the release of three opinions of the European Food Safety Authority (EFSA) on the comparative safety of MON 810 (in relation to its conventional counterpart). Finally, company stewardship activities and issue alerts did not reveal any adverse effects related to MON 810 cultivation in 2008.

Taken together, these results demonstrate that there were no adverse effects attributed to the cultivation of MON 810 in Europe in 2008.

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1. GENERAL INFORMATION

1.1	**Crop/event**	Maize/MON 810
1.2	**Member state consent number under Directive 2001/18/EC**	N/A
1.3	**Decision authorisation number under Regulation (EC) No 1829/2003**	N/A
1.4	**Unique identifier**	MON-ØØ81Ø-6
1.5	**Reporting Period**	July 08-July 09
1.6	**Other monitoring reports have been submitted in respect of:**	
	• **Import and Processing**	Yes (December 08)
	• **Food/Feed**	Yes (December 08)

2. INTRODUCTION AND BACKGROUND

Using modern biotechnology, Monsanto Company has developed insect-protected YieldGard® Corn Borer maize MON 810 (hereafter referred to as MON 810 maize) that produces the naturally occurring *Bacillus thuringiensis (Bt)* protein, Cry1Ab. MON 810 maize 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 favourable opinion. The other EU Member States raised objections. The European Commission sought the opinion of the Scientific Committee on Plants (SCP) which 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] (Commission Decision, 1998). France, as *rapporteur*, ratified the Commission Decision on 3 August 1998. According to this Decision, Monsanto is required to inform the European Commission and the competent authorities of the European Union Member States about the results of monitoring for insect resistance.

On 4 May 2007, Monsanto submitted an application for renewal of authorisation of MON 810 maize products to the European Commission in accordance with Article 20(1)(a)[3] of Regulation (EC) No 1829/2003 on genetically modified food and feed. In support of this

® 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 30 July, 2009)

[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.

[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.

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 EFSA overall opinion, confirming the conclusions of the original safety assessment, was adopted on 15 June 2009 (and published 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 2008, *Bt* maize was planted in the EU on 107 719 hectares across seven countries: Czech Republic (8 380 ha), Germany (3 173 ha), Poland (3 000 ha), Portugal (4 851 ha), Romania (7 146 ha), Slovakia (1 900 ha) and Spain (79 269 ha) (James, 2008).

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 strategies for insect-protected maize.

Following this example, Monsanto along with three other companies[5] have established the European Union Working Group on Insect Resistance Management (EUWGIRM). This group, formed in 2001, has developed a harmonised Insect Resistance Management (IRM) plan specific for the EU (Appendix 1), that enables 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 (Appendix 1) is based on published research, current EU legislation, the European Commission's Scientific Committee on Plants (SCP) opinion on IRM (SCP, 1999) 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. The harmonised IRM plan contains guidance on the following key elements:

- refuge;
- baseline studies and monitoring of the target pests;
- communication and education.

This report describes the components and results of the IRM plan for the 2008 season.

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

[4] 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 theuse 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 30 June, 2009)

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

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 (Commission Decision, 1998)).

The objective of general surveillance is to identify the occurrence of unanticipated adverse effects of the genetically modified plants on human health or the environment that were not anticipated in the 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].

Taking the above factors into consideration, the general surveillance monitoring program implemented by Monsanto for MON 810 consists of four elements:

- a Farmer Questionnaire designed to assess any unusual observations in the areas where the product is cultivated;
- data collected from scientific publications or reports relating to the cultivated product and its environmental safety;
- 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.

Results of Insect Resistance Management are provided to the European Commission on an annual basis (*i.e.* this report) along with the results of the general surveillance monitoring described above. 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.

[6] Opinion of the Scientific Panel on Genetically Modified Organisms on the Post Market Environmental Monitoring (PMEM) of genetically modified plants, The EFSA Journal (2006) 319, 1-27 – http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620769727.htm (Accessed 30 July, 2009)

3. IMPLEMENTATION OF THE INSECT RESISTANCE MANAGEMENT PLAN

The success of the IRM plan is ensured by the implementation of three key aspects. These are (1) refuge, (2) baseline studies and monitoring of the target pests, and (3) communication and education. These different aspects are reviewed in the following sections:

3.1 REFUGE

According to the *Harmonised insect resistance management (IRM) plan for cultivation of Bt maize in the EU* (Appendix 1), 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 explain to farmers the importance of implementing IRM measures (*see* Section 3.2). 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 2008 planting season, through a survey sponsored by ANTAMA (Spanish Foundation supporting the use of new technologies in agriculture). The survey, as in previous years, was carried out in the Ebro Valley (Huesca, Lérida and Zaragoza), which is where most of the *Bt* maize is currently planted in Spain. The survey involved 200 farmers and half of them (100) had planted more than 5 hectares of maize[7]. The 100 farmers planting *Bt* maize collectively planted 3 077 hectares. The conclusions from the answers delivered by the 100 farmers growing *Bt* maize are detailed below.

