Annual monitoring report on the cultivation of MON 810 in 2009

Czech Republic, Portugal, Slovakia, Poland, Romania 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 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 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

® 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 August 11, 2010)

[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 August 11, 2010)

[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 August 11, 2010)

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 2009, *Bt* maize was planted in the EU on 94 850 hectares across six countries: Czech Republic (6 480 ha), Poland (ca. 3 000 ha), Portugal (5 094 ha), Romania (3 344 ha), Slovakia (875 ha) and Spain (76 057 ha) (*see* Appendix 7).

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. 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; Monsanto Europe S.A., 2006; Monsanto Europe S.A., 2007; Monsanto Europe S.A., 2008; Monsanto Europe S.A., 2009).

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

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 2009-July 2010

1.6 Other monitoring reports have been submitted in respect of:

- **Import and Processing** Yes (December 2009)
- **Food/Feed** No

[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 August 11, 2010)

[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 August 11, 2010)

2. EXECUTIVE SUMMARY

In 2009, *Bt* maize was planted in the EU on 94 850 hectares across six countries. As part of stewardship of the technology, industry has implemented an Insect Resistance Management (IRM) plan to proactively avoid and/or delay the potential development of pest resistance to the Cry protein, as well as a voluntary general surveillance monitoring program. The adherence to these stewardship measures in the context of the 2009 cultivation of MON 810 maize in Europe is detailed in this report.

The planting of MON 810 in the 2009 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 the high levels of compliance with requirements for refuge implementation observed in the 2009 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 2009.

In 2009, 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 240 questionnaires from a survey of farmers cultivating MON 810 in six European countries in 2009 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 30 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 2009.

Taken together, these results demonstrate that there are no adverse effects attributed to the cultivation of MON 810 in Europe in 2009.

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]).

3.1.1 Description of general surveillance

In 2009, 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.

[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 August 20, 2010)

The general surveillance monitoring program performed by Monsanto in 2009 consisted of four elements:

- a farmer questionnaire designed to assess unusual observations in the areas where MON 810 has been cultivated (farmer questionnaire was amended on the basis of past experience);
- 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

As mentioned in Section 3.1.1, several methodologies are followed in the frame of general surveillance including a farmer questionnaire, data collected from scientific publications (*see* Section 3.1.6), company stewardship activities and alerts on environmental issues.

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[7]. 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

[7] 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 August 11, 2010)

to create a new version for 2006. The current version of the questionnaire has been used since 2006. As appropriate, in the 2009 season, adjustments were made to improve the statistical relevance of the collected data (*see* 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 2009 (Czech Republic, Poland, Portugal, Romania, Slovakia and Spain) and thus was performed in areas reflecting the range and distribution of farming practices and environments exposed to MON 810 plants and their cultivation. This allows for cross-checking of information indicative of an unanticipated effect, and the possibility to establish correlations either by comparing questionnaires between regions, or associating answers to observations made by existing networks, such as meteorological services (weather conditions) or extension services (pest pressure).

In 2009, 49 farmers in the Czech Republic, 42 farmers in Portugal, 6 farmers in Slovakia, 3 farmers in Poland, 40 farmers in Romania and 100 farmers in Spain were asked to complete the questionnaire. 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* Annex 7 for details on methodology). In Spain, where the largest acreage was planted, the survey was performed by Markin[8]. In Portugal surveys were performed by Agro.Ges[9]. In Romania survey were performed by MIA[10]. In Czech Republic and Slovakia the surveys were performed by the Czech Agriculture University[11]. In Poland, Monsanto and Monsanto's licensees' field representatives interviewed the farmers.

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',

[8] Instituto Markin, SL; c/ Caleruega, 60 4° D – 20833 Madrid, Spain

[9] Agro.Ges - Sociedade de Estudos e Projectos ; Av. da República 412, 2750-475 Cascais, Portugal

[10] MIA Marketing Institute Ltd.; 17 Unirii Blvd., Bucharest, Romania

[11] Czech Agricultural University; Kamýcká 129, Praha 6 – Suchdol, 165 21, Czech Republic

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.

3.1.2.2 Company stewardship activities

Monsanto is committed to the management of its products in a responsible and ethical way throughout their entire life cycle, from the stages of discovery to their ultimate use. It includes *1)* assessment of the safety and sustainability of the products, *2)* absolute respect of all the regulations in place, and *3)* support to the products by explaining and promoting the proper and responsible use of those products and technologies.

As part of product stewardship and responsible use, Monsanto urges user / licensees to notify of any unexpected potential adverse effects observed that might be linked to the use of its products. This can be done through the phone, fax or mail contact information given in the technology user guides (*see* Appendices 2.1 to 2.6). Alternatively, the Monsanto website offers a contact point[12].

