Annex 3 of EFSA (2021) – Scientific publications relevant to the food and feed and environmental safety of maize MON 810 assessed by EFSA as part of the 2019 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. du Pisanie A, du Preez L, van den Berg J and Pieters R, 2019. The rate of release of Cry1Ab protein from Bt maize leaves into water. Short Communication, 45, 710-715. The results indicate a general trend to increased leaching of Cry1Ab at higher temperatures and that concentrations increased at all temperatures and water types until the end of the experiments after 384 hours. No measurement of Cry1Ab in leaves was conducted and therefore it is not possible to derive exact leaching values and makes it also difficult to compare the outcome with results from other studies published in literature. 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. Fernandes MG, Costa EN, Dutra CC and Raizer J, 2019. Species Richness and Community Composition of Ants and Beetles in Bt and non-Bt Maize Fields. Environmental Entomology, 48(5), 1095–1103. The methods applied are appropriate for the aim of the study. The statistical evaluation supports the conclusion that Bt and non-Bt maize fields were not different in regard to species richness of ants and coleoptera. 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. Shogren AJ, Tank JL, Rosi EJ, Martha MD, Shannon LS, Bolster D and Scott PE, 2019. Transport and instream removal of the Cry1Ab protein from genetically engineered maize is mediated by biofilms in experimental streams. Plos One, 1-22. The methods applied are suitable for the aim of the study and the statistical evaluation supports the conclusions of the authors. 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.

4. **Shu Y, Du Y and Wang J, 2019. Presence of Cry1Ab in the Bt maize - aphid (*Rhopalosiphum maidis*) - ladybeetle (*Propylea japonica*) system has no adverse effects on insect biological parameters. Entomologia Experimentalis et Applicata, 167, 1-8.**

   The methodology followed by Shu et al. (2019) is suitable to evaluate the prey-mediated effects of Cry1Ab expressed in the maize events MON 810 and Bt11 on the predatory ladybird beetle *P. japonica*. The results reported by Shu et al. (2019) show that the insecticidal protein Cry1Ab expressed in the maize events Bt11 and MON 810 does not have negative effects after *P. japonica* larvae when preying aphids maintained on maize Bt11 and MON 810 plants. The findings presented in the publication are consistent with those reported in similar tritrophic experimental set ups. Thus, the results do 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.

5. **Szoboszlay M, Naether A, Mullins E and Tebbe CC, 2019. Annual replication is essential in evaluating the response of the soil microbiome to the genetic modification of maize in different biogeographical regions. Plos One, 1-23**

   The methodology is appropriate and well described. The statistical evaluation of the data supports the conclusions of the authors. 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.

6. **Visser A, du Plessis H, Erasmus A and van den Berg J, 2019. Preference of Bt-resistant and susceptible *Busseola fusca* moths and larvae for Bt and non-Bt maize. Entomologia Experimentalis et Applicata, 167, 849-867.**

   EFSA notes that the noctuid *B. fusca* is currently not present in Europe. EFSA concludes that the publication by Visser et al. (2019) does not change former risk assessment conclusions on and risk management recommendations for maize MON 810. 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. (2019) on this maize pest are of no direct relevance to the cultivation of maize MON 810.

7. **Visser A, Du Plessis H, van den Berg J and Erasmus A, 2020. Plant Abandonment by *Busseola fusca* (Lepidoptera: Noctuidae) Larvae: Do Bt Toxins Have an Effect? Insects, 77, 1-11.**

   EFSA notes that the noctuid *B. fusca* is currently not present in Europe. EFSA concludes that the publication by Visser et al. (2020) does not change former risk assessment conclusions on and risk management recommendations for maize MON 810. 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. (2020) on this maize pest are of no direct relevance to the cultivation of maize MON 810.

8. **Xu H, Wang X, Chi G, Tan B and Wang J, 2019. Effects of *Bacillus thuringiensis* Genetic Engineering on Induced Volatile Organic Compounds Emission in Maize and the Attractiveness to a Parasitic Wasp. Frontiers in Bioengineering and Biotechnology, 1-9.**

   The methods applied are appropriate for the objective of the study and the statistical evaluation supports the conclusions of the authors. The same volatile compounds were observed in BT and non-BT maize cultivars. The amounts of volatile compounds were greater in one of the two BT maize cultivars. However, no difference was observed regarding the attractiveness of the different cultivars when damaged and treated with caterpillar regurgitant or jasmonic acid to the parasitoid *T. ostriniae*. 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.

Relevant area: Comparative assessment

9. **Holderbaum DF, Traavik TI, Onofre Nodari R and Guerra MP, 2019. Comparison of in vitro callus-cultures from transgenic maize AG-5011YG (MON810) and conventional near-isogenic maize AG-5011. Crop Breeding and Applied Biotechnology, 19, 169-175.**

   The approach aims at identifying under *in-vitro* conditions phenotypic differences between maize hybrid carrying MON810 event and the near isogenic line. It is considered that potential unintended effects identified in MON810 maize hybrid under *in-vitro* conditions, cannot be considered directly relevant for the ERA and FF RA of maize MON810. It is considered that the identified effects on an undifferentiated tissue (callus) cannot be translated to a plant grown under natural conditions. It cannot be excluded that the observed changed response to 2,4-D is attributed to differences elsewhere in the genome and not specifically to the alteration due to the recombinant construct's integration. In conclusion the article does not provide information pointing to new hazards, modified exposure, or new scientific uncertainties that would change former risk assessment conclusions on and risk management recommendations

Relevant area: Food and feed safety assessment

10. **Al-Harbi A, Sahira L, Edwards MG, Qusti S, Cockburn A, Poulsen M and Gatehouse AMR, 2019. A proteomic-based approach to study underlying molecular responses of the small intestine of Wistar rats to genetically modified corn (MON810). Transgenic Res, 28, 479-498**.