Farmer responses demonstrated the effectiveness of communication regarding IRM requirements. 93% (93/100) of the farmers planting *Bt* knew about the recommendation to plant a refuge. In this group, 72% considered themselves to be "well informed", 17% "somehow informed", "little informed" and 3% "not informed". The farmers responses regarding the clarity of the recommendations about the implementation of refuges were as follows: 87% considered the recommendations "very clear/quite clear", while only 10% considered them "little clear/unclear". 51% of the interviewees considered that it is "very easy/quite easy" to follow the recommendations while 46% considered that it is "little easy/not easy at all". 3% of the farmers didn't know or wouldn't answer these questions.

The survey also revealed a high level of compliance with refuge requirements indicating that 82% of the 100 farmers included in the final survey planted a conventional maize refuge on their farm. The remaining farmers surveyed (*i.e.* 18%) did not plant a refuge. Reasons given

[7] The IRM states that no refuge is required if there is less than 5 hectares planted on the farm.

by the farmers for not planting a refuge were: (1) corn borers (*Ostrinia nubilalis*) cause significant economic losses, (2) the sowing is easier (with *Bt* maize), (3) they want to try *Bt* maize on the whole surface they have for this crop, or (4) they consider their farms as small farms (*i.e.* less than 5 hectares and therefore no refuge required).

In addition, this survey analysed the satisfaction of the growers. The survey indicated that 95% (95/100) of the farmers are very or quite satisfied, 4% a little satisfied, and 1% not satisfied at all. The main advantage/benefit, reported by 91% of the farmers, was the effective protection against corn borers, followed by the plant health (plants / ear of maize do not collapse (34%) and healthier plants (38%)), peace of mind (42%) and good yield (41%).

Apart from the ANTAMA survey in Spain, in the context of Monsanto's 2008 general surveillance, 297 farmers across seven countries where MON 810 was commercially cultivated were surveyed for their implementation of a refuge (*see* Appendix 7). 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. 90.5% of the farmers who answered the question indicated that they followed the technical guidelines regarding the implementation of a refuge (85.1% planted a refuge and 5.4% had less than 5 ha planted with MON 810 on their farm[8]). Most countries reported a very high level of compliance with refuge requirements. The results of this survey are discussed in further detail in Section 4.2.1.

The results of the Monsanto 2008 Farmer Questionnaire Survey showed that in Spain, where 74% of the total EU MON 810 acreage was planted, among the farmers who were required to plant a refuge (*i.e.* farm growing more than 5 ha of maize), 85.4% of the farmers participating in the survey declared that they implemented the refuge. This shows an improvement in the number of compliant farmers since the 2007 survey (77% of farmers participating in the 2007 survey in Spain were compliant with refuge requirements), which reflects the communication efforts undertaken prior to the 2008 growing season by the Asociación Nacional de Obtentores Vegetales (ANOVE or National Breeding Association) by organising additional information sessions on the importance of the planting a refuge for all Monsanto licensees (Section 3.2). Several of the farmers that did not comply mentioned that they considered the neighbouring fields where conventional maize was planted to be an appropriate refuge.

Responses of the Monsanto 2008 Farmer Questionnaire Survey show that while 76.3% of the farmers in Portugal planted a refuge, some farmers (*i.e.*, 9 of 38) indicated they did not plant a refuge. In Portugal, the farmers that reported they did not plant a refuge, indicated that they were part of a production area. The organisation in production areas allows for collective compliance with refuge requirements. Compliance with refuge requirements was audited by Portuguese officials in an inspection of 50% of farmers growing MON 810 in 2008. The

[8] The IRM states that no refuge is required if there is less than 5 hectares planted on the farm.

official report states that "no case of lack of compliance was found and in many cases it has been verified that refuge areas were bigger than requested"[9].

