Annex 3 of EFSA (2022) – Scientific publications relevant to the food and feed and environmental safety of maize MON 810 assessed by EFSA as part of the 2020 post-market environmental monitoring report. Table 1 provides an overview on the articles and their evaluation by EFSA. For further details with summaries of the articles are provided in the text following the table.

Table 1: List of relevant scientific publications and their evaluation by EFSA

| Relevant area: Environmental safety assessment |
|------------------------------------------------|
| **1. Camargo AM, Arias-Martin M, Castanera P and Farinos GP, 2020.** Performance of *Sesamia nonagrioides* on cultivated and wild host plants: Implications for Bt maize resistance management. Pest Management Science, 1-10. |
| The assessment of this publication does not point to new hazards, modified exposure, or new scientific uncertainties that would change former risk assessment conclusions on and risk management recommendations for maize MON 810. |
| **2. Garcia-Ruiz E, Cobos G, Sanchez-Ramos I, Pascual S, Chueca MC, Escorial MC, Santin-Montanya I, Loureiro I and Gonzalez-Nunez M, 2020.** Dynamics of canopy-dwelling arthropods under different weed management options, including glyphosate, in conventional and genetically modified insect-resistant maize. Insect Science, 00, 1-18. |
| The assessment of this publication does not point to new hazards, modified exposure, or new scientific uncertainties that would change former risk assessment conclusions on and risk management recommendations for maize MON 810. |
| **3. Visser A, du Plessis H, Erasmus A and van de Berg J, 2020a.** Larval migration behaviour of *Busseola fusca* (Lepidoptera: Noctuidae) on Bt and non-Bt maize under semi-field and field conditions. Insects, 11, 16, 1-24. |
| EFSA concludes that the Lepidoptera *B. fusca* is not currently present in the EU and, thus, it is not targeted by maize MON 810 plants cultivated in the EU. Therefore, the findings reported by Visser et al. (2020a) on this maize pest are of no direct relevance to the cultivation of maize MON 810. The publication by Visser et al. (2020a) does not change former risk assessment conclusions on and risk management recommendations for maize MON 810. |
| **4. Visser A, Du Plessis H, van den Berg J and Erasmus A, 2020b.** Plant Abandonment by *Busseola fusca* (Lepidoptera: Noctuidae) Larvae: Do Bt Toxins Have an Effect? Insects, 77, 1-11. |
| The study was evaluated previously in EFSA 2021¹. |

¹ EFSA (European Food Safety Authority), Álvarez F, Georgiadis M, Messéan A and Streissl F, 2021. Assessment of the 2019 post-market environmental monitoring report on the cultivation of genetically modified maize MON 810 in the EU. EFSA Journal 2021:19(7):6683, 39 pp. [https://doi.org/10.2903/j.efsa.2021.6683](https://doi.org/10.2903/j.efsa.2021.6683)
5. Lohn AF, Trtikova M, Hilbeck AC, Van den Berg IJ and du Plessis H, 2020. Transgene behavior in *Zea mays* L. crosses across different genetic backgrounds: Segregation patterns, cry1Ab transgene expression, insecticidal protein concentration and bioactivity against insect pests. *Environmental Toxicology and Chemistry*, 00, 1-9.

The methodology is appropriate and well described. The statistical evaluation of the data supports the conclusions of the authors. Cry1Ab transgene can successfully outcross to other maize varieties with stable expression of bioactive BT toxins. No correlation was found between gene expression, protein concentrations and insect mortality. The assessment of this publication does not point to new hazards, modified exposure, or new scientific uncertainties that would change former risk assessment conclusions on and risk management recommendations for maize MON 810.

Supporting information, Annex 3, [https://efsa.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.2903%2Fj.efsa.2021.6683&file=efs26683-sup-0003-Annex_3.pdf](https://efsa.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.2903%2Fj.efsa.2021.6683&file=efs26683-sup-0003-Annex_3.pdf)
Environmental safety assessment

Camargo AM, Arias-Martin M, Castanera P and Farinos GP, 2020. Performance of Sesamia nonagrioides on cultivated and wild host plants: Implications for Bt maize resistance management. Pest Management Science, 1-10.

Summary of the publication

The aims of the study were (i) to analyse the suitability of cultivated (rice and sorghum) and wild (Johnsongrass, cattail, common reed and giant reed) plants for larval development and oviposition of the corn borer Sesamia nonagrioides compared to maize, for which is considered to be a major pest in the Mediterranean basin, and (ii) to evaluate their potential role in delaying resistance development to genetically modified insect-resistant maize varieties (Bt maize).

