TECHNICAL REPORT

APPROVED: 21 October 2015 PUBLISHED: 28 October 2015

Relevance of a new scientific publication (Trtikova et al., 2015) on previous EFSA GMO Panel conclusions on the risk assessment of maize MON 810 and other Cry1Ab-expressing *Bt*-maize events

European Food Safety Authority

Abstract

Following a request from the European Commission, the European Food Safety Authority (EFSA) assessed the findings reported by Trtikova et al. (2015). The relevance of these findings for the risk assessment of maize MON 810 and other Cry1Ab-expressing *Bt*-maize events for which the EFSA GMO Panel has already issued a scientific opinion was also assessed. The publication by Trtikova et al. (2015) does not reveal any new information that would invalidate the previous conclusions and risk management recommendations made on maize MON 810 or any other Cry1Ab-expressing *Bt*-maize events previously assessed by the EFSA GMO Panel. Therefore, EFSA considers that the previous GMO Panel risk assessment conclusions and risk management recommendations on all Cry1Ab-expressing *Bt*-maize events so far, including the only cultivated crop in the European Union, maize MON 810, remain valid and applicable.

Key words: Cry1Ab protein levels, maize MON 810, variability, resistance evolution, stressful environmental conditions

Requestor: The European Commission

Question number: EFSA-Q-2015-00382

Correspondence: gmo@efsa.europa.eu

Acknowledgements: EFSA wishes to thank Konstantinos Paraskevopoulos and Matthew Ramon for the preparatory work on this scientific output, and Fernando Álvarez-Alfageme, Yann Devos and Elisabeth Waigmann for the support provided to this scientific output.

Suggested citation: EFSA (European Food Safety Authority), 2015. Relevance of a new scientific publication (Trtikova et al., 2015) on previous EFSA GMO Panel conclusions on the risk assessment of maize MON 810 and other Cry1Ab-expressing *Bt*-maize events. EFSA supporting publication 2015:EN-878. 11 pp.

Summary

Following a request by the European Commission, the European Food Safety Authority (EFSA) assessed the scientific information in the publication by Trtikova et al. (2015), as well as the relevance of their findings for the risk assessment of maize MON 810 and other Cry1Ab-expressing *Bt*-maize events for which the EFSA GMO Panel has issued a scientific opinion.

Trtikova et al. (2015) studied *cry1Ab* transgene expression and levels of the newly expressed *Bt* Cry1Ab protein in the leaves of two maize MON 810 varieties grown under optimal and stressful (cold/wet and hot/dry) environmental conditions. Based on their experiments with plants grown in controlled environments (climate chambers, greenhouse), the authors claimed that genetic background and environmental conditions, especially abiotic environments, could affect *cry1Ab* transgene expression and *Bt* Cry1Ab protein levels in maize MON 810. Regarding *Bt* Cry1Ab protein levels in particular, Trtikova et al. (2015) concluded that these factors could alter the expression variability of this protein. The authors extrapolated their findings to field-grown maize MON 810 and postulated that the *Bt* Cry1Ab protein concentration in relevant plant tissues of maize MON 810 might not always be sufficiently high to kill a high proportion of heterozygous resistant genotypes to maintain any resistance allele in the target insect pest population functionally recessive. The authors considered that this may have implications on insect resistance management (IRM) measures, which aim at delaying insect resistance evolution to *Bt* proteins in the target insect pest populations such as the European corn borer and Mediterranean corn borer.

The EFSA GMO Panel has already considered that there can be variability in *Bt* protein levels of genetically modified (GM) plants. This aspect is already well known and extensively reported in the peer-reviewed literature. Moreover, *Bt* Cry1Ab protein content variability in maize MON 810 and other Cry1Ab-expressing maize *Bt*-events is clearly indicated by the data in GM plant market registration applications. Reported *Bt* Cry1Ab protein levels in these applications are derived from plants grown in field trials carried out in different seasons and across several locations, thereby accounting for diverse environmental conditions.