The message on the importance of refuge implementation will be repeated in all countries growing MON 810 in the 2009 growing season. It is important to continue educating the farmers on the necessity to implement refuges. In addition, the strict monitoring of the farmers in countries where the technology has been introduced more recently will be maintained.

3.2 COMMUNICATION AND EDUCATION

An extensive grower education program is essential for the successful implementation of the IRM plan.

Each purchaser of *Bt* maize receives a technical user guide that contains the latest information on the growers' IRM obligations. The user guide requires farmers to implement IRM measures, including refuge planting. Examples of these documents that were distributed in the 2008 season can be found in Appendix 2. 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 the 2008 planting season in Spain, a number of initiatives were taken leading up to and throughout the growing season 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, hybrid guides on packaging). Some examples include the good agricultural practices (GAP) leaflet (Appendix 3.1) and Guía Técnica YieldGard® (YieldGard Technical Guide) (Appendix 2.7) that are attached to each MON 810 bag sold in Spain.
2) Interviews with farmers complying with refuge requirements published in prominent agricultural magazines, *Vida Rural, AECV.SV* (Conservation Tillage Association) and *ASAJA Huesca* (Farmer Union) magazines.
3) Presentation by sales and marketing teams of IRM requirements in farmer meetings / farmer talks to reinforce the need for refuge compliance.
4) An IRM exhibit at a major national agricultural fair.
5) Advertisement about refuge compliance published in a key agricultural magazine (Appendix 3.2).
6) Sending a letter (on behalf of ANOVE: the National Breeder Association in Spain) from each company to their farmer's database in *Bt* maize areas reinforcing the key messages of refuge implementation (Appendix 3.3).

[9] DGADR. 2008. Coexistencia entre Culturas Genticamente Modificados e outros Modos de Produçao Agricola. Relatorio de Acompatiamiento de 2008. Pg 17 – http://www.dgadr.pt/wwwbase/wwwinclude/ficheiro.aspx?tipo=o&id=12029 (Accessed 1 July 2009)

7) Train the trainers: an IRM session was organised and a presentation on IRM was jointly created and followed by all companies operating in the market to ensure common messages (Appendix 3.4).
8) Posters and stickers distributed among seed distributors and point of sales to be used with invoices and letters (Appendices 3.5 and 3.6).
9) Communication plan for cooperatives, small points of sales outlets and farmers: trained ANOVE inspectors completed 48 visits to inform them, distribute material and ensure that farmers are well informed on refuge implementation when buying *Bt* maize seeds.

The ANTAMA survey conducted in Spain, and referred to in Section 3.1, demonstrates the effectiveness of the education program to raise awareness of refuge implementation. 93% of the farmers surveyed acknowledged they were made aware of the fact that they are required to plant a refuge. Efforts will be continued in the 2009 season to ensure ongoing awareness of IRM.

4. MONITORING RESULTS

4.1 CASE SPECIFIC MONITORING

4.1.1 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).

Upon request of Monsanto, additional baseline studies have been conducted within Europe during 2005-2006. In 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 the Insect-Plant Interaction lab, led by Dr. [redacted] and Dr. [redacted] (Department of Plant Biology, CIB-CSIS). *S. nonagrioides* was collected from the Midi-Pyrénées (France) and Bajo-Alentejo (Portugal) areas while *O. nubilalis* was sampled from the Bajo-Alentego area (Ortego, 2006b; Ortego, 2006c). These results have been reported previously in the 2005 monitoring report (Monsanto Europe S.A., 2006). In 2006, the same laboratory established the baseline susceptibility to Cry1Ab within the French field population of *S. nonagrioides* collected from Poitou-Charentes (France) area, reported in the 2006 Monitoring Report (Monsanto Europe S.A., 2006; Monsanto Europe S.A., 2007).

Overall, the susceptibility to Cry1Ab of the species studied in 2005-2006 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. No resistance to Cry1Ab has been observed in any of the analysed populations.

In addition to the baseline results described above on *S. nonagrioides* in France, Portugal and Spain, and on *O. nubilalis* in Portugal and Spain, BTL Bio-Test Labor GmbH (Sagerheide, Germany), led by Dr. [redacted], established the baseline susceptibility to Cry1Ab from 2005 – 2008 in major European maize growing regions. This baseline study analysed the susceptibility of one laboratory colony and 47 populations of *O. nubilalis* collected in maize fields in growing regions of Moravia/Czech Republic, Southwest and West France, Northern Germany/Southwest Poland, Southern Germany and East France, Italy, the Panonian region (Western Slovakia and North West Hungary), East Poland, Portugal, Romania and Northeast and Southwest Spain (Appendix 4; (Thieme, 2009)).