    The methods applied are appropriate for the aim of the study. The results support the conclusions of the authors. 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.

11. **Corujo M, Pla M, van Dijk J, Voorhuijzen M, Staats M, Slot M, Lommen A, Barros E, Nadal A, Puigdomènech P, La Paz JL, van der Voet H and Kok E, 2019. Use of omics analytical methods in the study of genetically modified maize varieties tested in 90 days feeding trials. Food Chemistry, 292, 359-371.**

    The study was evaluated previously in EFSA 2020[1]

12. **Coumoul X, Servien R, Juricek L, Kaddouch-Amar Y, Lippi Y, Berthelot L, Naylies C, Morvan ML, Antignac JP, Desdoits-Lethimonier C, Jegou B, Tremblay-Franco M, Canlet C, Debrauwer L, Le Gall C, Laurent J, Gouraud PA, Cravedi JP, Jeunesse E, Savy N, Dandere-Abdoulkarim K, Arnich N, Fourès F, Cotton J, Broudin S, Corman B, Moing A, Laporte B, Richard Forget F, Barouki R, Rogowsky P and Salles B, 2019. The GMO90+ Project: Absence of Evidence for Biologically Meaningful Effects of Genetically Modified Maize-based Diets on Wistar Rats After 6-Months Feeding Comparative Trial. Toxicological Sciences, 168(2), 315-338.**

    The study was evaluated previously in EFSA 2020[1]

13. **Mesnage R, Biserni M, Antoniou MN, Le Roy C and Salles B, 2019. Relationship between faecal microbiota and plasma metabolome in rats fed NK603 and MON810 GM maize from the GMO90+ study. Food and Chemical Toxicology, 131, 1-8.**

    The authors used appropriate methodologies. 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.

14. **Stein T, GuangYao R, Bohmer M, Sharbati S and Einspanier R, 2019. Expression profiling of key pathways in rat liver after a one-year feeding trial with transgenic maize MON810. Scientific Reports, 9, 1-10.**

    The methods applied are appropriate for the objective of the study. The results support the conclusion that long-term feeding of MON810 does not lead to significant alterations in rat liver gene expression. 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

[1] EFSA (European Food Safety Authority), Álvarez F, Georgiadis M, Messéan A and Streissl F, 2020. Assessment of the 2018 post-market environmental monitoring report on the cultivation of genetically modified maize MON 810 in the EU. EFSA Journal 2020;18(10):6245, 42 pp. https://doi.org/10.2903/j.efsa.2020.6245, Supporting information, Annex 4, https://efsa.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.2903%2Fj.efsa.2020.6245&file=efs26245-sup-0004-Annex-4.pdf

Environmental safety assessment

du Pisanie A, du Preez L, van den Berg J and Pieters R, 2019. The rate of release of Cry1Ab protein from Bt maize leaves into water. Short Communication, 45, 710-715.

Summary of the publication

Aim:

The study was conducted as a pilot study for more comprehensive studies on aquatic exposure to Cry proteins. The release rate of Cry1 Ab proteins from water-submerged Bt maize leaves was investigated under different temperature regimes and water conditions.

Material and methods:

Pieces of dried BT and non-BT maize leaves (24 g) were submerged in water (1L) (distilled or borehole water) at temperatures of 10, 21 and 30 °C for 1 - 384 hours. The content of Cry protein in the water was measured with an ELISA kit.

Results:

The Cry1Ab concentrations in both water types increased over time until the end of the study after 384 hours. The concentrations were throughout the experiment the highest in the 30° C ambient temperature reaching the maximum concentration of 39.8 ng/mL and 54.8 ng/mL in borehole water and deionised water, respectively. The maximum concentrations at 21 °C and 10°C were 16.6 ng/mL and 14.8 ng/mL in deionised water and 23.8 ng/mL and 10.5 ng/mL. No significant differences in measured concentrations were found between the two water types. The concentrations were statistically significantly higher at 30°C compared to 21°C and 10°C.

Evaluation by EFSA

The results indicate a general trend to increased leaching of Cry1Ab at higher temperatures and that concentrations increased at all temperatures and water types until the end of the experiments after 384 hours. No measurement of Cry1Ab in leaves was conducted and therefore it is not possible to derive exact leaching values and makes it also difficult to compare the outcome with results from other studies published in literature.

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.

Fernandes MG, Costa EN, Dutra CC and Raizer J, 2019. Species richness and community composition of ants and beetles in Bt and non-Bt maize fields. Environmental Entomology, 48: 1095–1103.

Summary of the publication

Aim

The aim of the study was to investigate potential differences in ant and coleoptera species richness and composition of Bt maize fields and conventional maize fields.