Laboratory bioassays with excised parts or whole plants were performed to assess the suitability for S. nonagrioides oviposition of the above-mentioned plants. Additional bioassays (choice and no choice) were conducted to evaluate feeding preference by S. nonagrioides larvae.

The bioassays conducted with plant pieces or whole plants showed that the larval cycle could only be completed in the three cultivated plants and in Johnsongrass. Females showed a strong preference for ovipositing on maize in comparison with sorghum or rice. Although young larvae consumed more sorghum than maize in two-choice bioassays, both larvae and adults had a better performance in terms of shorter larval period and higher pupal weight, fecundity and fertility) when larvae fed on maize throughout their larval stage than when they fed on sorghum or rice.

The authors concluded that none of the alternative hosts of S. nonagrioides tested in the study should be considered as natural unstructured refuges within the high-dose refuge (HDR) strategy for Bt maize to prevent/delay resistance evolution in target species.

Evaluation by EFSA

The study of Camargo et al. (2020) is relevant to assess some of requirements that support the current resistance management strategy implemented in the EU. The methodology is appropriate and well-described.

The assessment of this publication does not point to new hazards, modified exposure, or new scientific uncertainties that would change former risk assessment conclusions on and risk management recommendations for maize MON 810. The findings of the study confirm that refuge areas should continue to be composed of non-Bt maize plants and support the some of the EFSA’s recommendations on insect resistance management, i.e., the importance of increasing growers’ compliance with refuge requirements, specially in areas of high adoption rate of Bt maize varieties like north-eastern Spain (Ebro valley).
Summary of the publication

The authors investigated potential impacts of genetically modified maize varieties, i.e., herbicide-tolerant and insect-resistant, in combination with different weed management options, including glyphosate application, on non-target arthropods under field conditions. The study included conventional and Bt maize varieties derived from the event MON 810, which produces the insecticidal Cry1Ab protein from Bacillus thuringiensis (Bt). Plant-dwelling arthropods were sampled by visual inspection of entire maize plants and yellow sticky traps from June/July until October/November over three consecutive growing seasons (2012-2014) in maize fields located in central Spain. In addition, the occurrence of the target corn borers Sesamia nonagrioides and Ostrinia nubilalis (target pests of Bt maize varieties derived from event MON 810) and secondary lepidopteran was evaluated in 2013 and 2014 in targeted samplings.

The composition of phytophagous, predatory and parasitic communities in the study was very similar to other maize field studies in Spain. No significant differences were observed in the overall abundance of non-target arthropods between conventional (non-Bt) and Bt maize varieties. The authors concluded that the Bt maize variety had no effect on any group of studied arthropods, excepted for the corn borers.

Evaluation by EFSA

For this mandate, the publications by Garcia-Ruiz et al. (2020) is relevant to assess potential effects of maize MON 810 plants on canopy-dwelling non-target arthropods under field conditions. The methodology is appropriate and well-described. The statistical evaluation of the data supports the conclusions of the authors. The findings of the study are in line with other maize field studies.

The assessment of this publication does not point to new hazards, modified exposure, or new scientific uncertainties that would change former risk assessment conclusions on and risk management recommendations for maize MON 810.
Visser A, du Plessis H, Erasmus A and van de Berg J, 2020a. Larval migration behaviour of *Busseola fusca* (Lepidoptera: Noctuidae) on Bt and non-Bt maize under semi-field and field conditions. *Insects*, 11,16, 1-24.

Summary of the publication

**Aim**

The authors investigated the effects of the presence of *Bt* toxin and plant growth stage on the migration behaviour of Cry1Ab-resistant *B. fusca* larvae under semi-field conditions and the effect of *Bt* toxin and plant density on the migration behaviour of *B. fusca* larvae under field conditions with the aim to inform IPM strategies.

**Material and methods**

Larvae were placed on a central plant and the damage and distribution of larvae over time in the inoculated plant and the plants in the same row and the adjacent rows was monitored weekly until the end of the experiment after 5 weeks (semi-field experiment) and 3 weeks (field experiment) respectively.

**Results**

The number and severity of damaged plants increased over the first three weeks after inoculation of larvae onto plants. Larvae did not migrate beyond week 3 to other plants in the greenhouse experiment. The inoculated plant and one or two plants adjacent were affected until the end of the experiment. The distance of affected plants from the inoculated plant was always higher in the same row as the inoculated plant and less in the adjacent rows. In the field experiments no larvae were found in adjacent rows. The growth stage of the plants also affected the migration as older plants are less likely to be successfully colonised by *B. fusca* larvae. Higher damage (although not statistically significant) was observed in non-Bt maize plots in the greenhouse and no difference in damages was observed in the field.