Differences between the most susceptible and most tolerant field-collected subpopulations were 9.1-fold. No concentration response was observed for certain *O. nubilalias* collections from France (Miradoux dans le Gers, Lezat Sur Leze en Ariege and Pamproux) apparently because of bacterial infections in the collections from the Miradoux dans le Gers and Pamproux and for unknown reasons for the collection from Lezat Sur Leze en Ariege. Subsequent collections from the same regions exhibited normal levels of susceptibility to Cry1Ab.

Results for 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 (Appendix 1).

Although variation in susceptibility to Cry1Ab was found among populations and among regions, the magnitude of the variation was small (*i.e.* 3.9-fold, 8.3-fold, 2.6-fold and 7.0-fold for *O. nubilalis* collected in 2005, 2006, 2007 and 2008, respectively). The results of the populations pooled according to geographic and climatic conditions were similar and differed 2.4-fold, 2.2-fold, 1.6-fold and 2.8-fold for *O. nubilalis* collected in 2005, 2006, 2007 and 2008, 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).

These results indicate that the observed population variation in susceptibility reflects natural variation in *Bt* susceptibility among *O. nubilalis* populations. Therefore, European populations of *O. nubilalis* are uniformly susceptible to Cry1Ab without any obvious genetic differentiation linked to geographic or other factors. In the future, other regional sources may be added to ensure that the monitoring program continues to represent the Cry1Ab maize market in Europe.

4.1.2 Monitoring for insect resistance

As mentioned previously, 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 *Bt* 176 from Syngenta, that also expresses a Cry1Ab protein (Farinós *et al.*, 2004).

During 2004-2007, the laboratory of Dr. performed monitoring for *O. nubilalis* and *S. nonagroides* resistance to Cry1Ab exp MON 810 (Ortego, 2005; Ortego, 2006a; Ortego, 2006b; Ortego, 2006c). Different geographical areas where the commercial growing of MON 810 varieties is considerable were selected. According to the protocol, each target population is monitored every two years; this is assumed to be an acceptable interval for the early detection of resistance in a field population, if it were to occur. In 2007, the monitoring was carried out in Spain (Ebro Valley, Albacete, and the Extremadura-Andalusia regions) and France (Midi-Pyrénées) (Hernández-Crespo, 2008a; Hernández-Crespo, 2008b).

In 2008, this monitoring was continued and samples were collected from the MON 810 growing areas in Central Iberia, Southwest Iberia and Northeast Iberia (Appendix 5; (Hernández-Crespo, 2009). The susceptibility of French populations from Poitout-Charentes and Midi-Pyrénées could not be evaluated due to low levels of the pest found in these regions in 2008. The monitoring studies performed with *O. nubilalis* and *S. nonagrioides* collected during the 2008 season did not reveal any resistance to Cry1Ab among the regions. The susceptibility to Cry1Ab of *S. nonagrioides* from Central Iberia is comparable with the

susceptibility to the toxin showed by the laboratory strain, and within the range obtained in populations of this species collected from the same geographical area during the period 2004-2007. Results obtained from the study show that the susceptibility to Cry1Ab were consistent with the susceptibility of populations evaluated between 2004 and 2007.

4.2 GENERAL SURVEILLANCE

In 2008, Monsanto continued the general surveillance monitoring program initiated in 2005 on a voluntary basis, in anticipation of a 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 of the MON 810 consent (Commission Decision, 1998)).

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. It is largely based on routine observation and involves the collection, scientific evaluation and reporting of reliable scientific evidence, in order to be able to identify whether unanticipated, direct or indirect, immediate or delayed adverse effects might have been caused by the placing on the market of a genetically modified crop in its receiving agricultural or non-agricultural environment.

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 2008 consisted of five 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 the cultivated product and its comparative safety (to conventional counterparts) with respect to human, and animal health and environmental;
- 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;
- an analysis of publicly available resources of networks in Germany to assess whether they report or indicate any potential adverse effects that have occurred as a result of MON 810 cultivation.