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)[13].

[12] Monsanto product stewardship webpage - http://www.monsanto.com/products/techandsafety/stewardship.asp (Accessed August 11, 2010)

[13] Channels of communication to external stakeholders include the Monsanto *for the record* website - http://www.monsanto.com/monsanto_today/for_the_record/ and Monsanto *for the record - Science* website - http://www.monsanto.com/products/techandsafety/fortherecord_science/ (Accessed August 11, 2010)

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[14]; 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 2009 (Please note that such report was not developed this year as MON 810 was not planted in Germany in 2009).

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 is currently coordinating a more general effort to map EES networks in Europe and to set up a unique reporting system. Harmonisation of effort allows improving the quality of the data collection and reporting, bringing consistency across criteria for the selection of networks, methodology in the assessment of data and translations of surveillance reports and having a single interface with networks. In what follows, a general approach is described on how these EES networks could be selected and used in PMEM of GM crops.

EuropaBio will identify a list of EES networks operating in the field of environmental monitoring as part of the harmonized EU approach for the General Surveillance of cultivated GM crops. These networks will be selected from a pool of national or EU-wide obligatory monitoring activities (according to EU directives 92/43/EEC and 2004/35/EC) and other existing national or EU-wide environmental monitoring programs. This approach was also proposed by Bartsch *et al.* (2008). An annual report will be provided based on the review of the publications from those networks.

Identification of the EES networks

Firstly, the initial list of available EES networks will be classified according to the protection goals they are addressing and to their geographical coverage. No specific protection goals are defined in Directive 2001/18/EC or other GMO legislation. Directive 2002/811/EC only states that 'the monitoring plan should […] incorporate general surveillance for unanticipated or unforeseen adverse effects'. Therefore, protection goals for GM monitoring have to be sought in other EU and national legislation as already mentioned above, such as Directives 92/43/EEC and 2004/35/EC. Examples of these protection goals could be, biodiversity, human health, animal health, plant health, soil

[14] 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.

function, water quality, and sustainable agriculture. These will form the basis for categories of EES networks identified.

Inclusion of the EES networks for the PMEM plan

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

Analysis of the reports from the included EES networks

On an annual basis, the reports of selected EES networks will be analysed by a third party for Europabio. However, it should be noted that the "non-hypothesis" driven observation of the networks can only provide insights on important unanticipated adverse effects (or lack thereof) and cannot deliver final conclusions.

3.1.3 Details of information and/or training provided to operators and users, etc.

Each purchaser of *Bt* maize receives a technical user guide 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 2009 season can be found in Appendix 2. 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 previous years surveys (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 9).

3.1.4 Results of general surveillance

3.1.4.1 Farmer questionnaires

Methodology is described in section 3.1.2.1. The analysis of 240 questionnaires from the survey of farmers cultivating MON 810 maize in six European countries in 2009 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.

3.1.4.2 Company stewardship activities

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

Methodology is described in section 3.1.2.3. No potential adverse effects related to MON 810 were reported in 2009.

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 2009 - 2010

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 2009, and beginning of June 2010, more than 30 publications related to MON 810 and/or Cry1Ab were published in peer reviewed journals[15]. Those references were obtained by running a search using ISI Web of Knowledge™ (search terms: (maize or corn or Zea-mays) and (((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)); (Cry1Ab or CryIab or Cry-1Ab or CryI-Ab or Cry1A-B or CryIA-B); (MON810 or MON-810); (Bt-Maize or Bt-corn or Yieldg* or Yield-gard or Yield-guard). Search results were screened, and relevant publications to the risk assessment were subsequently assessed. Publications were classified into the categories of food/feed (Molecular characterization; Animal feeding study; Toxicology/Allergenicity; Composition/Nutrition study - *see* Appendix 8.1) and environment (Non-target Organisms; Insect resistance/impact of management practices; protein/DNA fate in soil; Spillage and consequences of thereof - *see* Appendix 8.2). The detailed analysis of these peer reviewed publications is presented in Appendix 8.