**Conclusions**

The study showed that migration of larvae occurs but that it is limited and only on short distances and that larval mortality is high. The results can be used to develop targeted integrated resistance management measures for *B. fusca*.

**Evaluation by EFSA**

The lepidoptera *B. fusca* is not currently present in the EU and, thus, it is not targeted by maize MON 810 plants cultivated in the EU. Therefore, the findings reported by Visser et al. (2020a) on this maize pest are of no direct relevance to the cultivation of maize MON 810. The publication by Visser et al. (2020a) does not change former risk assessment conclusions on and risk management recommendations for maize MON 810.
Visser A, Du Plessis H, van den Berg J and Erasmus A, 2020b. Plant Abandonment by Busseola fusca (Lepidoptera: Noctuidae) Larvae: Do Bt Toxins Have an Effect? Insects, 77, 1-11.

The study of Visser et al. 2020b was already evaluated by EFSA previously and no new hazards, modified exposure, or new scientific uncertainties that would change former risk assessment conclusions on and risk management recommendations for maize MON 810 were identified (see Annex 3, supporting information to EFSA 2021²).

² EFSA (European Food Safety Authority), Álvarez F, Georgiadis M, Messéan A and Streissl F, 2021. Assessment of the 2019 post-market environmental monitoring report on the cultivation of genetically modified maize MON 810 in the EU. EFSA Journal 2021:19(7):6683, 39 pp. https://doi.org/10.2903/j.efsa.2021.6683
Supporting information, Annex 3,
https://efsa.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.2903%2FFj.efsa.2021.6683&file=efs26683-sup-0003-Annex_3.pdf
Food and feed safety assessment

Lohn AF, Trtikova M, Hilbeck AC, Van den Berg IJ and du Plessis H, 2020. Transgene behavior in Zea mays L. crosses across different genetic backgrounds: Segregation patterns, cry1Ab transgene expression, insecticidal protein concentration and bioactivity against insect pests. Environmental Toxicology and Chemistry, 00, 1-9.

Summary of the publication

Aim

The relationship between transgene expression rates and the concentrations of the Bt protein and its bioactivity against insect pests in non-GM maize hybrids following transgene flow from GM hybrid maize into the non-GM maize (near-isogenic maize and open pollinating varieties).

Materials and methods

Crosses and successive backcrosses between MON810/AG5011YG, non-GM near isogenic maize (AG5011) and open pollinating variety (OPV) Pixurum 5 from Brazil and between MON 810, variety PAN6Q 308B, non-GM near isogenic maize variety PAN6P-110 and OPV Kalahari were generated. Seedlings of F1 and backcrosses with confirmed Bt expression were chosen for the experiments.

Samples were taken to determine Cry1Ab concentrations by ELISA and transgene expression by qRT-PCR two weeks after planting. Leaf pieces collected from the same plants were used for the bioassay feeding trials with second instar larvae of the two lepidopteran species Helicoverpa armigera and Spodoptera littoralis. Larvae mortality was recorded four days after the start of the experiment.

Results

The Cry1Ab transgene outcrossed and was stably expressed in both ISO and OPV genetic backgrounds. In South African crosses significant differences were observed in relative transgene expression but not in Brazilian crosses. The Bt toxin concentrations were variable but similar to the parental GM maize. The mortality in H. armigera and S. littoralis larvae were >92.19% and ≥ 40.63%, respectively. No significant correlation was found between the quantity of mRNA for Cry1Ab and corresponding Cry1Ab protein concentrations and between Cry1Ab concentrations and insect mortality rates.

Conclusions

The authors conclude that Cry1Ab transgene can successfully outcross into different maize varieties and genetic backgrounds with most segregations following a Mendelian segregation pattern. The transgene is stably expressed and the Bt toxins were expressed in a bioactive form. No consistent correlation could be found between gene expression, protein concentrations and insect mortality. The results obtained with the crosses from South African plants suggest an effect of the genetic background on transgene expression. The concentration of mRNA does not seem to be a reliable indicator of the Bt protein product concentration or its bioactivity. Further work is needed to investigate the underlying causes of the observations.
**Evaluation by EFSA**

The methodology is appropriate and well-described. The statistical evaluation of the data supports the conclusions of the authors. Cry1Ab transgene can successfully outcross to other maize varieties with stable expression of bioactive *Bt* proteins. No correlation was found between gene expression, protein concentrations and insect mortality. The assessment of this publication does not point to new hazards, modified exposure, or new scientific uncertainties that would change former risk assessment conclusions on and risk management recommendations for maize MON 810.