4.2.1 Farmer questionnaires

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 given 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[10]. The questionnaire approach has also proven its applicability with other industries, *e.g.* the pharmaceutical industry.

A farmer questionnaire has been developed as the 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 2006, however in the 2008 season an additional question was added on corn borer control practices to assess to what extent change in insecticide regime is linked to a change in the control method for corn borers (Appendix 6). 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 2008 (Czech Republic, Germany, 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 2008, 51 farmers in the Czech Republic, 44 farmers in Germany, 40 farmers in Portugal, 9 farmers in Slovakia, 10 farmers in Poland, 43 farmers in Romania and 100 farmers in Spain

[10] Opinion of the Scientific Panel on Genetically Modified Organisms on the Post Market Environmental Monitoring (PMEM) of genetically modified plants, The EFSA Journal (2006) 319, 1-27 – http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620769727.htm (Accessed 30 July, 2009)

were asked to complete the questionnaire. The farmers / fields were randomly selected between the countries depending on the grade of 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). In Spain, where the largest acreage was planted, the survey was performed Markin[11], a contractor specialised in agricultural surveys. In Czech Republic and Slovakia the surveys were performed by the Czech Agriculture University[12] and in Germany by BioMath GmbH. In the other countries, Monsanto and Monsanto's licensees' field representatives interviewed the farmers. To assist the interviewers with the questionnaire, a 'user manual' was developed and provided to the people conducting the interviews. This manual clarifies the objectives of each question.

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, fertiliser, etc.

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, etc.

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.

The analysis of 297 questionnaires from the survey of farmers cultivating MON 810 maize in seven European countries in 2008 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 7.

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 maize cultivation that require risk mitigation, these will be reported immediately.

[11] Instituto Markin, SL; c/ Caleruega, 60 4° D – 20833 Madrid – SPAIN.
[12] Czech Agricultural University, Kamýcká 129, Praha 6 – Suchdol, 165 21 – CZECH REPUBLIC.

4.2.2 Peer reviewed publications on the safety of MON 810 and/or the Cry1Ab protein published in 2008 - 2009

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. July 2008, and beginning of June 2009, more than 50 publications related to MON 810 and/or Cry1Ab were published in peer reviewed journals. Those references were obtained by running a search using the ISI Web of Knowledge™ (search terms: ((lepidoptera* resistan*) or (lepidoptera* tolerant) or (insect resist*) or (insect toleran*)) and (maize or corn); ((genetically modified or genetically transformed) and (corn or maize)); (GM maize or GM corn or transgenic maize or transgenic corn or Bt maize or BT corn); Cry1Ab; (MON 810 or MON810 or Bt176 or Bt11)). Search results were manually screened, and relevant publications were subsequently assessed. Publications were classified into the categories of environment (NTOs; gene flow; impact of management practices; protein/DNA fate in soil; others) and food/feed (nutritional equivalence; allergenicity; toxicology; compositional equivalence; others).

Between July 2008 and the beginning of June 2009, 9 studies relevant to the food-feed safety of MON 810 and 43 peer reviewed publications relevant to the environmental effects of MON 810 were obtained in the search. The detailed analysis of these peer reviewed publications is presented in Appendix 8.

Nine food/feed publications were analysed (Appendix 8.1). A study by Coll *et al.*, (2008) confirmed the substantial equivalence of MON 810 at the level of transcriptomics, while De Luis *et al.*, (2008) demonstrated that the Cry1Ab protein expressed in MON 810 is denatured through heat treatment. Analysis of two studies assessing the allergen or immune responses of mice fed Cry1Ab protein or MON 810 (Finamore *et al.*, 2008; Guimaraes *et al.*, 2008) either indicated that there was no response, or any small changes observed would not be expected to be associated with any known health effects and would not lead to any potential cause allergies or other immunotoxic effects, either in humans or animals. Mycotoxin levels of harvested grain and field debris were analysed in two studies and indicated that *Bt* maize grain had lower mycotoxin levels than other hybrids (Abbas *et al.*, 2008; Polisenska *et al.*, 2008). Three studies presented the results of animals fed diets containing MON 810. Two papers by Guertler *et al.* (Guertler *et al.*, 2008; Guertler *et al.*, 2009) demonstrated that no transgenic DNA or Cry1Ab protein was detected in milk of lactating cows fed MON 810 maize, while a slight increase in mRNA expression of a putative salmon homologue to a proton dependent, high affinity oligopeptide transporter was observed between fish fed diets containing MON 810 or conventional control maize (FrØystad-Saugen *et al.*, 2009). Further analysis of this study reveals discrepancies between diets of test and control groups, and that changes reported are of questionable biological and physiological relevance.