Regarding possible implications on IRM measures due to insufficient levels of *Bt* Cry1Ab protein in relevant tissues of maize MON 810 as hypothesised by Trtikova et al. (2015), EFSA considers that this aspect would be relevant only for GM plant market registration applications for cultivation. However, the *Bt* Cry1Ab protein levels reported in Trtikova et al. (2015) for the two maize MON 810 varieties grown under optimal or stressful conditions are well within the range of values determined for maize MON 810 and Bt11 in the GM plant market registration applications for cultivation for which the EFSA GMO Panel has issued a scientific opinion. Moreover, at present, EFSA is not aware of early warning signs indicating increases in tolerance to Cry1Ab-expressing *Bt* maize in field populations of the European corn borer and Mediterranean corn borer.

Taken together, the findings reported by Trtikova et al. (2015) present no new scientific information that would invalidate the EFSA GMO Panel's previous risk assessment conclusions and recommendations on risk management of maize MON 810 or any other Cry1Ab-expressing *Bt*-maize events for which it has issued a scientific opinion. Therefore, EFSA is of the opinion that the previous risk assessment conclusions and risk management recommendations on all Cry1Ab-expressing *Bt*-maize events so far, including maize MON 810, remain valid and applicable.

Table of contents

1. Introduction

Following a request by the European Commission, the European Food Safety Authority (EFSA) assessed the scientific information reported by Trtikova et al. (2015), as well as the relevance of these findings for the risk assessment of maize MON 810 and all other Cry1Ab-expressing *Bt*-maize events for which the EFSA GMO Panel has issued a scientific opinion.

1.1. Background and Terms of Reference as provided by the requestor

EFSA is requested to evaluate the scientific paper published by Trtikova et al. (2015) and provide the European Commission with a response indicating whether *"the new scientific information contains elements that could lead the GMO panel to reconsider the outcome of its previous opinions for GM maize MON 810 and possibly other Bt crops*".

2. Data and Methodologies

2.1. Data

In delivering this technical report, EFSA considered the data and conclusions reported in the scientific publication by Trtikova et al. (2015) along with data in all Cry1Ab-expressing *Bt*-maize events for which the EFSA GMO panel has already issued a scientific opinion.

2.2. Methodologies

EFSA took into account the appropriate principles described in its guidelines for the food and feed (EFSA, 2006, 2011a) and environmental risk assessment (EFSA, 2010a) of GM plants.

3. Assessment

The EFSA assessment described in this section is composed of two parts. In the first part, the data and conclusions on maize MON 810 as reported by Trtikova et al. (2015) are presented. In the second part, the relevance of the results and discussion of this publication for the risk assessment of maize MON 810 and all other Cry1Ab-expressing *Bt*-maize events for which the EFSA GMO Panel has already delivered a scientific opinion is considered.

Maize MON 810 contains the *cry1Ab* gene (derived from *Bacillus thuringiensis* (*Bt*)) and produces the *Bt* Cry1Ab protein conferring resistance to specific lepidopteran pests such as the European corn borer (ECB), *Ostrinia nubilalis*, and the Mediterranean corn borer (MCB), *Sesamia nonagrioides* (EFSA, 2009a). Maize MON 810 is the only GM crop that is currently cultivated in the European Union.

3.1. Summary of the scientific publication

In their publication, Trtikova et al. (2015) reported on the *cry1Ab* transgene expression and protein content of the newly expressed *Bt* Cry1Ab protein in the leaves of two maize MON 810 varieties. These aspects were further analysed in the context of environmentally stressful conditions where the two maize MON 810 varieties were exposed to cold/wet and hot/dry conditions under controlled environments (climate chamber, greenhouse).

Seeds of the two maize MON 810 varieties (white *Bt*-PAN 6Q-321B and yellow *Bt*-PAN 6Q-308B, hereafter referred to as 'white maize MON 810' and 'yellow maize MON 810', respectively) were first grown under 'optimal conditions'[1] for six weeks. Following this period, they were either kept further under optimal conditions or exposed to hot/dry[2] or cold/wet[3] stressful conditions. Leaves from the same plants were sampled before stressful conditions were applied and after one week in the hot/dry and cold/wet environments. RNA and proteins were extracted from these samples and analysed to determine *cry1Ab* transgene expression and *Bt* Cry1Ab protein levels respectively.