Ten publications were analysed in terms of food/feed safety[15] (Aguilera *et al.*, 2008; Barros *et al.*, 2010; Coll *et al.*, 2010; de Luis *et al.*, 2010; de Vendomois *et al.*, 2009; Guimaraes *et al.*, 2010; La Paz *et al.*, 2010; Sissener *et al.*, 2010; Swiatkiewicz *et al.*, 2010; Szekacs *et al.*, 2010). A statistical reevaluation of data from three 90 day rat feeding studies with GM maize varieties MON 810, MON 863 and NK603 maize grain by de Vendomois *et al.* (2009)

[15] Note that the publications on food/feed published between July 2009 and December 2009 were already covered in the *2009 Annual General Surveillance Report for MON 810 undertaken on a voluntary basis.* Report submitted in December 2009 by Monsanto Europe.

claimed to find evidence of kidney and liver toxicity. However, EFSA[16] and the Haut Conseil des Biotechnologies (HCB)[17] concluded that there was no new evidence of harmful effects in these studies. Two studies (de Luis *et al.*, 2010; Guimaraes *et al.*, 2010) tested the rapid *in vitro* digestion of the Cry1Ab protein. Also, a broiler feeding study (Swiatkiewicz *et al.*, 2010) confirmed the nutritional equivalence of MON 810 to conventional maize. Overall, the ten studies support the conclusion of equivalence of MON 810 to its conventional counterpart in terms of food/feed safety. For the detailed analyses of all food/feed studies reviewed, refer to Appendix 8.1.

Twenty-two publications related to environmental effects of MON 810 were reviewed (Arenas *et al.*, 2010; Badea *et al.*, 2010; Bohn *et al.*, 2010; Cancino-Rodezno *et al.*, 2010; Crespo *et al.*, 2009; Daudu *et al.*, 2009; Dorhout and Rice, 2010; Erasmus *et al.*, 2010; Goldstein *et al.*, 2010; Icoz *et al.*, 2009; Jensen *et al.*, 2010; Kramarz *et al.*, 2009; Lopez *et al.*, 2010; Park *et al.*, 2010; Perry *et al.*, 2010; Peterson *et al.*, 2009; Porcar *et al.*, 2009; Prasifka *et al.*, 2010; Raubuch *et al.*, 2010; Swan *et al.*, 2009; Xu *et al.*, 2010; Zurbrugg *et al.*, 2010). Studies related to non-target organisms confirm that there is no harmful effect of Cry1Ab on pea aphid, ground beetles, non-target lepidopterans, moths and other species (Erasmus *et al.*, 2010; Jensen *et al.*, 2010; Perry *et al.*, 2010; Peterson *et al.*, 2009; Porcar *et al.*, 2009). Studies by Kramarz *et al.* (2009) and Bohn *et al.* (2010) claim to have demonstrated negative effects on snails water fleas and daphnia. However, further analyses of these studies revealed that adverse observations were more likely related to methodology, and not direct effects of the Cry1Ab protein. Studies on the fate of the Cry1Ab protein or *Bt* maize straw in soil did not reveal accumulation of Cry1Ab protein in the soil that would cause concerns (Badea *et al.*, 2010; Daudu *et al.*, 2009; Icoz *et al.*, 2009; Swan *et al.*, 2009; Zurbrugg and Nentwig, 2009) or was inconclusive (Raubuch *et al.*, 2010). Overall, the twenty-two publications support the conclusion of equivalence of MON 810 to its conventional counterpart in terms of impacts to the environment. For the detailed analyses of all environmental studies, refer to Appendix 8.2.

Letters to the editor relative to the recent cultivation ban of MON 810 in Germany were also identified in the search results (Rauschen, 2010; Ricroch *et al.*, 2010). The authors examined the justifications invoked by the German government in April 2009 to suspend the cultivation of MON 810 and carried out a critical examination of the alleged new data on potential environmental impacts. The authors did not find any justification for this suspension in an extensive survey of the scientific literature regarding possible effects under natural field conditions on non-target animals. These reviews are not presented in Appendix 8.

Finally, several review papers on *Bt* crops were identified in the search output. No adverse effects were reported (La Reesa Wolfenbarger *et al.*, 2008; Naranjo, 2009; Tabashnik *et al.*, 2009). Lang and Otto (2010) and Then (2010) reviewed publications that reported on the toxic

[16] 55th plenary meeting of GMO Panel (Annex 1), EFSA (2010) - http://www.efsa.europa.eu/en/events/event/gmo100127.htm (Accessed August 11, 2010)

[17] Avis relatif à la saisine du 15 décembre 2009 de Monsieur le Député François Grosdidier, HCB (2009) - http://www.ogm.gouv.fr/article.php3?id_article=115 (Accessed August 11, 2010)

effects of Bt-maize and/or Cry proteins. However, these two reviews fail to cite any of the comprehensive field data accumulated over the last 10 years showing the lack of harm of *Bt* crops on non-target organisms or rely heavily only on controversial articles that were conducted only in the laboratory. Therefore, these five reviews (not presented in Appendix 8) do not change the conclusions of negligible risk of the initial risk assessment.

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 assessment of the recent scientific opinion from EFSA on MON 810[4].

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 strategies for insect-protected maize.