The analyses of publications related to environmental effects of MON 810 support the equivalence of MON 810 to its conventional counterpart in terms of impacts to the environment (Appendix 8.2). NTO related studies confirm there is no effect of Cry1Ab on

bees, lady beetles, non-target aphids, coccinellid beetles and other species (Álvarez-Alfageme *et al.*, 2008; Dhillon and Sharma, 2009; Konrad *et al.*, 2009; Moser *et al.*, 2008; Priestley and Brownbridge, 2009; Ramirez-Romero *et al.*, 2008a; Ramirez-Romero *et al.*, 2008b). Several studies also concluded that the Cry1Ab protein did not affect fauna in soil (Honemann *et al.*, 2008; Oliveira *et al.*, 2008) nor have any unexpected residual presence in soil (Fu *et al.*, 2009; Gruber *et al.*, 2008; Margarit *et al.*, 2008; Schrader *et al.*, 2008).

Studies by Bohn *et al.*, (2008) and Schmidt *et al.*, (2009) claim to have demonstrated negative effects on water fleas and ladybirds. However, further analyses of these studies revealed that adverse observations were more likely related to methodology, and not direct effects of the Cry1Ab protein. A 'meta study' by Lövei *et al.*, (2009) summarises 80 peer reviewed studies published in the literature about the impact of *Bt* crops on arthropod natural enemies in laboratory settings and claims *Bt* crops may have substantial negative impacts on NTOs. A rebuttal by a number of prominent scientists (Shelton *et al.*, 2009) argues that the analysis by the authors of this study used statistically non-significant data for comparing results, made no distinction between reported impacts, and their conclusions lack field relevance. Douville *et al.*, (2009) suggest that horizontal gene transfer (plant to bacteria) had occurred between freshwater mussels in areas of *Bt* corn cultivation; a conclusion that is not supported by the data presented.

For the analyses of other environmental and food/feed studies reviewed, please refer to Appendix 8.

In addition, three scientific opinions regarding MON 810 were issued by EFSA during the year from July 2008 to June 2009. These included two opinions regarding the safety of MON 810 following the invocation of the safeguard clause under Article 23 of Directive 2001/18/EC by Austria and France[13,14]. In both cases, EFSA concluded that no specific evidence was provided by either country that would justify the invocation of the safeguard clause. Furthermore, on 30 June 2009, EFSA published its opinion on the applications for renewal of authorisation for the continued marketing of existing food and food ingredients produced from MON 810; feed consisting of and/or containing MON 810, including the use of seed for cultivation; and food and feed additives and feed materials produced from MON 810. In its opinion, EFSA concluded that "maize MON 810 is as safe as its conventional counterpart with respect to potential effects on human and animal health". The EFSA GMO Panel also concluded that "maize MON 810 is unlikely to have any adverse effect on the environment in the context of its intended uses, especially if appropriate

[13] Scientific Opinion of the Panel on Genetically Modified Organisms on a Request from the European Commission related to the safeguard clause invoked by France on maize MON 810 according to Article 23 of Directive 2001/18/EC and the emergency measure according to Article 34 of Regulation (EC) No 1829/2003. The EFSA Journal (2008) 850, 1-45 - http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902156394.htm (Accessed 30 July, 2009)

[14] Scientific Opinion of the Panel on Genetically Modified Organisms on a request from the European Commission related to the safeguard clause invoked by Austria on maize MON 810 and T25 according to Article 23 of Directive 2001/18/EC. The EFSA Journal (2008) 891, 1-64 - http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902209965.htm (Accessed 30 July, 2009)

management measures are put in place in order to mitigate possible exposure of non-target Lepidoptera".[15]

The publications identified by this literature search reinforce our knowledge of MON 810 and its safety. 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 assessment of the available scientific opinions.

4.2.3 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 of any unexpected potential adverse effects observed that might be linked to the use of its products. This can be done through a number of available means including a hotline, contact phone numbers for Monsanto representatives in each country, a general Monsanto contact phone number or email address and a contact point on the Monsanto website.