Material and methods

Thirteen different maize growing sites in Brazil were investigated over two years. Fields were cultivated according to good agricultural practise including the choice of appropriate insecticides to control the target pests. Insecticides were sprayed when the target pests reached levels of 30% above the control level established for maize IPM to minimize effects of insecticides on the studied insects. Bt and non-Bt maize was grown next to each other on the same field. Insects were collected with modified pitfall traps and determined by taxonomic specialists. A paired t-test was applied to compare BT and non-BT maize for their species richness (Chao 2 index). Nonmetric multidimensional scaling (NMDS) was conducted to examine the gradient of species composition of ants and ground beetles in maize crops. A multivariate analysis of variance (MANOVA) was performed to check whether there was any influence of site (county), cropping season (first vs second) and technology (Bt vs non-Bt) on the species composition.

Results

In total 25 and 26 ant species were found. Few ant species were found only in Bt or in non-Bt maize. Seventy-four coleoptera species were collected in total. With 5 (7 in the second growing season) species occurring only in Bt maize and 12 (8 in the second growing season) species occuring only in non-Bt maize. No statistically significant difference was observed between Bt and non-Bt maize regarding species richness. No separation of ants or coleoptera species in Bt and non-Bt maize was observed by NMDS analysis and MANOVA did not indicate an influence of Bt on ant or coleoptera species composition. The MANOVA resulted in significant differences in coleoptera species in the different counties and cropping season.

Conclusion

No difference was observed for Bt and non-Bt maize regarding species richness of ants and coleoptera. For coleoptera species there seems to be the characteristics of the farms, and abiotic factors which can explain differences in species composition. The greater variety and number of insecticides applications in non-Bt maize did not result in a significant difference between BT and non-BT which could be due to the compliance with IMP principles.

Evaluation by EFSA

The methods applied are appropriate for the aim of the study. The statistical evaluation supports the conclusion that Bt and non-Bt maize fields were not different in regard to species richness of ants and coleoptera.

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.

Shogren AJ, Tank JL, Rosi EJ, Martha MD, Shannon LS, Bolster D and Scott PE, 2019. Transport and instream removal of the Cry1Ab protein from genetically engineered maize is mediated by biofilms in experimental streams. Plos One, 1-22.

Summary of the publication

Aim

The aim of the study was to investigate the influence of biofilm on the degradation and transport of Cry1Ab protein from GMO maize in experimental streams.

Material and methods

Cry1Ab proteins were released monthly from June to October in four experimental streams with sand, pea gravel, cobble and an equal mix of all substrates. All substrates were bare when the experiment started in June. After 159 days of undisturbed biofilm growth manual sloughing was perfomed to simulate a disturbance event where all biofilm was removed. The Cry1Ab release was continued after the disturbance event. Stream width, water veolicity were measured daily. Benthic samples of were taken before each Cry1Ab protein release to estimate the algal biomass and organic matter content as ash free dry mass (AFDM). The percent cover of filamentous green algae, terrestrially-derived organic matter and benthic algal biofilm was estimated. At the termination of the experiment the total organic matter was estimated using manual collection as well as nets placed at the bottom of each experimental stream section. Water temperature and dissolved oxygen were recorded every 10 minutes. The primary productivity, ecosystem respiration and gas exchange rate (K) was calculated using the oxygen concentrations upstream and downstream and the photosynthetic active radiation.

Water samples were taken every 10 m from the release site. The samples were concentrated and the Cry1Ab content measued by ELISA. The removal of Cry1Ab from the water column was calculated as uptake of Cry1Ab over a certain distance of travelling in the stream (uptake length Sw). The uptake length (Sw) is strongly dependent on the flow. Therefore, it was converted to an uptake velocity (m^2s^{-1}). ANOVAs and Tukey`s HSD post-hoc test were used to evaluate significant differences among streams. Cry1Ab removal rates were calculated only for releases with significant uptake regressions and substrate driven differences were evaluated by comparing the uptake metrics across streams with ANCOVA.

Results

Significant Cry1Ab uptake was found during times when biofilm had colonized substrate in each stream. This suggests that streams can remove Cry1Ab from the water column either via physical retention (e.g. sorption) or via biological removal (e.g. as a source of dissolved organic matter for heterotroph micro-organisms). Biofilm chlorophyll a and % cover of filamentous green algae and increasing heterotrophic metabolism were positively correlated with uptake velocity. No Cry1Ab uptake was measured on the first day of the experiment and after the disturbance. The highest Cry1Ab uptake was observed at the peak of biofilm colonization. The substrate itself had limited effects on Cry1Ab removal and Cry1Ab uptake was strongly influenced by biofilm colonization.

The authors modelled water concentrations by combining the empirically derived removal of Cry1Ab and previously published degradation coefficients from mesocosm studies. The starting concentration was 200 ng L^{-1} Cry1Ab which represents Cry1Ab concentrations in a Midwestern stream. The model resulted when considering only degradation in concentrations of Cry1Ab below analytical detection limits after 2 and 14 days in the high and low biofilm

scenario, respectively. When accounting for the additional role of biological uptake (degradation + uptake) then the concentrations were below the detection limits after 1 and 6 days in the high and low biofilm scenario, respectively.

Conclusions

The empirical measurement and the modelling support the hypothesis that instream removal of Cry1Ab may be an important mechanism for removal of leached Cry1Ab from the water column, reducing the available pool of the protein susceptible to downstream transport.

Evaluation by EFSA

The methods applied are suitable for the aim of the study and the statistical evaluation supports the conclusions of the authors.

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.