[1] Optimal conditions: 16/8 h light/dark (L/D), 25/20 °C, relative humidity (RH) 50/65 %.
[2] Hot/dry conditions: 16/8 h L/D, 21–45 °C, RH 39–67 %, watered sparsely.
[3] Cold/wet conditions: 16/8 h L/D, 16/13 °C, 65/80 % RH, watered heavily for 24 h; afterwards soil kept saturated with water.

The results and analyses on *cry1Ab* transgene and *Bt* Cry1Ab protein levels as reported by Trtikova et al. (2015) for the white and yellow maize MON 810 varieties are summarised below:

- White maize MON 810: The data derived from the white maize MON 810 variety indicated that there was no significant difference in *cry1Ab* transgene expression levels between optimal and cold/wet conditions. In contrast, a significant reduction was observed for plants grown in a hot/dry environment. Regarding *Bt* Cry1Ab protein levels, the data indicated that, under optimal conditions, white maize MON 810 contained on average, ~40 % less protein than the yellow maize MON 810 variety. Overall *Bt* Cry1Ab protein levels remained stable for plants grown under hot/dry conditions compared with optimal conditions but increased by ~4 fold when plants were grown in a cold/wet environment.
- Yellow maize MON 810: The data obtained for the yellow maize MON 810 variety indicated that both *cry1Ab* transgene expression and overall *Bt* Cry1Ab protein levels were similar in plants grown in optimal conditions and those grown in hot/dry or cold/wet environmentally stressful conditions.

The authors concluded from their studies that, in controlled environments (climate chambers, greenhouse), *cry1Ab* transgene expression and *Bt* Cry1Ab protein levels might be influenced by factors such as genetic background and environmental conditions. Trtikova et al. (2015) extrapolated their findings to maize MON 810 grown in the field, by postulating that "*field-grown Bt maize plants might therefore not always produce high enough dose of Bt protein to kill the intermediate (heterozygous) resistant insect pests*". Based on this consideration, they hypothesised that there could be possible implications for IRM measures established in *Bt* crop cultivation areas to delay the evolution of insect resistance to *Bt* proteins (Bates et al., 2005).

3.2. Relevance of the scientific publication for the risk assessment of maize MON 810 and other Cry1Ab-expressing *Bt*-maize events

During its evaluation of the data and findings in the Trtikova et al. (2015) publication, EFSA noted that the main issues relevant for the risk assessment of GM plants concern (1) variability in *Bt* Cry1Ab protein levels in maize MON 810 and (2) the possible implications of this variability on IRM programmes for Cry1Ab-expressing *Bt*-maize plants. EFSA therefore considers that the findings in this publication are mostly relevant for the environmental risk assessment (ERA) of Cry1Ab-expressing *Bt*-maize events. EFSA's assessment below therefore focuses on the relevance for the ERA of maize MON 810 and all other Cry1Ab-expressing *Bt*-maize events for which the EFSA GMO Panel has delivered a scientific opinion (see Table 1).

1) Regarding variability in the *Bt* Cry1Ab protein levels of GM maize events producing this protein, EFSA acknowledges that several factors could affect *Bt* protein levels, including genetic background, environmental conditions and agricultural practices. Variability in *Bt* Cry1Ab protein levels is indicated by the data in GM plant market registration applications containing maize events that produce this protein, including maize MON 810, and has been considered by the EFSA GMO Panel during its assessment (Table 1). Reported *Bt* Cry1Ab protein levels in these GM maize events are derived from plants grown in field trials carried out in different seasons and across several locations, thereby accounting for diverse environmental conditions. Moreover, variability in *Bt* protein levels in maize, including Cry1Ab protein in maize MON 810, has been extensively documented in the peer-reviewed literature (Nguyen and Jehle, 2007, 2009; Adamczyk et al., 2009; Székács et al., 2010).