Following this example, Monsanto along with three other companies[18] 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 (*see* 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 is based on published research, current EU legislation, the European Commission's Scientific Committee on Plants (SCP) opinion on IRM[19] and practical experience gained during the implementation of IRM plans in other parts of the world. The purpose of the IRM plan is to proactively avoid where possible, and in all cases delay the potential development of pest resistance to the Cry protein expressed in *Bt* maize. The harmonised IRM plan (see Appendix 1) contains guidance on the following key elements:

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

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

[19] SCP (1999), Opinion of the Scientific Committee on Plants on Bt resistance monitoring (Opinion expressed on March 04, 1999), *Document SCP/GMO/094-Rev.5* - http://ec.europa.eu/food/fs/sc/scp/out35_en.print.html (Accessed August 11, 2010)

3.2.1.1 Refuge

According to the *Harmonised insect resistance management (IRM) plan for cultivation of Bt maize in the EU* (*see* 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 3.2.1.3). 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 2009 planting season, through a survey sponsored by ANTAMA (Spanish Foundation supporting the use of new technologies in agriculture[20]). 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 had planted more than 5 hectares of MON 810 maize. 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. 96% of the farmers planting *Bt* knew about the recommendation to plant a refuge. In this group, 70% considered themselves to be "well informed", 23% "somehow informed", 6% "little informed" and 1% "not informed". The farmers responses regarding the clarity of the recommendations about the implementation of refuges were as follows: 88% considered the recommendations "very clear/quite clear", while only 12% considered them "little clear/unclear". 69% of the interviewees considered that it is "very easy/quite easy" to follow the recommendations while 31% considered that it is "little easy/not easy".

The survey also revealed a high level of compliance with refuge requirements indicating that 81% of the 100 farmers included in the final survey planted a conventional maize refuge on their farm. The remaining farmers surveyed (*i.e.* 19%) did not plant a refuge. Reasons given 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).

[20] ANTAMA - http://fundacion-antama.org/ (Accessed August 11, 2010)

In addition, this survey analysed the satisfaction of the growers. The survey indicated that 95% of the farmers are very or quite satisfied and 5% a little satisfied. The main advantage/benefit, reported by 95% of the farmers, was the effective protection against corn borers, followed by peace of mind (53%), good yield (46%), the plant health (plants / ear of maize do not collapse (45%) and healthier plants (30%)).

Apart from the ANTAMA survey in Spain, in the context of Monsanto's 2009 general surveillance, 240 farmers across six 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. 90.4% of the farmers who answered the question indicated that they followed the technical guidelines regarding the implementation of a refuge (87.1% planted a refuge and 3.3% had less than 5 ha planted with MON 810 on their farm[21]). Most countries reported a very high level of compliance with refuge requirements. The results of this survey are discussed in further detail in Section 3.1.4.1.

The results of the Monsanto 2009 farmer questionnaire survey showed that in Spain, where 80% 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), 91.4% 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 2008 survey (85.4% of farmers participating in the 2008 survey in Spain were compliant with refuge requirements), which reflects the communication efforts undertaken prior to the 2009 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 (*see* Section 3.2.1.3). 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 2009 Farmer Questionnaire Survey show that while 73.8% of the farmers in Portugal planted a refuge, some farmers (*i.e.*, 11 of 42) 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 in Portugal by the General Directory of Agriculture and Rural Development (DGADR) together with the Regional Direction of Agriculture and Fishery (DRAP). The survey for the 2009 planting season involved 105 farmers, representing 45% of the total number of farmers who declared being planting *Bt* maize and 43.2 % of the area planted with *Bt* maize. Additionally, five farmers cultivating conventional maize within the production zone of GM maize were surveyed. Farmer responses demonstrated the effectiveness of communication regarding IRM requirements. The majority (99%) of the farmers declared that the training they received to be able to plant *Bt* maize was sufficient and clear. In addition, the information in the seed bags

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

was comprehensive for all farmers (100%). There was full compliance with refuge requirements indicating that the area planted with the refuge was even higher than that required by legislation. The survey also revealed that 85% of the farmers decided to plant *Bt* maize to assure a better control of *O. nubilalis* and to reduce insecticide applications[22].

The message on the importance of refuge implementation will be repeated in all countries growing MON 810 in the 2010 growing season. It is important to continue educating the farmers on the necessity to implement refuges.

3.2.1.2 Baseline studies and monitoring of the target pests

Baseline studies

Baseline studies with Cry1Ab were performed in Spain with *S. nonagrioides* and *O. nubilalis* populations collected in the three major regions where insect pressure would justify the use of MON 810 (Ebro Valley, centre of Spain and Extremadura-Andalusia) prior to the introduction of *Bt* maize in Spain (Gonzalez-Nunez *et al.*, 2000). These results were reported in the 2003-2004 Monitoring Report (Monsanto Europe S.A., 2005).