To date, no unexpected potential adverse effects related to MON 810 have been reported or confirmed.

4.2.4 Alerts on environmental issues

Since the commercial introduction of MON 810, attention to potential environmental issues have been raised through a number of sources.

An issue management process has been put in place 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);
- Analysis of the potential issue and its relevance to the safety assessment of the product;

[15]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 theuse 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 30 June, 2009)

- Sharing of expert commentary with regulators and other stakeholders (if warranted);
- Communication of conclusions to internal and external stakeholders (if warranted).

Although a number of issues were raised in 2008, including claims from both Luxemburg and Germany that new scientific evidence was available that warranted an invocation of the safeguard clause in these countries, no potential adverse effects related to MON 810 were confirmed through this process in 2008. See also Appendix 8 for an assessment of the main studies cited in the Luxemburg and German bans.

4.2.5 Network monitoring in Germany

On 27 April 2007, the German Competent Authority, the Federal Office of Consumer Protection and Food Safety (BVL; Bundesamt für Verbraucherschutz und Lebensmittelsicherheit), temporarily suspended the authorisation to distribute MON 810 maize seeds for commercial planting in Germany. The suspension remained valid until Monsanto, as authorisation holder, submitted a monitoring plan for MON 810 cultivation in Germany that was acceptable to the BVL. An agreement on this monitoring plan was the basis for the lifting of the German suspension. While Farmer Questionnaires remain the central element of the monitoring plan for MON 810, the use of available information from defined existing networks was an additional new element that was proposed for incorporation into the general surveillance in Germany.

The report presenting Monsanto's analysis of German monitoring networks for the 2008 season is presented in Appendix 9. Two categories of networks were monitored, the first providing information on relevant monitoring characters, while the second category looked at relevant influencing characters which play a critical role in determining the context in which the crop was cultivated.

Under the first category (relevant monitoring characters), five networks were assessed; game species, birds, butterfly population dynamics, bees and soil. None of the five networks specifically mentioned MON 810 as an influencing factor in any observed variation in data. Furthermore, an analysis of the available information to determine if the data indicated any effects that may have been caused by the cultivation of MON 810 was conducted. While some fluctuations and variations in populations of game species, birds, butterflies, and honeybees were reported in the data, in no instances could any differences be explained as an effect of the planting of MON 810. In most instances, differences were attributed to the impacts of weather, land use, predators, land disturbance, expansion of agriculture and urbanisation into pristine areas, disease or pesticides. It must also be acknowledged that in some instances, methods of data collection may have had an impact on observed fluctuations from region to region and year to year.

The network measuring indicators of biodiversity in agriculture and the Plant Protection Service were also assessed to determine if they reported any adverse effects attributed to the cultivation of MON 810 in Germany. There was no information reported in either of these networks that indicated any adverse effects in relation to planting of MON 810 in Germany.

This information confirms that validity of the assumptions and conclusions laid down in the environmental risk assessment, that the product is as safe as conventional corn when cultivated in an agricultural environment.

5. SUMMARY OF RESULTS AND CONCLUSIONS

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 (Section 3). 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 learning's (Section 3.2).

For the 2008 planting season, no issues related to Insect Resistance were experienced (Section 4). Following the establishment and reinforcement of an effective education and communication programme in countries where MON 810 was grown in 2008, the percentage of farmers implementing refuges in their fields was very high (Section 3.1).

The results of the analysis of 2008 farmer questionnaires did not identify any potential adverse effects that might be related to MON 810 plants and their cultivation (Section 4.2.1), nor did the analysis of several German networks (Section 4.2.5). A review of peer reviewed publications confirmed the negligible potential of MON 810 and/or the Cry1Ab protein to cause adverse effects (Section 4.2.2). Finally, company stewardship activities and issue alerts did not reveal any adverse effect related to MON 810 cultivation (Sections 4.2.3 and 4.2.4).

These results demonstrate that there are no adverse effects attributed to the cultivation of MON 810 in Europe.

6. ADAPTATIONS FOR FUTURE MONITORING PLANS

The information provided in this report is not considered confidential in accordance with Article 25 of Directive 2001/18/EC.

This does not prevent the lead competent authority and the Commission from requiring additional information from the notifier, both confidential and non-confidential.

In the case of confidential data, it should be provided in an annex to the report format, with a non-confidential summary or general description of these data, which will be made available to the public.

Signed: 

Date: 6/8/2009

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