Table 1: Overview of GM maize applications producing the *Bt* Cry1Ab protein transgene previously assessed by the EFSA GMO Panel

GM Plant Application	Event	Scope	Reference	Range of Cry1Ab protein levels in leaves (μg/g dw)[(a),(b)]
EFSA-GMO-RX-MON 810 (8.1-ab and 20-1ab)	MON 810	Import/Processing/ Cultivation	EFSA (2009a)	25.3–34.5[(a)]
EFSA-GMO-UK-2004-01	NK603 × MON 810	Import/Processing	EFSA (2005a)	(–)
EFSA-GMO-DE-2004-03	MON863 × MON 810	Import/Processing	EFSA (2005b)	32.7–51.3[(a)]
EFSA-GMO-BE-2004-07	MON863 × MON 810 × NK603	Import/Processing	EFSA (2005c)	65.0–110.0
EFSA-GMO-CZ-2006-33	MON88017 × MON 810	Import/Processing	EFSA (2009b)	89.0–130.0
Notification C/F/96/05.10	Bt11	Cultivation	EFSA (2005d)	12.0–154.0[(b)]
EFSA-GMO-RX-Bt11 (8-1ab and 20-1ab)	Bt11	Import/Processing	EFSA (2009c)	12.0–154.0[(b)]
EFSA-GMO-UK-2007-49	Bt11 × GA21	Import/Processing	EFSA (2009d)	9.1–49.1
EFSA-GMO-UK-2007-50	Bt11 × MIR604	Import/Processing	EFSA (2010b)	12.2–29.0
EFSA-GMO-UK-2008-56	Bt11 × MIR604 × GA21	Import/Processing	EFSA (2010c)	26.1–40.6

–: no information available.
(a): In the case of values reported as μg/g fresh weight (fw), a suggested value of 70 % relative water content was used to convert to μg/g dry weight (dw) (as estimated in Gui-Rui et al., 2000).
(b): Data in these GM applications are from the same study.

2) Possible implications for insect resistance evolution due to insufficient levels of *Bt* Cry1Ab protein expressed in relevant tissues of maize MON 810, as hypothesised by Trtikova et al. (2015), would be relevant only in the context of GM plant market registration applications for cultivation. Thus, EFSA focuses its assessment of the Trtikova et al. (2015) publication on GM applications whose scope includes cultivation in the European Union and that contain Cry1Ab-expressing maize *Bt*-events, namely MON 810 and Bt11 (see Table 1). As Trtikova et al. (2015) do not present *Bt* Cry1Ab levels in a numerical form, they were estimated by EFSA from the graphical representation of the analysed protein samples. The lowest reported levels were 20–30 μg/g dw and were similar for both maize MON 810 varieties grown under optimal or stressful conditions. These values are well within the range of those reported for maize MON 810 and Bt11 events by applicants in the frame of their GM plant market registration applications for cultivation for which the EFSA GMO Panel has issued a scientific opinion (Table 1).

In addition, to ensure effective long-term ECB/MCB pest management and the sustainable use of *Bt* maize, the EFSA GMO Panel advocated an integrated pest management approach in which *Bt* maize is only one of many management options. Moreover, resistance monitoring to detect early warning signs indicating resistance evolution in the field, compliance monitoring to assess farmers' compliance with IRM requirements and education (training) programmes aiding farmers to understand the importance of adhering to IRM requirements are essential to the success of the high-dose refuge (HDR) strategy and should therefore continue to form an integral part of IRM plans for *Bt*-maize.

At present, EFSA is not aware of early warning signs indicating increases in tolerance to Cry1Ab-expressing *Bt* maize in field populations of the ECB/MCB. Annual assessments of ECB/MCB susceptibility to *Bt* Cry1Ab protein in the USA and EU have not revealed any significant change in susceptibility or identified populations that survive on Cry1Ab-expressing *Bt*-maize plants (after more than 10 years' exposure to the *Bt* Cry1Ab protein in the USA) (Farinós et al., 2004, 2011; Stodola et al., 2006; Andreadis et al., 2007; Siegfried et al., 2007; Crespo et al., 2009, 2010; EFSA, 2011b, 2012, 2013, 2014, 2015; Siegfried and Hellmich, 2012).