The baseline susceptibility to Cry1Ab was established for the French and Portuguese field populations of *S. nonagrioides* and for the Portuguese populations of *O. Nubilalis* in 2005 and again for the French samples of *S. Nonagrioides* in 2006 (Monsanto Europe S.A., 2006; Monsanto Europe S.A., 2007). Overall, the susceptibility to Cry1Ab of theses species was within the range obtained in baseline studies and subsequent monitoring performed after Bt176 maize cultivation (Farinós *et al.*, 2004; Gonzalez-Nunez *et al.*, 2000), prior to MON 810 introduction.

In addition to the above, the BTL Bio-Test Labor GmbH[23], led by Dr. [redacted], expanded the baseline of susceptibility of *O. nubilalis* to Cry1Ab from 2005 to 2007 based on adoption of MON 810 in major European maize growing regions. Thus far, levels of susceptibility to Cry1Ab have been determined for one laboratory colony and populations collected in maize fields in Czech Republic, France, Germany, Italy, Hungary, Slovakia, Poland, Portugal and Romania (see Appendix 4).

Monitoring of the target pests

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

During 2004-2008, monitoring for *O. nubilalis* and *S. nonagroides* resistance to Cry1Ab expressed in MON 810 was performed. Different geographical areas with considerable commercial growing of MON 810 varieties were selected. The monitoring studies performed with *O. nubilalis* and *S. nonagrioides* showed that the susceptibility of the population samples

[22] DGADR, Informações referentes a campanha de 2009, page 23 - http://www.dgadr.pt/ (Accessed August 11, 2010)

[23] BTL Bio-Test Labor GmbH, Birkenallee 19, 18184 Sagerheide, Germany

to Cry1Ab were within what is considered a normal range, demonstrating no development of resistance.

In 2009, this monitoring was continued and samples were collected from the MON 810 growing areas in Northeast Iberia by [redacted] (*see* Appendix 5). Although planned for evaluation, *S. nonagrioides* from Southwest Iberia could not be evaluated due to the low levels of the pest found in this area during the 2009 campaign. Because a high degree of variability in susceptibility of *S. nonagrioides* to Cry1Ab using lethal concentrations has been demonstrated, which is not an evidence of resistance development (Farinós *et al.*, 2004), Molting Inhibition Concentration (MIC) (a different methodology), was used to determine the development of resistance in populations collected in 2009 (*see* Appendix 5). Results showed that the MIC_{50} values were very similar for both the laboratory and field collected strains. Furthermore, all the susceptibility data of *S. nonagrioides* to Cry1Ab gathered since 2004 were reanalyzed using the MIC_{50} and MIC_{90} as comparators among populations. Results showed a variation of 4 folds between the highest and lowest MIC_{50} calculated indicating that this methodology is optimal for the monitoring of susceptibility of *S. nonagrioides* to Cry1Ab.

In addition to the baseline results described above, the BTL Bio-Test Labor GmbH determined the susceptibility of *O. nubilalis* to Cry1Ab from 2005 to 2009 in major European maize growing regions. The susceptibility of 15 populations with 98 samples (including replicates and assays without concentration response relationship) of *O. nubilalis* were analysed. Thus far, susceptibility to Cry1Ab have been assessed for one laboratory colony and populations collected in maize fields in Czech Republic, France, Germany, Italy, Hungary, Slovakia, Poland, Portugal, Romania, and Spain. *O. nubilalis* larvae were exposed to artificial diet treated with increasing Cry1Ab concentrations, and mortality and growth inhibition were evaluated after 7 days (*see* Appendix 4).

Results for *O. nubilalis* populations were pooled according to geographic and climatic conditions. These pooled populations correspond to homogenous regions based on available knowledge of insect biology and geography. This approach follows the IRM industry working group guidelines (*see* Appendix 1). The results of the *O. nubilalis* populations pooled according to geographic and climatic conditions were similar and differed 1.8-fold, 6.6-fold, 2.6-fold, 4.2-fold and 3.2-fold for *O. nubilalis* collected in 2005, 2006, 2007, 2008, and 2009 respectively. A similar degree of variability was reported for *O. nubilalis* susceptibility to Cry1Ab for populations from three broad geographic areas in the US, chosen based on market penetration for *Bt* maize. Similar levels of variability were also observed in a study that included populations of different voltine ecotypes and pheromone strains (Marçon *et al.*, 1999). For the current study, the pheromone races were not distinguished.

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.