Shu Y, Du Y and Wang J, 2019. Presence of Cry1Ab in the Bt maize - aphid (*Rhopalosiphum maidis*) - ladybeetle (*Propylea japonica*) system has no adverse effects on insect biological parameters. Entomologia Experimentalis et Applicata, 167, 1-8.

Summary of the publication

Aim

The authors performed several laboratory bioassays under environmental controlled conditions to evaluate lethal and sub-lethal effects of two Cry1Ab-expressing maize events (i.e. MON 810 and Bt11) on (i) the non-target aphid *Rhopalosiphum maidis* in comparison with conventional (non-Bt) maize, and (ii) the predatory ladybird beetle *Propylea japonica* via the predation of *R. maidis*.

Material and methods

The development, longevity and fecundity of aphids feeding on Bt or non-Bt maize leaves were studied for three consecutive generations. In tritrophic assay, P. japonica (larvae and adults) were fed *ad libitum* aphids maintained on Bt maize MON 810, Bt11 or non-Bt maize (control) plants. Pre-imaginal developmental time, mortality, weight and reproductive parameters were estimated and compared between treatments. In addition, the transfer of Cry1Ab through the food chain (*Bt* maize $\rightarrow$ *R. maidis* $\rightarrow$ *P. japonica*) was determined using enzyme-linked immunosorbent assays (ELISA).

Results

The results of the ELISA showed that Cry1Ab concentrations in maize Bt11 were higher than those in MON 810 at all developmental stages. Also, Cry1Ab levels in Bt11 leaved increased as the plants developed from the 5-/6- to 12-/13-leaf stage, whereas in MON 810 did not. Cry1Ab levels in the aphid *R. maidis* decreased significantly, and no traces of Cry1Ab were detected in *P. japonica* preying on aphids fed Bt maize. In the bioassays with *R. maidis*, both Bt maize events had no negative effects on any of the biological parameters measured. Likewise, for *P. japonica*, no statistically significant differences were observed in lethal and sublethal endpoints between Bt and non-Bt treatments.

Conclusions

The authors concluded that the study *revealed no unexpected acute lethal or sublethal effects of Cry1Ab maize on P. japonica during their life cycle in a tritrophic system*.

Evaluation by EFSA

The methodology followed by Shu et al. (2019) is suitable to evaluate the prey-mediated effects of Cry1Ab expressed in the maize events MON 810 and Bt11 on the predatory ladybird beetle *P. japonica*. The results reported by Shu et al. (2019) show that the insecticidal protein Cry1Ab expressed in the maize events Bt11 and MON 810 does not have negative effects after *P. japonica* larvae when preying aphids maintained on maize Bt11 and MON 810 plants. The findings presented in the publication are consistent with those reported in similar tritrophic experimental set ups. Thus, the results do 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.

Szoboszlay M, Naether A, Mullins E and Tebbe CC, 2019. Annual replication is essential in evaluating the response of the soil microbiome to the genetic modification of maize in different biogeographical regions. Plos One, 1-23

Summary of the publication

The objective of this study was to evaluate the influence of field site locations in different geographical zones on the rhizosphere microbial community of Bt-maize MON810 in comparison to near isogenic control varieties. This was done already in previous studies but in the current study a new highly sensitive method was applied which allows the characterisation of structural and functional diversity of rhizomicrobiomes in more depth than previously.

Materials and methods

Four field sites located in Denmark, Slovakia, Spain and Sweden. At each site 10 plots of 10x10m width were sown with BT maize and near-isogenic non-BT cultivar. Each plot was surrounded by 5m wide strips of conventional maize. Rhizosphere samples were taken during flowering over 3 consecutive years. Soil was removed by shaking from the roots and the roots washed with with sterile saline solution to collect soil particles and microbial cells from the roots. 16S rRNA genes, *nir*K, *nir*S and fungal internal transcribed spacer (ITS) sequences were determined by quantitative PCR.

Statistical analysis of sequencing data.

Simpson´s diversity index was calculated for the 16S rRNA gene, fungal ITS sequence variants (SVs) and the nirK T SVs. BT and non-BT maize from the same site and year were compared by t-tests and differences between sites and years were analysed with ANOVA and Tukey`s HSD post hoc tests. 16S rRNA gene sequence variants (SVs) and nirK translated sequences variants (TSVs) which didn´t have at least 0.1% relative abundance and fungal ITS SVs with less than 1% abundance in at least one of the samples were removed from the PCA (Principal Component Analysis). To obtain sequence counts of taxa, SVs were merged according to their classification at a given taxonomic level. Analysis of Differential Abundance Taking Sample Variation Into Account (ALDEx2) was applied to bacterial and archaeal taxa which had at least 100 sequences in total in the compared samples.

Results

No statistically significant difference was observed in the abundance of bacterial 16S rRNA and nirS genes at any site in any of the sampling years. Greater abundance of archaeal 16S rRNA in non-BT in one year on one site. Fungal ITS sequences were more abundant in BT samples in one year in Sweden. The nirK copy number was higher in non-BT samples in one year in Slovakia and in Sweden. No significant difference in the Simpson diversity index was observed between BT and non-BT maize for 16SrRNA gene SVs and fungal ITS SVs. A lower Simpson diversity of nirK TSVs was observed in one year at one site. The Simpson index for 16S RNA showed no significant variation between sites and years with the exception of one site in one year. The diversity of nirK TSVs was significantly different in some sites in some years. The Simpson index values were lower for one site in two years. The principal component analysis (PCA) for 16S rRNA and ITS SVs did not separate BT and non-BT samples from the same site and year indicating that the microbial community structure was not influcenced by the maize genotype. A considerable year to year variation was observed in community structure and a clear separation was observed in the PCA between years in some sites. Similar results were obtained for the nirK TSVs. However, in one site the nirK samples for the non-BT

maize separate from previous years and from the BT maize in that year. Some genera had a statistically significant lower abundance in BT samples compared to non-BT samples in two sites in some years. These differences were not found in other locations. The community structure patterns were similar between BT and non-BT samples and differed most between the different sites.