Based on the available information, EFSA considers that there is no scientific evidence indicating that the maize MON 810 and Bt11 events fail to meet the high-dose condition of the HDR strategy and would therefore diminish the efficacy of the HDR strategy to delay resistance evolution; the *Bt* Cry1Ab protein concentration is sufficiently high to kill a high proportion of heterozygous resistant genotypes, resulting from the mating between individuals emerging from the refuge and from the *Bt* maize field, to maintain any resistance allele in ECB/MCB populations functionally recessive.

4. Conclusions

The findings and discussion of Trtikova et al. (2015) relevant for the risk assessment of GM plants concern variability in *Bt* Cry1Ab protein levels in maize MON 810 and the possible implications of this variability on IRM measures for Cry1Ab-expressing *Bt*-maize plants.

Concerning *Bt* Cry1Ab protein expression levels, EFSA acknowledges that there can be variability in *Bt* protein levels of GM maize plants producing this protein, including maize MON 810, arising from several factors including genetic background, environmental conditions or agricultural practices. This aspect is already extensively documented in the peer-reviewed literature. It is also indicated by the data in GM plant market registration applications for Cry1Ab-expressing *Bt*-maize events derived from plants grown in field trials in a range of environmental conditions and agricultural practices and has been considered by the EFSA GMO Panel during its assessment (see Table 1 for an overview).

Regarding possible implications on IRM measures due to insufficient *Bt* Cry1Ab protein levels in *Bt*-maize plants, EFSA notes that the lowest *Bt* Cry1Ab protein levels reported by Trtikova et al. (2015) for maize MON 810 are well within the range of the levels reported for maize MON 810 and Bt11 events in the GM plant market registration applications for cultivation for which the EFSA GMO Panel has issued a scientific opinion (Table 1). Moreover, after more than 10 years' exposure to the *Bt* Cry1Ab protein in the USA and EU, no early warning signs indicating increases in tolerance to Cry1Ab-expressing *Bt*-maize in field populations of the ECB/MCB have been identified.

Taken together, the findings reported by Trtikova et al. (2015) present no new scientific information that would invalidate the EFSA GMO Panel's previous risk assessment conclusions and recommendations on risk management of maize MON 810 or any other Cry1Ab-expressing *Bt* maize events for which it has issued a scientific opinion (see Table 1 for an overview). Therefore, EFSA is of the opinion that the previous risk assessment conclusions and risk management recommendations on maize MON 810 and all other Cry1Ab-expressing *Bt*-maize events remain valid and applicable.

Documentation provided to/by EFSA

1. Letter from the European Commission to the EFSA Executive Director dated 21 May 2015, requesting scientific assistance from EFSA on new scientific information in relation to the risk assessment of GM maize MON 810 and possibly other Bt crops.

2. Letter from the EFSA Executive Director to the European Commission dated 16 June 2015, acknowledging the reception of the mandate and proposing a deadline for publication of the output.

3. Letter from the European Commission to the EFSA Executive Director dated 6 July 2015 agreeing with EFSA's publication deadline proposal.

References

Adamczyk JJ Jr. and Perera Meredith WR, 2009. Production of mRNA from the *cry1Ac* transgene differs among Bollgard® lines which correlates to the level of subsequent protein. Transgenic Research, 18, 143–149.

Andreadis SS, Álvarez-Alfageme FA, Sánchez-Ramos I, Stodola TJ, Andow DA, Milonas PG, Savopoulou-Soultani M and Castañera P, 2007. Frequency of resistance to *Bacillus thuringiensis* toxin Cry1Ab in Greek and Spanish population of *Sesamia nonagrioides* (Lepidoptera: Noctuidae). Journal of Economic Entomology, 100, 195–201.

Bates SL, Zhao JZ, Roush RT and Shelton AM, 2005. Insect resistance management in GM crops: past, present and future. Nature Biotechnology 23, 57–62.

Crespo ALB, Spencer TA, Alves AP, Hellmich RL, Blankenship EE, Magalhães LC and Siegfried BD, 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.

Crespo ALB, Spencer TA, Tan SY and Siegfried BD, 2010. Fitness costs of Cry1Ab resistance in a field-derived strain of *Ostrinia nubilalis* (Lepidoptera: Crambidae). Journal of Economic Entomology, 103, 1386–1393.