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

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 *Bt* maize receive a technical user guide (*see* appendix 2). 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. In the 2009 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, hybrid guides on packaging). Some examples include the good agricultural practices (GAP) leaflet (*see* Appendix 3.1) and Guía Técnica YieldGard® (YieldGard Technical Guide) (*see* Appendix 2.6) that are attached to each MON 810 bag sold in Spain.
2) Interviews with farmers complying with refuge requirements published in prominent agricultural magazines as Vida Rural.
3) Presentation by sales and marketing teams of IRM requirements in farmer meetings / farmer talks to reinforce the need for refuge compliance.
4) IRM information exhibited at different national and regional agricultural fairs.
5) Advertisement about refuge compliance published in key agricultural magazines (Vida Rural and Phytoma) (*see* 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 (*see* Appendix 3.3).
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 (*see* Appendix 3.4).
8) Posters and stickers distributed among seed distributors and point of sales to be used with invoices and letters (*see* Appendices 3.5 and 3.6).
9) Communication plan for cooperatives, small points of sales outlets and farmers: trained ANOVE inspectors completed several visits in *Bt* maize growing areas 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 3.2.1.1, demonstrates the effectiveness of the education program to raise awareness of refuge implementation. 96% of the farmers surveyed acknowledged they were made aware of the fact that they are required to plant a refuge.

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 7), the scientific literature (*see* Section 3.1.6 and Appendices 8.1 and 8.2), 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.

A copy of the manual to assist farmers completing the questionnaire is provided in Appendix 9.

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 learning's. 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 programme in countries where MON 810 was grown in 2009, the percentage of farmers implementing refuges in their fields was very high.

The results of the analysis of 2009 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 peer reviewed 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 2009 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 2009.

All together, these results demonstrate that there are no adverse effects attributed to the cultivation of MON 810 in Europe. The result of the 2009 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 Farmer Questionnaire can be improved based on experience from year to year. Also, in anticipation of the mandatory request in all applications or renewals for deliberate release submitted under Directive 2001/18/EC and Regulation (EC) No. 1829/2003, the monitoring of existing environmental networks on an EU scale is in development phase (*see* Section 3.1.2.1).

Signed: 

Date: 20 August 2010

REFERENCES

Aguilera, M., Querci, M., Balla, B., Prospero, A., Ermolli, M. and Van den Eede, G. (2008) A qualitative approach for the assessment of the genetic stability of the MON 810 trait in commercial seed maize varieties, *Food Analytical Methods*, **1**, 252-258.

Arenas, I., Bravo, A., Soberon, M. and Gomez, I. (2010) Role of alkaline phosphatase from *Manduca sexta* in the mechanism of action of *Bacillus thuringiensis* Cry1Ab toxin, *Journal of biological chemistry*, **285**, 12497-12503.

Badea, E. M., Chelu, F. and Lacatusu, A. (2010) Results regarding the levels of Cry1Ab protein in transgenic corn tissue (MON810) and the fate of Bt protein in three soil types, *Romanian Biotechnological Letters*, **15**, 55-62.

Barros, E., Lezar, S., Anttonen, M. J., van Dijk, J. P., Rohlig, R. M., Kok, E. J. and Engel, K. H. (2010) Comparison of two GM maize varieties with a near-isogenic non-GM variety using transcriptomics, proteomics and metabolomics, *Plant Biotechnology Journal*, **8**, 436-451.

Bartsch, D., Gathmann, A., Koziolek, C., Vaasen, A. and Buhk, H. J. (2008) Implication of the Environmental Liability Directive for General Surveillance - What to protect?, *Journal für Verbraucherschutz und Lebensmittelsicherheit*, **3**, S2:8-11.

Bohn, T., Traavik, T. and Primicerio, R. (2010) Demographic responses of *Daphnia magna* fed transgenic *Bt*-maize, *Ecotoxicology*, **19**, 419-430.

Cancino-Rodezno, A., Alexander, C., Villasenor, R., Pacheco, S., Porta, H., Pauchet, Y., Soberon, M., Gill, S. S. and Bravo, A. (2010) The mitogen-activated protein kinase p38 is involved in insect defense against Cry toxins from *Bacillus thuringiensis*, *Insect Biochemistry and Molecular Biology*, **40**, 58-63.

Coll, A., Nadal, A., Collado, R., Capellades, G., Kubista, M., Messeguer, J. and Pla, M. (2010) Natural variation explains most transcriptomic changes among maize plants of MON810 and comparable non-GM varieties subjected to two N-fertilization farming practices, *Plant Molecular Biology*, **73**, 349-362.

Crespo, A. L. B., Spencer, T. A., Alves, A. P., Hellmich, R. L., Blankenship, E. E., Magalhaes, L. C. and Siegfried, B. D. (2009) On-plant survival and inheritance of resistance to Cry1Ab toxin from *Bacillus thuringiensis* in a field-derived strain of European corn borer, *Ostrinia nubilalis*, *Pest Management Science*, **65**, 1071-1081.