Conclusions

The study shows that neither the abundance nor the diversity of markers characterizing the rhizobiome was affected by maize MON810. Distinct microbiomes were found at each site suggesting that the environmental conditions have a predominant influence on community structure. The biogeographical zone with the combined effects of soil type and climate was obviously more important in shaping the rhizomicrobiome than the maize cultivars. The response of vascular arbuscular (VA) mycorrhizal fungi to BT which was found in one greenhouse study could not be confirmed under field conditions. These fungi were found in very low abundance. The lack of mycorrhization is not unexpected because of excessive fertilization of maize.

In one year a significant difference between BT and non-BT was found in nirK TSVs on one site. In the other sites and years these differences were not observed. If only the date from that year would have been observed in isolation then it could wrongly be concluded that BT has an impact on the bacterial community involved in nitrification depending on the environmental factors at the test sites. To avoid such misconcpetions it is important to investigate not only different sites but also to include annual replication in such studies. The study demonstrates that BT maize MON810 has no tangible effects on the composition of the rhizomicrobiome. Sporadic differences found in abundance and sommunity structure were minor and isolated cases which were not consistently observed in subsequent years. The results suggest that annual replication is even more important than the consideration of field sites from different biogeographical zones.

Evaluation by EFSA

The methodology is appropriate and well described. The statistical evaluation of the data supports the conclusions of the authors.

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 den Berg J, 2019. Preference of Bt-resistant and susceptible *Busseola fusca* moths and larvae for Bt and non-Bt maize. Entomologia Experimentalis et Applicata, 167, 849-867.

Summary of the publication

Aim

In their study, Visser et al. (2019) tested one of the assumptions of the high-dose/refuge strategy on the lepidopteran pest of maize *Busseola fusca* (Lepidoptera: Noctuidae), i.e., that *B. fusca* adults and larvae do not show any preference for either *Bt* or non-*Bt* maize plants.

Material and methods

The authors assessed the feeding and oviposition preference of *B. fusca* larvae and gravid females using *Bt*-susceptible and resistant populations and *Bt* (events MON 810 and MON 89034) and non-Bt maize plants in choice test bioassays in the laboratory.

Results

The results of the choice tests assays with *B. fusca* females revealed no differential oviposition preference for either resistant or susceptible individuals whereas *B. fusca* neonated were able to detect *Bt* proteins and avoided feeding on *Bt* maize leaves.

Conclusion

The authors of the study concluded that migration behaviour of *B. fusca* larvae and not oviposition preference will determine the choice of refuge structure in insect resistance management plants for delaying *Bt*-resistance evolution of B. fusca in South Africa. They recommended that the larval migration behaviour in *Bt* and non-*Bt* maize should be further assessed to determine the best refuge strategy (structured refuges or seed blends/refuge-in-the bag).

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. (2019) on this maize pest are of no direct relevance to the cultivation of maize MON 810. Therefore, the publication by Visser et al. (2019) 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, 2020. Plant abandonment by *Busseola fusca* (Lepidoptera: Noctuidae) larvae: Do Bt toxins have an effect? Insects, 77, 1-11.

Summary of the publication

Aim

In their study, Visser et al. (2020) determined whether larvae of *Busseola fusca* (Lepidoptera: Noctuidae) are more likely to abandon genetically modified plants expressing insecticidal Cry1Ab protein from *Bacillus thuringiensis* (Bt) than conventional non-Bt plants, and if resistance to the Cry1Ab protein affects this behavior.

Material and methods

Two *B. fusca* populations were used in the study: a Cry1Ab-susceptible population collected in the Eastern Cape region (South Africa) and a a Cry1Ab-resistant population collected in the Harrismith region (South Africa). Three maize hybrids representing two different Bt treatments and a control were used: a single-gene Cry1Ab-expressing variety derived from event MON 810), a pyramid hybrid expressing Cry1.105 + Cry2Ab2 proteins derived from event MON 89034), and a near-isogenic non-Bt hybrid serving as a control. Preliminary bioassays confirmed that approximately 90% of early instars from the resistant population could survive when feeding on maize MON 810 plants for 10 days. To determine the avoidance behavior of B. fusca larvae on *Bt* maize, 30 neonates larvae of either the Cry1Ab-resistant or the susceptible population were placed in individual potted maize plants. The number of larvae that abandoned plants by means of ballooning onto the sticky trap surface was recorded daily for four consecutive days.

Conclusion

For the susceptible and the resistant populations, the number of *B. fusca* larvae abandoning the inoculated plant was higher on plants of both *Bt* events when compared to the conventional maize plants. The authors attributed such differences to the Bt toxin avoidance behavior displayed by the larvae. Visser et al. (2020) recommend that the potential impact of *B. fusca* migration within maize fields on current insect resistance management strategies implemented in South Africa should be further investigated.