EFSA (European Food Safety Authority), 2005a. Scientific Opinion of the Panel on Genetically Modified Organisms (GMO Panel) on an application (Reference EFSA-GMO-UK-2004-01) for the placing on the market of glyphosate-tolerant and insect-resistant genetically modified maize NK603 x MON 810, for food and feed uses under Regulation (EC) No 1829/2003 from Monsanto. The EFSA Journal 2005, 309, 1-22. doi:10.2903/j.efsa.2005.309

EFSA (European Food Safety Authority), 2005b. Scientific Opinion of the Panel on Genetically Modified Organisms (GMO Panel) on an application (Reference EFSA-GMO-DE-2004-03) for the placing on the market of insect protected genetically modified maize MON 863 x MON 810, for food and feed use, under Regulation (EC) No 1829/2003 from Monsanto. The EFSA Journal 2005, 252, 1-25. doi:10.2903/j.efsa.2005.252

EFSA (European Food Safety Authority), 2005c. Scientific Opinion of the Panel on Genetically Modified Organisms (GMO Panel) on an application (Reference EFSA-GMO-BE-2004-07) for the placing on the market of insect-protected glyphosate-tolerant genetically modified maize MON863 × MON810 × NK603, for food and feed uses, and import and processing under Regulation (EC) No 1829/2003 from Monsanto. The EFSA Journal 2005, 256, 1-25. doi:10.2903/j.efsa.2005.256

EFSA (European Food Safety Authority), 2005d. Scientific Opinion of the Panel on Genetically Modified Organisms (GMO Panel) related to the notification for the placing on the market of insect resistant genetically modified maize Bt11, for cultivation, feed and industrial processing under Part C of Directive 2001/18/EC from Syngenta Seeds. The EFSA Journal 2005, 213, 1-33. doi:10.2903/j.efsa.2005.213

EFSA (European Food Safety Authority), 2006. Guidance document of the Panel on Genetically Modified Organisms (GMO Panel) for the risk assessment of genetically modified plants and derived

food and feed - including draft document updated in 2008. The EFSA Journal 2006, 99, 1-100. doi:10.2903/j.efsa.2006.99

EFSA (European Food Safety Authority), 2009a. Applications (EFSA-GMO-RX-MON 810) 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 of (3) food and feed additives, and feed materials produced from maize MON 810, all under Regulation (EC) No 1829/2003 from Monsanto. The EFSA Journal 2009, 1149, 1-85. doi:10.2903/j.efsa.2009.1149

EFSA (European Food Safety Authority), 2009b. Application (Reference EFSA-GMO-CZ-2006-33) for the placing on the market of the insect-resistant and glyphosate-tolerant genetically modified maize MON 88017 × MON 810, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. The EFSA Journal 2009, 1192, 1-27. doi:10.2903/j.efsa.2009.1192

EFSA (European Food Safety Authority), 2009c. Scientific Opinion on application reference EFSA-GMO-RX-Bt11 for renewal of the authorisation of existing products produced from insect-resistant genetically modified maize Bt11, under Regulation (EC) No 1829/2003 from Syngenta. The EFSA Journal 2009, 977, 1-13. doi:10.2903/j.efsa.2009.977

EFSA Panel on Genetically Modified Organisms (GMO), 2009d. Scientific Opinion on application (EFSA-GMO-UK-2007-49) for the placing on the market of the insect resistant and herbicide tolerant genetically modified maize Bt11xGA21 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal 2009;7(9):1319, 27 pp. doi:10.2903/j.efsa.2009.1319

EFSA Panel on Genetically Modified Organisms (GMO), 2010a. Guidance on the environmental risk assessment of genetically modified plants. EFSA Journal 2010;8(11):1879, 111 pp. doi:10.2903/j.efsa.2010.1879

EFSA Panel on Genetically Modified Organisms (GMO), 2010b. Scientific Opinion on application (Reference EFSA-GMO-UK-2007-50) for the placing on the market of insect resistant and herbicide tolerant genetically modified maize Bt11xMIR604, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Syngenta Seeds. EFSA Journal 2010;8(5):1614, 30 pp. doi:10.2903/j.efsa.2010.1614