Daudu, C. K., Muchaonyerwa, P. and Mnkeni, P. N. S. (2009) Litterbag decomposition of genetically modified maize residues and their constituent *Bacillus thuringiensis* protein (Cry1Ab) under field conditions in the central region of the Eastern Cape, South Africa, *Agriculture Ecosystems & Environment*, **134**, 153-158.

de Luis, R., Lavilla, M., Sanchez, L., Calvo, M. and Perez, M. D. (2010) Pepsin degradation of Cry1A(b) protein purified from genetically modified maize (*Zea mays*), *Journal of Agricultural and Food Chemistry*, **58**, 2548-2553.

de Vendomois, J. S., Roullier, F., Cellier, D. and Seralini, G. E. (2009) A comparison of the effects of three GM corn on mammalian health, *International Journal of Biological Sciences*, **5**, 706-721.

Dorhout, D. L. and Rice, M. E. (2010) Intraguild competition and enhanced survival of Western Bean Cutworm (Lepidoptera: Noctuidae) on transgenic Cry1Ab (MON810) *Bacillus thuringiensis* corn, *Journal of Economic Entomology*, **103**, 54-62.

Erasmus, A., Van Rensburg, J. B. J. and Van Den Berg, J. (2010) Effects of Bt maize on *Agrotis segetum* (Lepidoptera: Noctuidae): a pest of maize seedlings, *Environmental Entomology*, **39**, 702-706.

Farinós, G. P., 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.

Goldstein, J. A., Mason, C. E. and Pesek, J. (2010) Dispersal and movement behavior of neonate European corn borer (Lepidoptera: Crambidae) on non-Bt and transgenic Bt corn, *Journal of Economic Entomology*, **103**, 331-339.

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.

Guimaraes, V., Drumare, M. F., Lereclus, D., Gohar, M., Lamourette, P., Nevers, M. C., Vaisanen-Tunkelrott, M. L., Bernard, H., Guillon, B., Creminon, C., Wal, J. M. and Adel-Patient, K. (2010) In vitro digestion of Cry1Ab proteins and analysis of the impact on their immunoreactivity, *Journal of Agricultural and Food Chemistry*, **58**, 3222-3231.

Icoz, I., Andow, D., Zwahlen, C. and Stotzky, G. (2009) Is the Cry1Ab protein from *Bacillus thuringiensis* (*Bt*) taken up by plants from soils previously planted with Bt corn and by carrot from hydroponic culture?, *Bulletin of Environmental Contamination and Toxicology*, **83**, 48-58.

Jensen, P. D., Dively, G. P., Swan, C. M. and Lamp, W. O. (2010) Exposure and nontarget effects of transgenic *Bt* corn debris in streams, *Environmental Entomology*, **39**, 707-714.

Kramarz, P., de Vaufleury, A., Gimbert, F., Cortet, J., Tabone, E., Andersen, M. N. and Krogh, P. H. (2009) Effects of *Bt*-maize material on the life cycle of the land snail *Cantareus aspersus*, *Applied Soil Ecology*, **42**, 236-242.

La Paz, J. L., Vicient, C., Puigdomenech, P. and Pla, M. (2010) Characterization of polyadenylated *cryIA(b)* transcripts in maize MON810 commercial varieties, *Analytical and Bioanalytical Chemistry*, **396**, 2125-2133.

La Reesa Wolfenbarger, L., Naranjo, S., Lundgren, J. G., Bitzer, R. and Watrud, L. (2008) Bt crop effects on functional guilds of non-target arthropods: A meta-analysis, *PLoS ONE*, **3**, 1-11.

Lang, A. and Otto, M. (2010) A synthesis of laboratory and field studies on the effects of transgenic *Bacillus thuringiensis* (Bt) maize on non-target Lepidoptera, *Entomologia Experimentalis Et Applicata*, **135**, 121-134.

Lopez, M. D., Sumerford, D. V. and Lewis, L. C. (2010) *Nosema pyrausta* and Cry1Ab-incorporated diet led to decreased survival and developmental delays in European corn borer, *Entomologia Experimentalis Et Applicata*, **134**, 146-153.

Marçon, P. C. R. G., Taylor, D. B., Mason, C. E., Hellmich, R. L. and Siegfried, B. D. (1999) Genetic similarity among pheromone and voltinism races of *Ostrinia nubilalis* Hübner (Lepidoptera: Cramidae), *Insect Molecular Biology*, **8**, 213-221.