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. (2020) on this maize pest are of no direct relevance to the cultivation of maize MON 810. Therefore, the publication by Visser et al. (2020) does not change former risk assessment conclusions on and risk management recommendations for maize MON 810.

Xu H, Wang X, Chi G, Tan B and Wang J, 2019. Effects of *Bacillus thuringiensis* Genetic Engineering on Induced Volatile Organic Compounds Emission in Maize and the Attractiveness to a Parasitic Wasp. Frontiers in Bioengineering and Biotechnology, 1-9.

Summary of the publication

Aim

Herbivore-induced plant volatiles are an important defence mechanism of plants as these compounds attract natural enemies of the herbivorous species. The aim of the study was to investigate differences in the amounts of plant volatiles released after plant tissue damage by two BT maize cultivars and their near isogenic non-BT cultivar.

Materials and methods

Maize seedlings (14 d old) were damaged mechanically (scissors cuts 1cm long x 15 times on 2nd and 3rd leaves) and the regurgitant of *Spodoptera litura* was added to the damaged tissue. Control plants were also treated with the plant hormone jasmonic acid which triggers similar defence responses in plants. Each plant was placed into a glass container. The headspace air was pumped through a filter to collect and volatile organic compounds. The volatile organic compounds were identified by mass spectrometry and quantified by gas chromatography. Olfactory preference to the volatile compounds of the different maize plants were tested with the parasitoid *T. ostriniae* in a Y-tube olfactometer.

Results

The Bt maize plants were as attractive as their nearly isogenic non-Bt line to the parasitoid *T. ostriniae*, when they were applied with caterpillar regurgitant or treated by jasmonic acid. All three maize cultivars released the same 11 main volatile compounds. One of the BT maize cultivars released more volatile compounds compared to the other BT cultivar and non-BT mais when induced with caterpillar regurgitant and jasmonic acid.

Conclusion

In conclusion, transformations of foreign genes to crops may change their volatile organic compounds emission. However, the variations are normally less apparent than those among conventional cultivars. Importantly, the modifications of volatile organic components emission normally do not reduce the attractiveness of GM plants to natural enemies. The findings indicate that releasing natural enemies is still likely to be an effective way to control non-target or Bt-resistant pests in Bt fields.

Evaluation by EFSA

The methods applied are appropriate for the objective of the study and the statistical evaluation supports the conclusions of the authors. The same volatile compounds were observed in BT and non-BT maize cultivars. The amounts of volatile compounds were greater in one of the two BT maize cultivars. However, no difference was observed regarding the attractiveness of the different cultivars when damaged and treated with caterpillar regurgitant or jasmonic acid to the parasitoid *T. ostriniae.* 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.

Comparative assessment

Holderbaum DF, Traavik TI, Onofre Nodari R and Guerra MP, 2019. Comparison of in vitro callus-cultures from transgenic maize AG-5011YG (MON810) and conventional near-isogenic maize AG-5011. Crop Breeding and Applied Biotechnology, 19, 169-175.

Summary of the publication

Aim

Possible unintended effects due to the insertion of the recombinant gene were investigated comparing morphological parameters of calli generated from MON810 (AG5011YG) and its near isogenic line (NIH AG5011). In particular, the objective of this study was to compare induction rate, frequency of friable calli, and morphogenesis responses of AG5011YG and NIH AG-5011

Materials and methods

Mature zygotic embryos were excited from AG5011YG and NIH AG5011 seeds and placed on germination media. The obtained plantlets were used to explant thin cell layer (TCL) from the shoot apical meristem (SEM) and from root segments. These were transferred on nutrient media supplemented with a gradient of used 2,4D concentrations (0, 4 and 17 µM for SEM; 0, 13, 17 and 21 µM for root segments) to induce callus formation. 229 SEM TCLs and 576 root segments were inoculated. Callus friability (friable vs soft) and morphogenesis were evaluated at 4 and 8 weeks after induction. The collected data were analysed in a generalized linear mixed model.

Results

Callus induction: Calli were effectively induced by 2,4-D on SEM TCLs and root segments. A significant difference in the callus induction from root segments between AG5011YG and NIH AG5011 at the highest 2,4D concentrations.

Callus friability: Friability was enhanced by 2,4-D concentration with the NIH AG5011 showing a stronger response to 2,4-D producing significantly more friable calli.

Conclusion

The study indicates a different response in callus formation and friability to certain 2,4-D concentrations in explants collected from AG5011YG and NIH AG5011 seedlings. The authors conclude that this is a novel approach to perform comparison between GM maize and their non-GM counterparts that can provide information on off-target effects.

Evaluation by EFSA

The approach aims at identifying under *in-vitro* conditions phenotypic differences between maize hybrid carrying MON810 event and the near isogenic line. It is considered that potential unintended effects identified in MON810 maize hybrid under *in-vitro* conditions, cannot be considered directly relevant for the ERA and FF RA of maize MON810. It is considered that the identified effects on an undifferentiated tissue (callus) cannot be translated to a plant grown under natural conditions. It cannot be excluded that the observed changed response

to 2,4-D is attributed to differences elsewhere in the genome and not specifically to the alteration due to the recombinant construct's integration. In conclusion the article does not provide information pointing to new hazards, modified exposure, or new scientific uncertainties that would change former risk assessment conclusions on and risk management recommendations

Food and feed safety assessment

Al-Harbi A, Sahira L, Edwards MG, Qusti S, Cockburn A, Poulsen M and Gatehouse AMR, 2019: A proteomic-based approach to study underlying molecular responses of the small intestine of Wistar rats to genetically modified corn (MON810)

Summary of the publication

Aim

Differential gene expression in the epithelial cells of the small intestine of rats fed for 7 or 28 days diets containing MON810, its near isogenic line, two conventional corn varieties, and a commercial corn-based control diet were investigated using comparative proteomic profiling, to address this important knowledge gap and to gain insights into the underlying molecular responses in rat to MON810.