EFSA Panel on Genetically Modified Organisms (GMO), 2010c. Scientific Opinion on application (Reference EFSA-GMO-UK-2008-56) for the placing on the market of insect resistant and herbicide tolerant genetically modified maize Bt11 × MIR604 × GA21, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Syngenta Seeds. EFSA Journal 2010;8(5):1616, 30 pp. doi:10.2903/j.efsa.2010.1616

EFSA Panel on Genetically Modified Organisms (GMO), 2011a. Guidance for risk assessment of food and feed from genetically modified plants. EFSA Journal 2011;9(5):2150, 37 pp. doi:10.2903/j.efsa.2011.2150

EFSA Panel on Genetically Modified Organisms (GMO), 2011b. Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2009. EFSA Journal 2011;9(10):2376, 66 pp. doi:10.2903/j.efsa.2011.2376

EFSA Panel on Genetically Modified Organisms (GMO), 2012. Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2010. EFSA Journal 2012;10(3):2610, 35 pp. doi:10.2903/j.efsa.2012.2610

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2013. Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2011. EFSA Journal 2013;11(12):3500, 38 pp. doi:10.2903/j.efsa.2013.3500

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2014. Scientific Opinion on the annual post-market environmental monitoring (PMEM) report from Monsanto Europe S.A. on the

cultivation of genetically modified maize MON 810 in 2012. EFSA Journal 2014;12(6):3704, 29 pp. doi:10.2903/j.efsa.2014.3704

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2015. Scientific Opinion on the annual post-market environmental monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2013. EFSA Journal 2015;13(3):4039, 11 pp. doi:10.2903/j.efsa.2015.4039

Farinós GP, de la Poza M, Hernández-Crespo P, Ortego F and Castañera 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.

Farinós GP, Andreadis SS, de la Poza M, Mironidis GK, Ortego F, Savopoulou-Soultani M and Castañera P, 2011. Comparative assessment of the field-susceptibility of *Sesamia nonagrioides* to the Cry1Ab toxin in areas with different adoption rates of Bt maize and in Bt-free areas. Crop Protection, 30, 902–906.

Gui-Rui Y, Takuji M, Keiichi N, Nobuhiro M and Hisashi K, 2000. Proposal for universal formulas for estimating leaf water status of herbaceous and woody based on spectral reflectance properties. Plant and Soil 227(1), 47–58.

Nguyen HT and Jehle JA, 2007. Quantitative analysis of the seasonal and tissue-specific expression of Cry1Ab in transgenic maize MON 810. Journal of Plant Diseases and Protection, 114, 820–887.

Nguyen H and Jehle JA, 2009. Expression of Cry3Bb1 in transgenic corn MON88017. Journal of Agriculture and Food Chemistry, 57, 9990–9996.

Siegfried BD and Hellmich RL, 2012. Understanding successful resistance management: The European corn borer and Bt corn in the United States. GM Crops & Food: Biotechnology in Agriculture and the Food Chain, 3, 184–193.

Siegfried BD, Spencer T, Crespo AL, Storer NP, Head GP, Owens ED and Guyer D (2007). Ten years of Bt resistance monitoring in the European corn borer: what we know, what we don't know, and what we can do better. American Entomologist, 53, 208–214.

Stodola TJ, Andow DA, Hyden AR, Hinton JL, Roark JJ, Buschman LL, Porter P and Cronholm GB, 2006. Frequency of resistance to *Bacillus thuringiensis* toxin Cry1Ab in southern United States corn belt population of European corn borer (Lepidoptera: Crambidae). Journal of Economic Entomology, 99, 502–507.

Székács A Lauber E Juracsek J and Darvas B, 2010. Cry1Ab toxin production of MON 810 transgenic maize Environmental Toxicology and Chemistry, 29, 182–190.

Trtikova M, Wikmark OG, Zemp N, Widmer A and Hilbeck A, 2015.Transgene expression and Bt protein content in transgenic Bt maize (MON 810) under optimal and stressful environmental conditions. PLoS ONE, 10(4): e0123011.