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*

Naranjo, S. E. (2009) Impacts of *Bt* crops on non-target invertebrates and insecticide use patterns, *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, **4**, 1-11.

Park, K. W., Lee, B., Kim, C. G., Kim, D. Y., Park, J. Y., Ko, E. M., Jeong, S. C., Choi, K. H., Yoon, W. K. and Kim, H. M. (2010) Monitoring the occurrence of genetically modified maize at a grain receiving port and along transportation routes in the Republic of Korea, *Food Control*, **21**, 456-461.

Perry, J. N., Devos, Y., Arpaia, S., Bartsch, D., Gathmann, A., Hails, R. S., Kiss, J., Lheureux, K., Manachini, B., Mestdagh, S., Neemann, G., Ortego, F., Schiemann, J. and Sweet, J. B. (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.

Peterson, J. A., Obrycki, J. J. and Harwood, J. D. (2009) Quantification of Bt-endotoxin exposure pathways in carabid food webs across multiple transgenic events, *Biocontrol Science and Technology*, **19**, 613-625.

Porcar, M., Grenier, A. M., Federici, B. and Rahbe, Y. (2009) Effects of *Bacillus thuringiensis* delta-endotoxins on the Pea Aphid (*Acyrthosiphon pisum*), *Applied and Environmental Microbiology*, **75**, 4897-4900.

Prasifka, J. R., Hellmich, R. L., Crespo, A. L. B., Siegfried, B. D. and Onstad, D. W. (2010) Video-tracking and on-plant tests show Cry1Ab resistance influences behavior and survival of neonate *Ostrinia nubilalis* following exposure to Bt maize, *Journal of Insect Behavior*, **23**, 1-11.

Raubuch, M., Behr, K., Roose, K. and Joergensen, R. G. (2010) Specific respiration rates, adenylates, and energy budgets of soil microorganisms after addition of transgenic *Bt*-maize straw, *Pedobiologia*, **53**, 191-196.

Rauschen, S. (2010) A case of "pseudo science"? A study claiming effects of the Cry1Ab protein on larvae of the two-spotted ladybird is reminiscent of the case of the green lacewing, *Transgenic Research*, **19**, 13-16.

Ricroch, A., Berge, J. B. and Kuntz, M. (2010) Is the German suspension of MON810 maize cultivation scientifically justified?, *Transgenic Research*, **19**, 1-12.

Sissener, N. H., Johannessen, L. E., Hevry, E. M., Wiik-Nielsen, C. R., Berdal, K. G., Nordgreen, A. and Hemre, G. I. (2010) Zebrafish (*Danio rerio*) as a model for investigating the safety of GM feed ingredients (soya and maize); performance, stress response and uptake of dietary DNA sequences, *British Journal of Nutrition*, **103**, 3-15.

Swan, C. M., Jensen, P. D., Dively, G. P. and Lamp, W. O. (2009) Processing of transgenic crop residues in stream ecosystems, *Journal of Applied Ecology*, **46**, 1304-1313.

Swiatkiewicz, S., Swiatkiewicz, M., Koreleski, J. and Kwiatek, K. (2010) Nutritional efficiency of genetically-modified insect resistant corn (MON 810) and glyphosate-tolerant soybean meal (Roundup Ready) for broilers, *Bulletin of the Veterinary Institute in Pulawy*, **54**, 43-48.

Szekacs, A., Lauber, E., Takacs, E. and Darvas, B. (2010) Detection of Cry1Ab toxin in the leaves of *MON 810* transgenic maize, *Analytical and Bioanalytical Chemistry*, **396**, 2203-2211.

Tabashnik, B. E., Van Rensburg, J. B. J. and Carriere, Y. (2009) Field-evolved insect resistance to *Bt* crops: definition, theory, and data, *Journal of Economic Entomology*, **102**, 2011-2025.

Then, C. (2010) Risk assessment of toxins derived from *Bacillus thuringiensis*-synergism, efficacy, and selectivity, *Environmental Science and Pollution Research*, **17**, 791-797.

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.

Xu, L., Wang, Z., Zhang, J., He, K., Ferry, N. and Gatehouse, A. M. R. (2010) Cross-resistance of Cry1Ab-selected Asian corn borer to other Cry toxins, *Journal of Applied Entomology*, **134**, 429-438.

Zurbrugg, C. and Nentwig, W. (2009) Ingestion and excretion of two transgenic *Bt* corn varieties by slugs, *Transgenic Research*, **18**, 215-225.

Zurbrugg, C., Honemann, L., Meissle, M., Romeis, J. and Nentwig, W. (2010) Decomposition dynamics and structural plant components of genetically modified *Bt* maize leaves do not differ from leaves of conventional hybrids, *Transgenic Research*, **19**, 257-267.