Materials and methods

Omics-based technologies enable mechanistic understanding of toxicological or nutritional events at the cellular/receptor level.

Statistical analysis

Significant differences were tested using the parametric analysis of variance (one-way ANOVA) and the nonparametric analysis of variance (Kruskal–wallis test), followed by Tukey Test. The IBM SPSS statistics 20 and Minitab 16 programs were used to perform statistical analyses. Differences were considered to be statistically significant when the p value was < 0.05 and highly significant when the p value was < 0.001.

Results

Pairwise and five-way comparisons showed that the majority of proteins that were differentially expressed in the small intestine epithelial cells in response to consumption of the different diets in both 7-day and 28-day studies were related to lipid and carbohydrate metabolism and protein biosynthesis.

Conclusions

Irrespective of the diet, a limited number of stress-related proteins were shown to be differentially expressed between diets; these stress-related proteins are good indicators of a range of different types of physical and psychological stress, including first exposure to new food components prior to immune recognition/tolerance, infection, inflammation, exercise, exposure of the cell to toxins, starvation, hypoxia, water deprivation or anxiety. Therefore, results suggest that these stress-related proteins found in MON810-fed rats are not directly related to the consumption of MON810 corn diet, but are due to normal physiological variation between rats. These findings suggest that MON810 has negligible effects on the small intestine

of rats at the cellular level compared with the well-documented toxicity observed in susceptible insects.

Evaluation by EFSA

The methods applied are appropriate for the aim of the study. The results support the conclusions of the authors. 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.

Corujo M, Pla M, van Dijk J, Voorhuijzen M, Staats M, Slot M, Lommen A, Barros E, Nadal A, Puigdomènech P, La Paz JL, van der Voet H and Kok E, 2019 Use of omics analytical methods in the study of genetically modified maize varieties tested in 90 days feeding trials

Coumoul X, Servien R, Juricek L, Kaddouch-Amar Y, Lippi Y, Berthelot L, Naylies C, Morvan ML, Antignac JP, Desdoits-Lethimonier C, Jegou B, Tremblay-Franco M, Canlet C, Debrauwer L, Le Gall C, Laurent J, Gouraud PA, Cravedi JP, Jeunesse E, Savy N, Dandere-Abdoulkarim K, Arnich N, Fourès F, Cotton J, Broudin S, Corman B, Moing A, Laporte B, Richard Forget F, Barouki R, Rogowsky P and Salles B, 2019. The GMO90+ Project: Absence of Evidence for Biologically Meaningful Effects of Genetically Modified Maize-based Diets on Wistar Rats After 6-Months Feeding Comparative Trial. Toxicological Sciences, 168(2), 315-338

The studies of Corujo et al. 2019 and Coumoul et al. 2019 were 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 2, supporting information to EFSA 2020[2]).

[2] EFSA (European Food Safety Authority), Álvarez F, Georgiadis M, Messéan A and Streissl F, 2020. Assessment of the 2018 post-market environmental monitoring report on the cultivation of genetically modified maize MON 810 in the EU. EFSA Journal 2020;18(10):6245, 42 pp. https://doi.org/10.2903/j.efsa.2020.6245, Supporting information, Annex 4, https://efsa.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.2903%2Fj.efsa.2020.6245&file=efs26245-sup-0004-Annex-4.pdf

Mesnage R, Biserni M, Antoniou MN, Le Roy C and Salles B, 2019. Relationship between faecal microbiota and plasma metabolome in rats fed NK603 and MON810 GM maize from the GMO90+ study. Food and Chemical Toxicology,Volume 131,2019, 110547, ISSN 0278-6915, https://doi.org/10.1016/j.fct.2019.05.055.

Summary of the publication

Aim

The aim of the study was to investigate potential effects of NK603 and MON810 maize on the composition of the gut microbiota in Wistar rats. This study was conducted on rats from the GMO90+ study (Coumoul et al., 2019).

Materials and methods

Following 6-month feeding with diets containing NK603 (treated with conventional herbicides only or with Roundup) or MON810 maize at 11 and 33% incorporation rate, or diets containing the same quantities of the closest isogenic non-GM maize varieties, faecal samples were collected by placing rats in individual metabolism cages for 24 h at week 25/26 (total number of samples collected=189).

The composition of the faecal microbiota was evaluated by quantifying the relative abundance of 16S rRNA gene hypervariable regions. Faecal genomic DNA was extracted and quantified and used to generate amplicons covering V3 and V4 16S rDNA gene hypervariable regions of bacteria and Archaea. Sequencing libraries were validated and quantified. Sequencing was performed and image analysis and base calling conducted by dedicated software. Three technical replicates were performed. Plasma metabolomics was also conducted, as described in a preceding publication (Coumoul et al., 2019).

Statistical analysis

The data analysis was performed using centered log-ratio (clr) transformed relative abundance values. The statistical significance of differences in taxa abundance was conducted by fitting an ANOVA model, with the sex of the animal as covariate. Metabolomics data were mean centred and normalised prior to analysis. Gender-based analysis was conducted, since unsupervised principal component analysis (PCA) of plasma metabolome showed that male and female rats had very distinct metabolic profiles as previously reported (Coumoul et al., 2019).

Results

A total of 1530 Exact Sequence variants (ESV) were detected. These corresponded to 1529 bacterial ESV and 1 archea (Methanobacteria). A total of 6 different phyla were detected. Firmicutes represented most of the faecal microbiota, in particular Ruminococcaceae and Lachnospiraceae families. A total of 72 and 19 distinct genera and species were identified.

No differences in the faecal microbiota composition between control non-GM maize fed groups and GM maize test groups were seen. In addition, no effect on the faecal microbiota was seen when comparing groups given MON810 and NK603 diets.

Metabolomic gender separation was noted, mostly driven by higher carnitine, methylhistamine, butyril-carnitine, L-threonic acid, carnosine and acetylcholine in males and an higher lysine, 5-methyldeoxycytidine, corticosterone glycyl-L-leucine, N6-acetly-L-lysine, tryptophane, perillic acid and indoleacrylic acid in female animals.

The authors report that the faecal microbiota composition was closely correlated with the plasma metabolite profile in a gender-specific manner, with two genera in males (Coriobacteriaceae and Acetatifactor) and two in females (Bifidobacterium and Ruminococcus) significantly being able to predict the plasma metabolic profile in males and females respectively. Coriobacteriaceae relative abundance was significantly associated with 29 metabolites including L-anserine, carnosine, leucine/proline (positively) and allantoin cinnamic acid perillic acid (negatively). Acetatifactor was significantly associated with 30 metabolites including one bile acid (lithocholic acid-3 sulphate –positive-) and three N-acetyl amino acids (arginine, asparagine and glutamine –positive-). In female rats, Bifidobacterium was positively associated with four bile acids (deoxycholic acid, cholic acid, lithocholic acid and lithocholic acid 3 sulphate) and Ruminococcus with 39 metabolites.

Conclusions

The authors conclude that the consumption of maize NK603 and MON810 up to 33% dietary incorporation rate for 6 months had no effect on the status of the rat faecal microbiota community profile compared to non-GM near isogenic lines. This is similar to findings of a previous study (Li et al., 2018). In addition, no differences between rats fed the two GM diets were noted.

By integrating results from the faecal microbiota analysis and plasma metabolome data, the authors found that some bacterial taxa could be used as predictors of the rat plasma metabolite profile, in a gender specific manner. The metabolic profile is reported to be dominated by markers of protein metabolism in males (carnitine, carnosine and butyryl-carnitine) and positively associated with Coriobacteriaceae relative abundance. In females, Bifidobacterium an Ruminococcus were positively associated with several primary and secondary bile acids. Although it is not directly relevant for the effects of GM maize, this set of data can inform on general aspects of the interface between the gut microbiota and the blood metabolome in laboratory rodents.

Evaluation by EFSA

The authors used appropriate methodologies. 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.

Stein T, GuangYao R, Bohmer M, Sharbati S and Einspanier R, 2019: Expression profiling of key pathways in rat liver after a one-year feeding trial with transgenic maize MON810

Summary of the publication

Aim

The aim of the study was to characterise the liver transcript profiles of key cellular pathways to identify any possible gene expression changes not manifested on morphological level after long-term feeding of rats. The study was conducted as a follow up to a 1-year feeding study in Wistar Han RCC rats and MON810 where no adverse effects were observed while in previous studies potential hepatotoxic effects were found.

Material and methods

The analysis on the rat liver was focussed in particular on the expression of genes associated with the major stress pathways; expression of RNA coding for proteins of the apoptosis-, NF-kB-, DNA damage response- (DDR), and the unfolded-protein response (UPR) pathways were investigated by RT-qPCR on total RNA and partly by Western blot.

Statistical analysis

All data was expressed as mean ΔCt ± standard deviation (SD) from eight samples for each group and processed using Excel. Two-sided student T-Tests were used to test for significance of difference of the means. $P \leq 0.05$ was considered statistically significant. Unsupervised hierarchical (HOPACH) clustering of individual samples was performed and visualised using the default settings of the open-source ALTANALYZE software (http://www.AltAnalyze.org) based on the ΔCt values with in-row normalisation to the respective median.

Results

Consistent with the previous results, new data did not describe a gene expression pattern consistent with any short or long term adverse effect. With the exception of Birc2, none of the RNAs showed consistent changes in abundance of the non-stringent cut-off of ≥1.5-fold in both male and female rats. Despite the increase in Birc2 RNA, this did not translate into a corresponding protein increase. Because of this result, and the absence of any other corresponding significant transcript changes that reflect the changes seen for Birc2 in a positive or negative way, it was considered unlikely that this difference was indeed biologically relevant.

Conclusion

The new data provided do not detect consistent significant alterations in rat liver gene expression that would indicate an adverse effect after long-term feeding of MON810 compared to feeding with isogenic control maize.

Evaluation by EFSA

The methods applied are appropriate for the objective of the study. The results support the conclusion that long-term feeding of MON810 does not lead to significant alterations in rat liver gene expression. 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